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Effect of Inoculation with *Klebsiella oxytoca* '10 mkr 7' on *Striga* Suicidal Germination in *Zea mays*

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Abstract: The *Striga* species are obligate root parasitic plants. Plant growth promoting rhizobacteria could enhance *Striga* suicidal germination. In screen-house studies we quantified the effect of bacterial inoculation with increasing maize plant density on the parasitic weed *Striga hermonthica*. The design used was a randomized complete block with a factorial design (4 plant density levels, 2 *S. hermonthica* infestation levels, artificial and natural and 2 inoculation levels, with and without isolate). Interactions between bacteria and *Striga* count varied significantly (p=0.05). Seed germination differed significantly among plant densities from 6 to 8 WAP in the absence of bacteria. In bacterial inoculated pots significant differences were observed among plant densities from 5 WAP. Maize planted on soil inoculated with *Klebsiella oxytoca* '10mkr7' had higher *Striga* counts at harvest for all plant densities. Significant variation was observed for interactions between bacteria and Striga. These findings suggest that *K. oxytoca* '10mkr7' is a plant growth promoter and could stimulate *Striga* suicidal germination. Hence, there are good prospects for biological control of *Striga* using indigenous rhizosphere *K. oxytoca* '10mkr7'

Key words: Maize % plant density % *Klebsiella oxytoca* % *Striga hermonthica* % suicidal germination

INTRODUCTION

Studies conducted on plant competition to determine the optimum plant population density of maize [1-3] showed that there was no single recommendation for all environments because optimum plant density varies, depending on the environmental factors, such as soil fertility, planting date and planting pattern [4]. Proximity to adjacent plants affects maize plant yield [5]. Plant population above a critical density has a negative effect on yield per plant due to the effects of interplant competition for light, water, nutrition and other potential yield-limiting environmental factors [2, 5]. Moreover, higher plant densities are encouraged for germplasm improvement in order to facilitate foraging of the unwanted plants [6]. *Striga* and *Alectra*, which are agriculturally important genera of the family *Scrophulariaceae* originated from Africa [7]. The purpose of this work was to test the hypothesis that an increased host root system will support higher numbers of *Striga hermonthica* in the presence of potential stimulatory rhizosphere bacteria, with a view to developing effective biological control programs.

MATERIALS AND METHODS

Seeds of the local maize variety 'Nyamula' from western Kenya were stored in plastic containers in the refrigerator at 7°C. Seeds of *Striga hermonthica,* collected in Kenya (Kenya Agricultural Research Institute (KARI)-Kisumu), from maize fields (cultivars unknown) during the 2000 long rainy season, were surface sterilized in 1% NaOCl solution for 5 min with continuous stirring. Several changes of sterile water were used to remove the sterilant from the seeds and discarded the floating seeds. Air-dried seeds were stored in small bottles for use when required.

The bacterium '10mkr7' was isolated from the rhizosphere of sorghum plant (local variety Mokwa) in 1998. It had been maintained on 35% glycerol at -80°C and cloned in Petri dishes using King's Medium B (15 g agar, 15 g Proteose peptone, 1.5 g K_2HPO_4, 1.5g $MgSO_4$, 12.6 g glycerol, 1L of distilled water). Incubation was at 28±1°C for 24 hours. Experiments were planted on 31st January and 16th March, 2001 at KARI-Kisumu where the soils are naturally infested with *Striga*. *Klebsiella oxytoca* suspended in distilled water was used to

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inoculate planting holes, at approximately 2 cm deep and 1 cm wide, at a rate of 7.35 x 10^7 cfu ml^{-1} pot^{-1} at planting. Pots that served as blank control received sterile distilled water only.

Experimental design was a randomized complete block with a factorial design (4 plant density levels, 1, 2, 4 and 6; 2 *Striga* infestation levels, artificial and natural; and 2 isolate levels, with and without isolate) with 4 x 2 x 2 = 16 treatment combinations. There were 32 pots in the first experiment and 64 pots in the second. Each pot (32 x 32 cm) was sown at 4 density levels (1, 2, 3, or 4 maize plants pot^{-1}). Pots were arranged on screen-house floor. The experiment was performed twice. There were only two replications in the first experiment because of limited resources but four replications were performed in the second experiment The soil used was naturally infested but was also artificially infested (50 mg pot^{-1}) so that relative performance could be assessed. Precaution was taken in the management of the two infestation levels to avoid cross contamination by planting the naturally infested soil first before the planting of artificially infested soil. A period of 14 days was allowed for *S. hermonthica* preconditioning. At 2 weeks after planting (WAP), the seedlings were thinned to 1, 2, 4 or 6 plants pot^{-1}, depending on the allocated root density.

Data were collected on number of days to *S. hermonthica* emergence. At the end of the growing period, visual rating of *S. hermonthica* growth was evaluated on a scale of 1 to 6 at 12 WAP. Where,

1 = small plants, no flowering;
2 = medium plants, some flowering;
3 = medium plants, full flowering
4 = large plants, full flowering;
5 = large plants, some capsules;
6 = large plants, full capsules.

At 9 WAP, maize damage scores were evaluated on a scale of 1 to 9 (1 = no symptoms and 9 = all plants dead or dying). Details of the rating are as follows [8]

1 = Normal plant growth, no visible symptoms.
2 = Small and vague. Purplish-brown leaf blotches visible.
3 = Mild leaf blotching, with some purplish-brown necrotic spots.
4 = Extensive blotching and mild wilting. Slight but noticeable stunting and reduction in ear and tassel size.
5 = Extensive leaf blotching, wilting and some scorching. Moderate stunting, ear and tassel size reduction.
6 = Extensive leaf scorching with mostly grey necrotic spots. Some stunting and reduction in stem diameter, ear size and tassel size.
7 = Definite leaf scorching, with grey necrotic spots and leaf wilting and rolling. Severe stunting and reduction in stem diameter, ear size and tassel size, often causing stalk lodging, brittleness and husk opening at a late-growing stage.
8 = Definite leaf scorching, with extensive grey necrotic spots. Conspicuous stunting, leaf wilting, rolling, severe stalk lodging and brittleness. Reduction in stem diameter, ear size and tassel size.
9 = Complete scorching of all leaves, causing premature death or collapse of host plant and no ear formation.

The maize plants from each pot were removed and dried at 70°C to a constant weight. *Striga* count data were subjected to square root transformation for analysis. Correlations were calculated between the variables measured. Combined analysis of variance was conducted on data between experimental dates to identify a high and stable average effect. The linear model of SAS version 9.1 [9] was used to analyze the data collected. Means were separated using a Tukey HSD test. Significance level was P = 0.05 in all tests except otherwise stated.

RESULTS

Mean squares of *Striga* count from 5 to 8 WAP were highly significant (P<0.01) (Table 1). At 5 WAP no interaction was significant (Table 1). At 6, 7 and 8 WAP the mean squares for *Striga* counts for Bacteria x *Striga*, Plant density and *Striga* x plant density were significant. As bacteria became significant, the interaction of bacteria with *Striga* also became significant (Table 1).

All through the experiment, *Striga* emergence count increase from 5 to 8 WAP (Table 2). A similar trend of *Striga* counts was observed between host plant population densities: with *Striga* counts increasing as plant population increased (Table 2).

For plant population of 6, *Striga* emergence count differed significantly among plant densities from 6 to 8 WAP in the absence of *Klebsiella oxytoca* (Table 3). In inoculated pots, *Striga* counts at 7 WAP were not significantly different among plant densities under

Table 1: Mean squares and significant levels from analyses of variance of *Striga* emergence count at two bacteria levels, two *Striga* levels and four plant populations

		Mean squares and significance levels (WAP)			
Source	Df	5	6	7	8
Corrected total	95				
Bacteria	1	0.38	3.32	17.61**	28.71**
Striga	1	46.70**	202.45**	402.71**	497.98**
Bacteria x *Striga*	1	0.69	3.30*	14.04**	19.46**
Plant density	3	4.00	6.48**	4.92*	18.62**
Striga x plant density	3	3.89	6.32*	4.42*	11.83*
Error	80	0.54	1.01	1.08	1.24
R-square		0.64	0.75	0.84	0.85
CV (%)		51.4	44.9	32.41	29.56

Table 2: Mean *Striga* emergence counts of maize at two bacteria levels, two *Striga* levels and four plant populations from week 5 to 9 after planting

		Striga emergence counts (WAP)[a]				
Treatments	Levels	5	6	7	8	9
Bacteria	Control	1.36a	2.14a	2.78b	3.22b	3.40b
	Inoculated	1.49a	2.51a	3.63a	4.31a	4.25a
	LSD (P = 0.05)	0.30	0.42	0.42	0.45	0.43
Striga	Natural	0.73b	0.87b	1.16b	1.49b	1.89b
	Artificial	2.12a	3.78a	5.25a	6.04a	5.75a
	LSD (P=0.05)	0.29	0.42	0.42	0.45	0.43
Density	1	0.71b	1.72c	2.64b	3.19c	3.33b
(Plants/pot)	2	1.17b	2.08bc	3.04ab	3.51bc	3.81ab
	4	1.68a	2.64ab	3.55a	4.30a	4.34a
	6	1.85a	2.86a	3.59a	4.06ab	3.81ab
	LSD (P = 0.05)	0.42	0.60	0.59	0.64	0.61

[a]Values presented are log-transformed data

Table 3: Mean squares and LSD for *Striga* emergence counts made with or without artificial bacterial inoculation with *Klebsiella oxytoca* in the soil at planting for four plant populations

		Striga emergence count (WAP)[a]			
t	Plant population	5	6	7	8
	Inoculated				
	1	1.03b	2.60b	5.18a	6.24bc
	2	1.57b	3.25ab	5.13a	5.86c
	4	3.16a	5.33a	6.83a	8.18a
	6	3.32a	5.41a	7.11a	7.88ab
	LSD (0.05)	1.34	2.17	2.03	1.90
	Non-inoculated				
	1	1.55a	2.77b	3.69b	4.24b
	2	1.60a	3.06b	4.12b	4.83ab
	4	2.16a	3.46ab	4.55ab	5.38ab
	6	2.59a	4.34a	5.40a	5.72a
	LSD (0.05)	1.14	1.15	1.18	1.35

[a]Means with the same letter within column of the same bacterial treatment level are not significantly different from each other (P = 0.05) according to Tukey HSD test

Table 4: Mean squares from ANOVA of *Striga* indices and maize damage score from maize at four plant densities under screen-house conditions

		Mean squares			
	Df	Days to emergence	Visual rating of *Striga* (1 to 6)[b]	*Striga* fresh weight	Maize damage score (1 to 9)[c] Source 9 WAP
Corrected total	95				
Bacteria	1	2.99	1.50	2524.06*	2.04
Striga	1	8361.01***	42.67***	21454.75***	330.04***
Bacteria x *Striga*	1	2.16	0.37	3627.89**	7.04**
Plant density	3	96.91	2.74**	385.35	3.26*
Striga x plant density	3	22.92	1.19	596.18	1.04

Df = degree of freedom, [a]*, **, *** Significant at p = 0.05, 0.01 and 0.001, respectively according to Tukey's studentized range (HSD Test), [b]Visual rating of *Striga* growth (1 to 6): 1 = small plants, no flowering; 6 = large plants, full capsules, [c]Maize damage score on pot basis: 1 = no damage, 9 = highest damage deathG[l]

Table 5: Maize damage scores and yield components of maize (var. Nyamula) averaged over bacterial treatments, *Striga* infestation treatments and plant densities

Treatments	Levels of treatment	Plant height (cm)	Ear number (no/pot)	Maize damage score(1to9)[a]	Ear weight (g)	Stover dry weight (g)
Bacteria	Control	94.79b	0.75a	6.63a	10.06a	48.94b
	Inoculated	113.85a	0.77b	6.33b	10.65a	71.19a
	LSD $_{(0.05)}$	13.13	0.01	0.17	5.03	17.49
Striga infestation	Natural	153.79a	1.33a	4.63b	19.90a	96.30a
	Artificial	54.85b	0.19b	8.33a	0.81b	23.29b
	LSD $_{(0.05)}$	13.13	0.32	0.47	5.03	18.15
Density (Plants/pot)	1	121.04a	0.46a	6.08a	12.85a	51.75a
	2	108.75ab	0.83a	6.25a	13.29a	60.60a
	4	102.20ab	0.83a	6.75a	10.16a	52.87a
	6	85.29b	0.92a	6.83a	5.12a	75.53a
	LSD $_{(0.05)}$	24.49	0.59	0.87	9.38	33.85[a]

Maize damage score on pot basis: 1= no damage, 9 = highest damage

Means with the same letter within column of the same treatment level are not significantly different from each other (P = 0.05) according to Tukey HSD test

infestation. Data of *Striga* emergence only give a partial view of the complex interactions of factors [8]. Plants from soil inoculated with *K. oxytoca* had higher *Striga* counts at harvest for all plant densities examined (Table 3). In the non-inoculated control all plant densities examined showed a reduction in the number of *Striga* shoots (Table 3).

K. oxytoca '10mkr7' significantly ($P < 0.05$) enhanced *Striga* fresh weight. For the number of days to *Striga* emergence, only *Striga* infestation was significant (P<0.001) among the main effects. Significant variation was observed for interactions of bacteria X *Striga* infestation for *Striga* fresh weight (P<0.01) and maize damage score (P<0.01), (Table 4). The interactions of bacteria and plant density were not significant and therefore have not been presented.

Data from the repeated experiments showed that the presence of bacteria increased the host plant yield components over the non-inoculated control with consequent significant reduction in maize damage score (Table 5). This is particularly true of the artificially infested pots, where the average increases recorded were more than 100% for maize plant height, ear weight and stover biomass (Table 5). On the average, a 10.47% decrease was recorded for maize damage score in the inoculated pots. This is to suggest that *K. oxytoca* '10mkr7' confers strength on the host plant by increasing available nutrients (Table 5), as concluded previously by

Skipper *et al*. [10]. The increase in yield components brought about by bacteria averaged between 0.05 and 33.9% for the naturally infested pots over the four plant densities (Table 5).

Among the plant densities considered, the effect of plant height was significantly different (Table 5). This is consistent with the report of Singh *et al*. [11] that plant density is known to affect *Striga* infestation. As plant density increases, plant height decreases (Table 5). The tallest plant height was recorded in the treatment that had one plant per pot, however it supported the lowest ear number. Maize damage score increased as plant density increased from the population of 1/pot to 6/pot though not to a significant extent (Table 5). Plant density of 6/pot supported the greatest stover biomass but the biomass was not statistically significant. From this study, the optimal plant population density on maize is 2/pot (Table 5). In the presence of bacteria, under artificial *S. hermonthica* infestation, a consistent trend of decreasing yield components with increasing plant population was observed. For example, plant height decreased from 96.00 cm to 58.50 cm as plant population increased from one plant to 6 maize plants/pot. No consistency was observed in the trend of yield components among plant densities in the absence of bacteria (Data not shown).

DISCUSSION

Results from our screenhouse-grown plants are similar to the field findings of Kim [8] who reported an increase in *Striga* emergence count in maize from 5 to 8 WAP when the highest numbers of emerged *Striga* were observed. The one-week difference in the first day of emergence between our report and that of Kim [8] could be associated with the *Striga* depth in the soil. The coefficient of variation in *Striga* counts was observed to decrease consistently from 5 to 8WAP when the host plant was harvested. The most discouraging aspect of our study was the high CV but this CV is associated with *Striga* count data. Kim *et al*. [12] reported a much higher CV of 61% for *Striga* emergence on their study on Synthetic maize population.

Striga emergence counts varied little between the natural and the artificially infested pots for bacterial effect at 5 to 6 WAP. The insignificant difference observed between the inoculated and the non-inoculated pots for early *Striga* counts (5 WAP) might be due to reduced microbial activity in the subsurface horizons. However, from 5 to 8 WAP, *Striga* counts between the 2 levels of infection had significant differences. In the presence of bacteria, *Striga* counts were greater (Table 2). This was expected and the result is consistent with others findings [13]. The LSD between the naturally infested and the artificially infested pots widened as the plants approached maturity. The *Striga* plants in artificially infested pots outnumbered those in naturally infested pots. Again, the LSD gradually increased from 5 to 8 WAP. Maize plants grown in pots artificially inoculated with bacteria did not support increasing counts of *Striga* shoot to harvest, however the *Striga* count recorded was still significantly different from the number in the non-inoculated pots despite the numerical decrease in the *Striga* counts. One reason for the reduced efficacy might be that other soil bacteria outgrew the inoculated isolate. However, all evidence points toward the rhizosphere competence of *K. oxytoca* ‘10mkr7’.

Though *Striga* emergence count reduced for plant population 6 by harvest, the linear trend of increasing *Striga* counts with plant population was still observed. Results from this study support an earlier report by Carsky *et al*. [14] which stated that increasing maize plant density did not result in lower emerged *S. hermonthica*. In the two instances (January and March) investigated, the environmental soil was not *Striga*-free hence the naturally infested experimental soil. This has been established by another study by the authors. The repeated artificial infestation of the soil in the research station could also help to explain the high *Striga* count observed in the non-infested pots.

These results are in agreement with those of Singh *et al*. [11]. A singular factor might be the specific bacteria in question. Previous findings corroborate this research findings have been published by Babalola *et al*. [15] and Babalola *et al*. [16]. In our experiment, the bacterium seems to be a plant growth promoter. Several factors could be responsible for the performance of the isolate. Among such factors is competition between the introduced isolate and the already established bacteria in the rhizosphere, each of which is likely to have a different biotic potential. Environmental factors might also have contributed to the result obtained by retarding the carrying capacity of the isolate and thereby interfering with their profound need for survival.

Several authors [17] have reported reduced harvest index as plant density increased. It is evident from the above results that plant height, ear weight and stover dry matter decrease as the plant density increases from 1 to 6/pot. Increasing the plant density increases the proportion of plants without an ear. This could be due to plant competition for nutrient and available resources in the potted soil. Previous research works [18] confirmed this. Esechie [18] and [19] also previously reported that increasing maize plant density resulted in smaller maize ears.

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