
Volume 76, April 2014
ISSN 0929-1393
ELSEVIER
Applied Soil Ecology

Applied Soil Ecology 76 (2014) 68–77

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Compatibility of *Rhizobium* inoculant and water hyacinth compost formulations in Rosecoco bean and consequences on *Aphis fabae* and *Colletotrichum lindemuthianum* infestations

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ARTICLE INFO

Article history:
Received 10 September 2013
Received in revised form
16 December 2013
Accepted 18 December 2013

Keywords:
Aphis fabae
Compost
Lake Victoria
Nitrogen
Rhizobium
Water hyacinth

ABSTRACT

The common bean, *Phaseolus vulgaris* is an important crop for food security and nitrogen fixation through *Rhizobium* symbiosis. Commercial *Rhizobium* inoculants are being promoted to fix nitrogen and enhance bean production in the Lake Victoria basin. *Rhizobium* symbiosis depends on nutrients, especially phosphorus, which is widely applied as diammonium phosphate (DAP) in the Lake Victoria basin. Water hyacinth, *Eichornia crassipes* (Mart.) Solms-Laubach (Pontederiaceae) is being developed into compost, with perceived benefits of improving crop production and limiting its disastrous spread in Lake Victoria. High nutrient content in water hyacinth compost can stimulate *Rhizobium* nodulation and nitrogen fixation, consequently improving plant growth and pest resistance. However, it is not yet established whether *Rhizobium* inoculants and water hyacinth composts are compatible options for plant growth promotion and pest suppression in beans. A field experiment with two trials was conducted to assess the compatibility of commercial *Rhizobium* inoculant, DAP, cattle farmyard manure (FYM), and four formulations of water hyacinth compost i.e., water hyacinth only (H), with molasses (H+Mol), cattle manure culture (H+CMC) or effective microbes (H+EM). *Rhizobium* inoculated plants had high number of root nodules when grown with H+CMC and H+EM. Plants were large in size with short development period when grown with the composts, especially H+CMC and H+EM. Those grown with H+EM produced high number of flowers. *Rhizobium* inoculated plants had high anthracnose incidence than non-inoculated ones when grown with H+CMC. Those grown with H+EM had low anthracnose incidence, but was high in FYM. During flowering, *Rhizobium* inoculated plants had higher *Aphis fabae* population than non-inoculated ones when grown in FYM or without fertilizer. Those grown with H+EM had the lowest *A. fabae* population. Yields in water hyacinth compost were improved, especially for H+CMC in the second trial. DAP treated plants had more flowers and pods having heavy seeds, with low anthracnose and *A. fabae* infestations; but had low germination rates that reduced the yields. In conclusion, the commercial *Rhizobium* inoculant is predominantly compatible with water hyacinth compost formulations containing effective microbes and cattle manure culture, which could enhance tolerance of bean plants to aphids and possibly to anthracnose disease. These two water hyacinth compost formulations need further investigation for their potential in enhancing food production and alleviating the water hyacinth problem in the Lake Victoria basin.

1. Introduction

The common bean *Phaseolus vulgaris* L. (Fabaceae) is a very important crop for food security and nutrition worldwide. The crop is also important due to its symbiotic nitrogen fixing capacity, which contributes to improvement of soil fertility (Maingi et al., 2001; Bala et al., 2011). However, bean production in sub-Saharan

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http://dx.doi.org/10.1016/j.apsoil.2013.12.011

Africa has been declining over years. Such declines have been attributed to agronomic constraints such as inferior germplasms, low soil fertility, pests and diseases (Odendo et al., 2011). Bean varieties have been succumbing to anthracnose disease caused by *Colletotrichum lindemuthianum* (Beebe, 2012; Kharinda, 2013), as well as a complex of viruses transmitted by aphids (Omunyin et al., 1995; Beebe, 2012). Soils have been depleted of essential nutrients for bean production, particularly nitrogen and phosphorus (Kimani et al., 2007; Kimani and Tongoona, 2008).

Several strategies are being developed and applied to enhance bean production. These strategies include improving germplasm in terms of yield potential and pest resistance. For instance, high yielding bean varieties that are moderately resistant to anthracnose have been produced in Kenya (Wagara et al., 2003; Kharinda, 2013). Despite these efforts, abiotic stresses, especially low soil fertility has been hindering the performance of improved bean varieties (Jansa et al., 2011). Recently, there have been efforts to improve legume production through the application of symbiotic microbes such as *Rhizobium* species, which enhance bean production by fixing atmospheric nitrogen (Samac and Graham, 2007). Some symbiotic *Rhizobium* species also protect plants from pests and diseases through induced resistance (Elbadry et al., 2006; Dutta et al., 2008). However, the ability of *Rhizobium* species to fix nitrogen is limited by inadequate nutrients in soil, especially phosphorus (O'Hara et al., 1988; Graham and Vance, 2000). This has necessitated the application of synthetic fertilizers such as diammonium phosphate (DAP) and triple superphosphate (TSP) on beans. However, there have been reports of these synthetic fertilizers being incompatible with *Rhizobium* species through inhibition of legume nodulation (Waterer and Vessey, 1993). This has necessitated the use of organic soil amendments that are more compatible with *Rhizobium* species (Saini et al., 2004; Zengeni et al., 2006).

The overuse of synthetic fertilizers has also been linked to eutrophication of water bodies, causing problems such as the spread of water hyacinth *Eichhornia crassipes* (Mart.) Solms-Laubach (Pontederiaceae) in Lake Victoria within East Africa (Lung'ayia et al., 2001; Cavalli et al., 2009). To mitigate this problem of fertilizer-induced eutrophication, water hyacinth is being developed into compost for crop production, with an anticipated benefit of controlling the invasive weed in Lake Victoria while promoting organic farming. Water hyacinth-derived composts are rich in nutrients, especially nitrogen and phosphorus (Amoding et al., 1999; Okalebo et al., 2006; Gunnarsson and Petersen, 2007), which influence root colonization by *Rhizobium* species and enhance plant resistance to pathogens (Zahran, 1999). Four water hyacinth compost formulations have been developed for potential use within the Lake Victoria basin namely; water hyacinth compost only, water hyacinth compost enhanced with effective microbes (EM), water hyacinth compost with molasses, and water hyacinth compost with cattle manure culture (Naluyange, 2013; Osoro et al., 2014). Studies are being conducted on the identification of *Rhizobium* species native to potential areas for the application of water hyacinth compost within Lake Victoria basin (Muthini et al., 2014). However, we are not aware of any studies investigating compatibility of *Rhizobium* inoculants and the previously mentioned water hyacinth compost formulations.

The objective of the present study was to establish whether commercial *Rhizobium* inoculant and water hyacinth compost formulations are compatible in bean growth promotion, and whether they have any consequences on infestations by aphids and the anthracnose pathogen *C. lindemuthianum*.

2. Materials and methods

2.1. Experimental design

A field experiment was conducted at the Masinde Muliro University of Science and Technology (MMUST) farm (N 00 17.104', E 034° 45.874'; altitude 1561 m a.s.l.). The land had been fallow and colonized by the African couch grass *Digitaria scalarum* (Schweinf.) Chiov. (Poaceae) for over 5 years. Soils in this region have been classified as dystro-mollic Nitisols (FAO, 1974; Rota et al., 2006). The experiment was laid out in a randomized block design comprising a 2×6 factorial experiment with *Rhizobium* inoculum factor having two levels (with or without inoculation) and fertilizer factor with six levels i.e., no fertilizer (Non), diammonium phosphate fertilizer (DAP), water hyacinth compost only (H), water hyacinth compost+molasses (H+Mol), water hyacinth compost+effective microbes (H+EM), and water hyacinth compost+cattle manure culture (H+CMC). In the first trial, water hyacinth compost only was not applied, while in the second trial, cattle farmyard manure (FYM) prepared under the MMUST farm management replaced water hyacinth compost+molasses. Each of the resulting 12 treatment combinations (plots) had 25 plants (n) in 3 blocks (i.e., $N = 900$). Each plot was in form of a row containing the 25 plants spaced at 20 cm, with a distance of 40 cm between the plots, without border rows. The treatment rows were completely randomized to minimize non-experimental bias in sampling for natural infestations of aphids and anthracnose disease on bean plants. This experiment was conducted during the long rain season between 20th April to 30th July 2012, and then repeated between 30th May and 31st August 2012. The soil samples collected on 14th April 2012, and four water hyacinth manure formulations (Table 1) developed under the VicRes project NR-03 2010 were analyzed for their chemical characteristics based on Okalebo et al. (2002) at the Department of Soil Science, University of Nairobi.

2.2. Water hyacinth compost formulations

Four types of water hyacinth compost were prepared concurrently from the same batch of water hyacinth material using

Table 1
Chemical characteristics of the water hyacinth compost formulations and the soil from the experimental field.

Parameter	H[a]	H+Mol[b]	H+EM[c]	H+CMC[d]	Soil
Organic carbon (%)	12.2	5.4	13.5	13.4	2.5
Total nitrogen (%)	1.3	0.6	1	1.1	0.26
Total phosphorus (ppm)	280	265	270	375	18.9
Potassium ($cmol_c\ kg^{-1}$)	25	21.2	24.5	21	0.41
Sodium ($cmol_c\ kg^{-1}$)	2.1	1.8	1.7	1.9	0.1
Calcium ($cmol_c\ kg^{-1}$)	20.7	37.2	27.5	22.3	2.3
Magnesium ($cmol_c\ kg^{-1}$)	9.3	9.3	15.3	12	0.8
Zinc (ppm)	3.0	3	4	2	1.9
Iron (ppm)	1.3	1.3	1.7	1.9	0.37
pH	8.4	8.4	8.4	8.1	4.2

[a] Water hyacinth compost only.
[b] water hyacinth compost with molasses.
[c] water hyacinth compost with effective microbes.
[d] water hyacinth with cattle manure culture.

aboveground closed aerobic heap design in 4 replicates. Sixteen heap stands measuring $1 \times 1 \times 1.5$ m ($L \times W \times H$), made of wooden frame and chicken wire mesh, with a fitting polythene sack on the inner part were constructed. The top part of these heap stands was left open for turning of the compost. Water hyacinth was harvested manually, then taken to the composting site and sun-dried for seven days. It was chopped into small pieces of about 5 cm using a chaff cutter to increase the surface area for decomposition. Dried and chopped water hyacinth material (20 kg) was put into the heap stands to form a layer ~10 cm thick. In the preparation of water hyacinth compost+effective microbes (H+EM) formulation, this 10 cm layer was sprayed with 10 L of 2% effective microorganisms solution (EM^{TM}), containing photosynthetic bacteria (*Rhodopseudomonas palustris*), lactic acid bacteria (*Lactobacillus plantarum* and *L. casei*), yeast (*Saccharomyces cerevisae*), molasses, and water (EM Technologies Ltd, Embu, Kenya). This process was repeated until the heap reached 1.2 m high holding ~240 kg of the water hyacinth material in twelve layers. A similar approach was used in the preparation of water hyacinth compost+molasses (H+Mol), except that 10 L of 2% molasses solution was applied. For water hyacinth compost only (H), 10 L of water without any other ingredient was applied after every layer. For water hyacinth compost+cattle manure culture (H+CMC), a culture made of 5 kg decomposed cattle manure per 10 L of water was prepared as a source of saprophytic microbes. This cattle manure culture (CMC) was applied after every water hyacinth layer was spread onto the heap stand, amounting to ~60 kg of cattle manure per heap. The compost heaps were mixed every 10 days using forked shovels to increase aeration and facilitate uniformity of temperature for decomposition within 55 days. Moisture content (60%) in the compost heaps was monitored using moisture meter (Model PM-300, Shenzhen Hinet Electronic Co. Ltd, Guangdong, China). The 4 replicate heaps for each type of water hyacinth compost were then mixed thoroughly into composite heaps for use. This compost is dark in colour with particles of loamy texture. From each of the composite heaps, 250 g samples were randomly picked for nutrient analysis at the Department of Soil Science, University of Nairobi.

2.3. Seed inoculation and planting

Rosecoco bean seeds (GLP 2) (Kenya Seed Company Ltd) were inoculated with *Rhizobium* inoculant powder (BIOFIX®, MEA Ltd, Kenya) as per the manufacturer's directions. The seeds (250 g) were mixed in gum Arabic solution (0.5 gum Arabic/5 mL of sterile lukewarm water). The gum Arabic-coated seeds (250 g) were mixed with the *Rhizobium* inoculant powder (1 g). Controls were coated with the gum Arabic solution only.

Planting holes ~200 cm^3 volume (i.e., ~5 cm diameter and ~10 cm deep) were dug using a shovel. The composts and FYM were applied using containers of 150 mL volumes per hole as per the respective treatments and mixed with soil. For the DAP treatments, one leveled teaspoon was mixed with soil in the planting hole. One bean seed was sown in every planting hole at a depth of ~2 cm.

2.4. Plant growth, root nodulation, and yields

The emergence date of every seedling was recorded independently, and used to determine the duration for germination. The number of seedlings that germinated out of the total number of seeds that were planted was used to determine the germination percentage. The germination rate recorded was for seedlings that emerged within 20 days from the planting date. The date for formation of the first trifoliate leaf was recorded and used to calculate the duration in days from the date of planting. When the first trifoliate leaves were fully formed in ~80% of the seedlings, plant height (stem base to petiole), length of the middle leaf (base to apex) and its width (widest part) were recorded. The date when the first flower of every plant appeared was recorded and used to calculate the duration for flowering in days from the date of planting. The number of flowers on each plant was recorded every three days for a period of three weeks. Ten days from the onset of flowering, 5 bean plants were randomly selected from each treatment per block for the estimation of number of root nodules associated with *Rhizobium* colonization. The bean plants were dug out from the soil into plastic bags, and the number of root nodules per plant was recorded using a tally counter in the laboratory.

The date when the first bean pod per plant formed was recorded and used to calculate the duration for pod formation in days from the date of planting. The date of ripening of the first pod per plant was recorded and used to calculate the duration to maturity in days from the date of planting. Plants were harvested independently and the number of harvested pods per plant recorded. The harvested pods from every plant were packed in separate paper packets and sun dried for a period of five days. From every paper packet, three pods were randomly selected and the number of seeds in each of the pod recorded. The weight of all seeds per packet was recorded as seed weight per plant, which was used to estimate yield per unit area (ton ha^{-1}) using the formula:-Yield per unit area = $\sum \frac{y\times\alpha}{(x\times n)\beta}$Whereby:$y$ = seed weight per plant in grams; x = space occupied by one plant (0.08 m^2); n = number of plants per treatment; $\alpha = 10^{-6}$ (converts weight in grams to tonnes); and $\beta = 10^{-4}$ (converts area in m^2 to hectares)

2.5. Aphid and anthracnose incidences

Aphid infestations on bean plants were recorded at the vegetative and flowering stage of bean plants. Three screw-capped containers each containing 10 mL of 70% ethanol were placed on every treatment row of 25 plants. Aphids from every 8 plants per row were collected into each container using a camel hair brush from leaves and stems. The collected aphids were identified under a dissection microscope (Model Z45E, Leica Inc., USA) at $\times$ 10 magnifications using the features described in Martin (1983) and Holman (1998), and their absolute counts recorded using a tally counter.

At the vegetative stage, the bean plants were also scored for anthracnose disease incidence i.e., the proportion of plants having anthracnose symptoms, characterized by dark brown to black lesions on leaves (Hagedorn and Inglis, 1986; Buruchara et al., 2010).

2.6. Statistical analysis

Statistical analysis was conducted using SAS 9.1 software (SAS Institute Inc.) at $p < 0.05$ confidence level. Descriptive statistics such as means were generated using proc means, while frequencies (percentages) were generated using proc freq. Data on plant growth was checked for normality using proc univariate; while proc transreg was used to find appropriate Box–Cox power transformations for normalization of data. Proc glm was used for analyses of variance (ANOVA) among the treatments; and means were separated using LS-means when the effects of treatments were significant. Data on aphid populations and root nodule counts were analyzed using proc genmod (χ^2 test; Poisson) and the means separated using proc multtest with bonferroni adjustment. Anthracnose disease incidences and germination percentages were analyzed by proc genmod (χ^2 test; binomial) and percentages compared using proc multtest.

3. Results

3.1. Germination percentage and number of root nodules

Seeds grown with DAP had significantly lower germination percentage than the water hyacinth compost formulations and the controls in both trials ($p < 0.0001$) (Fig. 1a and b). Germination percentage was not different between *Rhizobium* inoculated plants and the respective controls (Fig. 1a and b).

There was significant difference in the number of root nodules between the twelve treatments ($p < 0.0001$) (Fig. 2). Number of root nodules was significantly higher in *Rhizobium* inoculated plants than in the non-inoculated ones when grown with water hyacinth compost containing cattle manure culture (H+CMC) and water hyacinth compost containing effective microbes (H+EM). Number of root nodules was significantly lower in *Rhizobium* inoculated plants than in the non-inoculated ones when grown with water hyacinth compost only (H) and cattle farmyard manure (FYM); but not different in DAP and controls (Fig. 2). Roots of *Rhizobium* inoculated plants grown with water hyacinth compost containing cattle manure culture (H+CMC) and water hyacinth compost containing effective microbes (H+EM) had significantly higher nodule counts than in the other four fertility treatments (Fig. 2). In the non-inoculated plants, those grown with water hyacinth compost only (H) and FYM had highest nodule counts, followed by water hyacinth compost containing cattle manure culture (H+CMC) and water hyacinth compost containing effective microbes (H+EM), but lowest in DAP and the controls (Fig. 2).

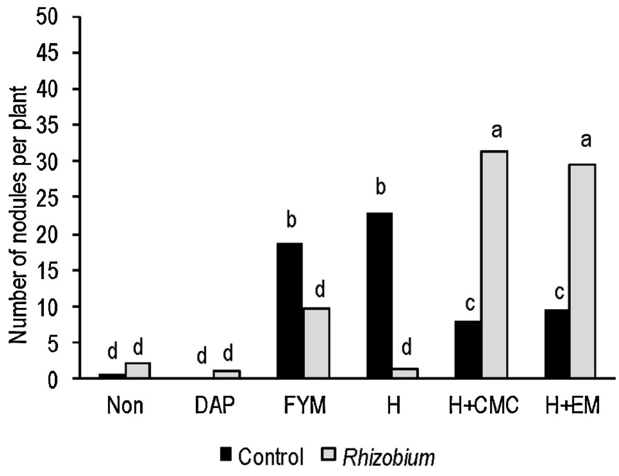

Fig. 2. Number of root nodules in Rosecoco bean plants as affected by commercial *Rhizobium* inoculant and soil fertility amendments in the second trial. Without fertilizer (Non), diammonium phosphate fertilizer (DAP), cattle farmyard manure (FYM), water hyacinth compost only (H), water hyacinth compost+cattle manure culture (H+CMC) and water hyacinth compost+effective microbes (H+EM). Bars with the same letter(s) are not significantly different (χ^2 test, $p > 0.05$).

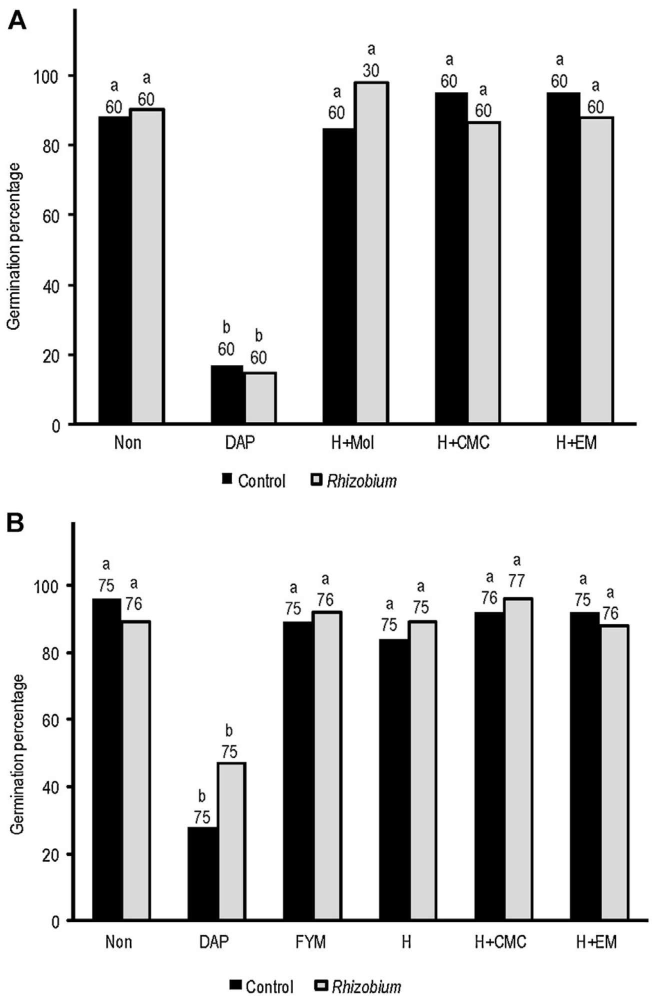

Fig. 1. Germination percentage of Rosecoco bean seeds as affected by commercial *Rhizobium* inoculant and soil fertility amendments in the first trial (A) and second trial (B). Without fertilizer (Non), diammonium phosphate fertilizer (DAP), cattle farmyard manure (FYM), water hyacinth compost only (H), water hyacinth compost+molasses (H+Mol), water hyacinth compost+cattle manure culture (H+CMC) and water hyacinth compost+effective microbes (H+EM). Numbers on top of bars represent sample sizes. Bars with the same letter(s) are not significantly different (χ^2 test, $p > 0.05$).

3.2. Developmental period

Plants grown with DAP had the longest developmental period, as exhibited in number of days to emergence through to the ripening of pods in both trials ($p < 0.05$) (Tables 2a and 2b). Those that were grown with water hyacinth compost containing effective microbes (H+EM) had the shortest developmental period, which was evident at emergence and numerically reflected through to the ripening of pods in the first trial (Table 2a). In the second trial, plants grown with the three water hyacinth compost formulations took a short period to germinate, which persisted to the ripening of pods in the water hyacinth compost with cattle manure culture (H+CMC) (Table 2b). Plants from *Rhizobium* inoculated seeds took a shorter period for emergence, formation of first trifoliate leaf and pods than the non-inoculated ones in the second trial (Table 2b).

3.3. Plant size, flower counts, and yields

Plants grown with water hyacinth compost containing effective microbes (H+EM) had larger size in terms of height and leaf length compared to those grown without fertilizer in the first trial (Table 3a). In the second trial, plants grown with the three formulations of water hyacinth compost and farmyard manure (FYM) were larger than those grown with DAP and without fertilizer in terms of height and leaf size (Table 3b). However, unlike in the first trial, those grown with water hyacinth compost containing effective microbes (H+EM) were smaller than the ones receiving water hyacinth compost only (H) and water hyacinth compost containing cattle manure culture (H+CMC) in the second trial (Table 3b).

In the first trial, plants grown with water hyacinth compost containing effective microbes (H+EM) and DAP produced more flowers than the other treatments (Table 3a). In the second trial, plants grown with the three formulations of water hyacinth compost and

Table 2a
Developmental periods of Rosecoco bean plants as influenced by *Rhizobium* inoculant and water hyacinth compost formulations during the first trial.

Source of variation	df	Emergence *F* values	First trifoliate	Flowering	Pod formation	Pod ripening
Rhizobium (*R*)	1	0.21	0.47	0.06	0.38	0.16
Fertilizer (*F*)	4	9.57***	7.21***	5.23***	4.41**	4.52***
R × *F*	4	0.63	4.07**	1.88	1.18	0.36
		Means (*first row are overall means for respective parameters*)				
		Days	Days	Days	Days	Days
		7.3	15.0	36.0	42.9	70.0
Inoculum						
Control		7.2	14.9	36.0	42.9	69.9
Rhizobium		7.3	15.0	35.9	42.9	69.9
Fertilizer						
Non		7.4 b	15.3	35.3 b	42.3 c	69.9 b
DAP		8.4 a	15.5	38.2 a	44.8a	71.9 a
H+Mol		7.4 b	15.0	36.8 a	43.3 b	70.1 b
H+CMC		7.1 bc	14.8	35.7 b	43.4 b	69.7 b
H+EM		6.9 c	14.7	35.8 b	42.4 bc	69.7 b
Control						
Non		7.3	14.9 bc	35.0	42.3	69.9
DAP		8.2	15.4 ab	38.0	44.0	72.0
H+CMC		7.2	14.9 bc	36.0	43.7	69.7
H+Mol		7.4	15.0 bc	36.3	42.9	70.1
H+EM		6.9	14.7 c	36.4	42.6	69.9
Rhizobium						
Non		7.5	15.6 a	35.5	42.4	69.9
DAP		8.6	15.5 ab	38.4	45.7	71.7
H+CMC		7.0	14.6 c	35.2	42.8	69.9
H+Mol		7.4	15.0 bc	37.2	43.6	70.2
H+EM		6.9	14.6 c	35.1	42.2	69.5

Without fertilizer (Non), diammonium phosphate fertilizer (DAP), water hyacinth compost+molasses (H+Mol), water hyacinth compost+cattle manure culture (H+CMC) and water hyacinth compost+effective microbes (H+EM). Asterisk indicates the significant effect, $^{***}p \leq 0.001$, $^{**}p \leq 0.01$, $^{*}p \leq 0.05$. Means with the same letter(s) are not significantly different. Interactions are considered over main effects wherever they are significant; no letters presented where there is no significant difference.

Table 2b
Developmental periods of Rosecoco bean plants as influenced by *Rhizobium* inoculant and water hyacinth compost formulations during the second trial.

Source of variation	df	Emergence *F* values	First trifoliate	Flowering	Pod formation	Pod ripening
Rhizobium (*R*)	1	6.57*	4.02*	2.87	5.21*	1.28
Fertilizer (*F*)	4	24.56***	12.86***	7.4***	5.37***	2.77*
R × *F*	4	1.55	1.66	1.93	1.67	0.98
		Means (*first row are overall means for respective parameters*)				
		Days	Days	Days	Days	Days
		7.3	15.0	41.0	47.6	74.4
Inoculum						
Control		7.4 a	15.1 a	40.9	47.9 a	74.1
Rhizobium		7.2 b	14.9 b	40.9	47.4 b	74.5
Fertilizer						
Non		7.4 b	15.1 b	40.8 bc	48.3 b	74.0 bc
DAP		9.7 a	16.6 a	43.8 a	50.4 a	75.8 a
FYM		7.2 bc	14.8 bc	40.6 bc	47.2 bc	74.6 ab
H		7.0 c	14.7 c	40.7 bc	47.2 bc	74.3 abc
H+CMC		6.9 c	14.6 c	40.4 c	46.7 c	73.6 c
H+EM		7.0 c	14.8 bc	41.3 b	47.9 b	74.8 ab

Without fertilizer (Non), diammonium phosphate fertilizer (DAP), cattle farmyard manure (FYM), water hyacinth compost only (H), water hyacinth compost+cattle manure culture (H+CMC) and water hyacinth compost+effective microbes (H+EM). Asterisk indicates the significant effect, $^{***}p \leq 0.001$, $^{**}p \leq 0.01$, $^{*}p \leq 0.05$. Means with the same letter(s) are not significantly different. Interactions are considered over main effects wherever they are significant; no letters presented where there is no significant difference.

FYM had more flowers than those without fertilizer, while the ones grown with DAP were intermediate (Table 3b).

Plants grown with DAP produced the highest number of pods, while those grown with water hyacinth compost containing cattle manure culture (H+CMC) had the fewest pods in the first trial (Table 3a). In the second trial, plants grown without fertilizer had fewer pods than the other five fertility treatments (Table 3b). *Rhizobium* inoculated plants produced fewer pods than those without the inoculum in the second trial (Table 3b); while seed count per pod did not vary between treatments in both trials (Tables 3a and 3b).

Plants grown with DAP had the highest seed weight, while those with water hyacinth compost containing molasses (H+Mol) and

Table 3a
Plant size, number of flowers and yield-related parameters of Rosecoco bean plants as influenced by *Rhizobium* inoculant and water hyacinth compost formulations during the first trial.

Source of variation	df	Plant size at the first trifoliate stage			Flowers	Yield-related parameters			
		Leaf length	Leaf width	Height		Harvested pods	Seeds per pod	Seed weight	Yield per unit area
		F values							
Rhizobium (*R*)	1	0.02	1.54	0.4	0.63	1.91	0.69	6.35*	1.63
Fertilizer (*F*)	4	2.48*	1.54	3.86**	4.3**	8.45***	1.97	6.8***	18.04***
$R \times F$	4	1.41	1.61	1.79	1.12	0.91	0.68	0.89	0.92
		Means (*first row are overall means for respective parameters*)							
		cm	cm	cm	Counts	Counts	Counts	Grams	tons ha^{-1}
		10.1	6.3	6.2	4.9	16.2	4.2	22.5	1.6
Inoculum									
Control		14.9	6.3	6.2	4.8	15	4.1	20.4 b	1.5
Rhizobium		15.0	6.3	6.1	4.9	15	4.2	24.6 a	1.7
Fertilizer									
Non		10.0 b	6.3	5.9 b	4.8 b	17.0 b	4.1	24.9 b	2.2 a
DAP		10.2 ab	6.8	5.8 b	6.3 a	25.8 a	4.4	32.8 a	0.4 c
H+Mol		9.6 b	6.1	6.1 b	4.6 b	15.3 bc	4.2	18.3 c	1.8 ab
H+CMC		10.1 ab	6.2	6.2 ab	4.4 b	13.4 c	4.0	20.4 c	1.5 b
H+EM		10.7 a	6.5	6.5 a	5.4 a	17.0 b	4.3	23.4 b	2.1 a

Without fertilizer (Non), diammonium phosphate fertilizer (DAP), water hyacinth compost+molasses (H+Mol), water hyacinth compost+cattle manure culture (H+CMC) and water hyacinth compost+effective microbes (H+EM). Asterisk indicates the significant effect, $^{***}p \le 0.001$, $^{**}p \le 0.01$, $^{*}p \le 0.05$. Means with the same letter(s) are not significantly different; those with more than one letter are intermediate. Interactions are considered over main effects wherever they are significant; no letters presented where there is no significant difference.

Table 3b
Plant size, number of flowers and yield-related parameters of Rosecoco bean plants as influenced by *Rhizobium* inoculant and water hyacinth compost formulations during the second trial.

Source of variation	df	Plant size at the first trifoliate stage			Flowers	Yield-related parameters			
		Leaf length	Leaf width	Height		Harvested pods	Seeds per pod	Seed weight	Yield per unit area
		F values							
Rhizobium (*R*)	1	0.6	0	0.29	1.31	4.53*	1.78	7.56**	3.03
Fertilizer (*F*)	5	19.83***	16.57***	4.87***	4.0**	5.11***	1.9	5.36***	5.82***
$R \times F$	5	2.04	1.82	1.49	1.29	1.18	1.08	0.99	0.51
		Means (*first row are overall means for respective parameters*)							
		cm	cm	cm	Counts	Counts	Counts	Grams	tons ha^{-1}
		9.1	5.7	4.8	2.5	6.1	2.9	4.0	0.24
Inoculum									
Control		15.1	5.7	4.8	2.6	6.7 a	2.9	4.5 a	0.26
Rhizobium		14.9	5.7	4.7	2.4	5.6 b	2.8	3.4 b	0.21
Fertilizer									
Non		7.9 d	4.9 c	4.5c	2.0 b	4.5 b	2.8	3.0 d	0.20 b
DAP		7.9 d	5.1 c	4.4 c	2.4 ab	6.4 a	2.6	3.6 cd	0.08 c
FYM		10.0 a	6.3 a	4.8 ab	2.6 a	6.8 a	2.9	4.4 ab	0.32 a
H		9.5 bc	6.0 a	4.9 a	2.4 a	6.2 a	2.8	3.9 bc	0.25 ab
H+CMC		9.8 ab	5.9 a	5.0 a	2.7 a	6.6 a	3.0	4.6 a	0.32 a
H+EM		8.9 c	5.6 b	4.6 bc	2.7 a	6.4 a	2.8	3.9 abc	0.24 ab

Without fertilizer (Non), diammonium phosphate fertilizer (DAP), cattle farmyard manure (FYM), water hyacinth compost only (H), water hyacinth compost+cattle manure culture (H+CMC) and water hyacinth compost+effective microbes (H+EM). Asterisk indicates the significant effect, $^{***}p \le 0.001$, $^{**}p \le 0.01$, $^{*}p \le 0.05$. Means with the same letter(s) are not significantly different; those with more than one letter are intermediate. Interactions are considered over main effects wherever they are significant; no letters presented where there is no significant difference.

water hyacinth compost containing cattle manure culture (H+CMC) had the lowest seed weight in the first trial (Table 3a). In the second trial, those grown with water hyacinth compost containing cattle manure culture (H+CMC) had the highest seed weight, while the ones grown with DAP and those without fertilizer had the lowest seed weight (Table 3b). *Rhizobium* inoculated plants had heavier seeds than the controls in the first trial (Table 3a); but their seed weights were lower in the second trial (Table 3b). Yield per unit area in plants grown with DAP was the lowest in both trials (Tables 3a and 3b).

3.4. Anthracnose disease incidences

In the first trial, there was no significant difference in anthracnose incidence between the ten treatment combinations ($p > 0.05$). In the second trial, *Rhizobium* inoculated plants had significantly higher anthracnose incidence than non-inoculated ones when grown with water hyacinth compost containing cattle manure culture (H+CMC) ($p < 0.05$), but was not different in the other fertility treatments (Fig. 3). Anthracnose incidence was high in *Rhizobium* inoculated plants grown with water hyacinth compost containing

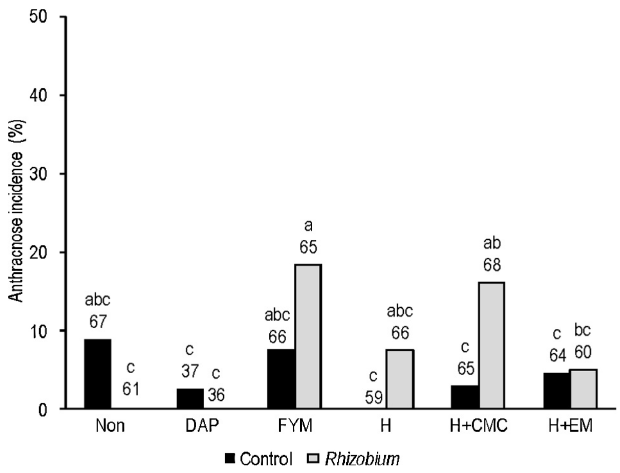

Fig. 3. Anthracnose incidences in Rosecoco bean plants as affected by commercial *Rhizobium* inoculant and soil fertility amendments in the second trial. Without fertilizer (Non), diammonium phosphate fertilizer (DAP), cattle farmyard manure (FYM), water hyacinth compost only (H), water hyacinth compost+cattle manure culture (H+CMC) and water hyacinth compost+effective microbes (H+EM). Numbers on top of bars represent sample sizes. Bars with the same letter(s) are not significantly different (χ^2 test, $p > 0.05$).

cattle manure culture (H+CMC) and FYM compared to the other fertility treatments ($p < 0.05$) (Fig. 3). Anthracnose incidence in non-inoculated plants was not different between the fertility treatments ($p > 0.05$) (Fig. 3).

3.5. Aphid density

The aphids on the bean plants were identified as *Aphis fabae* (black bean aphid) based on their dark colour (Martin, 1983; Holman, 1998). At the vegetative stage, *Rhizobium* inoculated plants had lower aphid density than non-inoculated ones when grown with water hyacinth compost only (H) and DAP; but was higher when grown in water hyacinth compost containing cattle manure culture (H+CMC), and not different in the other fertility treatments (Fig. 4a). Among the *Rhizobium* inoculated plants, those grown with water hyacinth compost containing cattle manure culture (H+CMC) had significantly high aphid density; while water hyacinth compost containing effective microbes (H+EM) had significantly low aphid counts among the non-inoculated plants (Fig. 4a).

At the flowering stage, *Rhizobium* inoculated plants had significantly higher aphid density than non-inoculated ones when grown with DAP, FYM or without fertilizer ($p < 0.05$) (Fig. 4b). Among the *Rhizobium* inoculated plants, aphid density was highest in those grown with FYM and without fertilizer, followed by water hyacinth compost containing cattle manure culture (H+CMC), but lowest in plants grown with DAP, water hyacinth compost only (H) and water hyacinth compost containing effective microbes (H+EM) (Fig. 4b). In the non-inoculated plants, aphid density was significantly high in plants without fertilizer but low in those grown with DAP and water hyacinth compost containing effective microbes (H+EM) (Fig. 4b).

4. Discussion

The production of beans in sub-Saharan has been declining partly due to low soil fertility. This has lead to the development of commercial *Rhizobium* inoculants for nitrogen fixation (Mugendi et al., 2011). Composts such as those produced from water hyacinth are also being developed to enhance crop production (Woomer et al., 2000; Gunnarsson and Petersen, 2007). Compatibility between such technologies in beans would be a sustainable factor in food production.

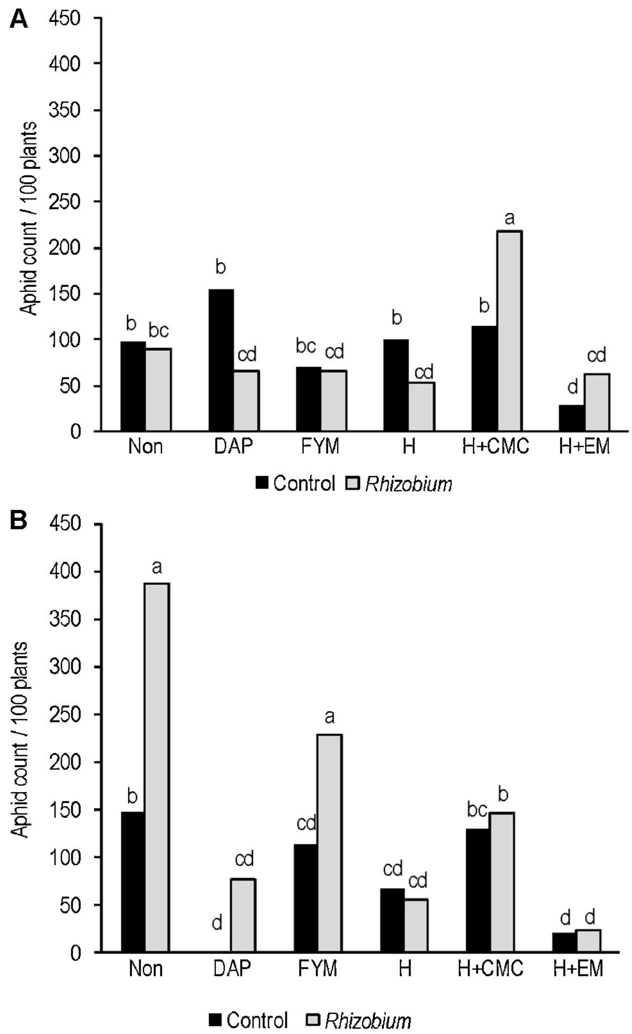

Fig. 4. Density of *Aphis fabae* on Rosecoco bean plants as affected by commercial *Rhizobium* inoculant and soil fertility amendments during the vegetative stage (A) and the flowering stage (B). Without fertilizer (Non), diammonium phosphate fertilizer (DAP), cattle farmyard manure (FYM), water hyacinth compost only (H), water hyacinth compost+cattle manure culture (H+CMC) and water hyacinth compost+effective microbes (H+EM). Bars with the same letter(s) are not significantly different (χ^2 test, $p > 0.05$).

Rosecoco bean seeds treated with water hyacinth compost+effective microbes took a short period for germination in the first trial; this also occurred in all the three formulations of water hyacinth compost in the second trial, especially when compared to DAP. Furthermore, hyacinth composts exhibited better percentage germination than those with DAP. Low seed germination has been reported in chickpea grown with phosphate fertilizer (TSP), and was attributed to phytotoxicity and high osmotic pressure that constrains water uptake by seeds (Zhang and Rengel, 2002; Kabir et al., 2010). However, the water hyacinth composts seemed to have no significant effect on percentage seed germination when compared to plants without fertilizer. The contribution of *Rhizobium* inoculant to seed germination in time and percentage was not evident. Other strains of *Rhizobium* such as *Bradyrhizobium japonicum* have been reported to enhance bean seed germination through production of Nod factors and phytohormones (Prithiviraj et al., 2003; Cassan et al., 2009).

Rhizobium nodulation was very poor in bean plants grown without fertilizers. This is because symbiosis between legumes and *Rhizobium* requires nutrients especially phosphorus for successful nodulation (Vance et al., 2002). The addition of DAP did not improve root nodulation. Although poor nodulation may be linked to toxicity of DAP on *Rhizobium* (Maheshwari et al., 2010), this cannot be fully supported by data in the present study, since nodule counts in plants without fertilizer were similar to those with DAP. The composts and cattle farmyard manure enhanced *Rhizobium* nodulation in a differential manner. Water hyacinth compost only and the farmyard manure appeared to favour nodulation by natural *Rhizobium* populations, because there were more nodules in plants that had not been inoculated. It has been suggested that native *Rhizobium* strains are better adapted for symbiosis within their area of origin than introduced commercial ones (Lopez-Garcia et al., 2002; Martinez-Romero, 2003; Dean et al., 2009). However, the adaptability of such strains is likely to be altered by soil amendments (Zahran, 1999; Andrade et al., 2002). This is because nodulation of the commercial *Rhizobium* inoculant was better in plants grown with water hyacinth compost enhanced with cattle manure culture (H+CMC) or effective microbes (H+EM). Therefore, based on nodulation, these two types of water hyacinth compost can be considered compatible with the commercial *Rhizobium* inoculant.

Plants treated with composts including those from water hyacinth (Tarkalson et al., 1998; Widjajanto et al., 2001, 2002; Lata and Veenapani, 2011), and those inoculated with *Rhizobium* are expected to be large in size (Carter et al., 1994; Karaca and Uyanoz, 2012). This seemed to be the case in the present study, as bean plants treated with water hyacinth composts were tall with longer leaves at the first trifoliate stage. This large size was particularly evident in plants that received water hyacinth compost+effective microbes (H+EM) and water hyacinth+cattle manure culture (H+CMC) in the first trial; and all formulations of water hyacinth compost in the second trial. The potential of DAP fertilizer in increasing bean plant size was not evident at the first trifoliate stage in the present study; although positive effects of phosphate fertilizers on plant size were observed by Araujo et al. (1997) and Hernandez et al. (2007). The effect of *Rhizobium* inoculant on plant size at the first trifoliate stage was not evident in both trials. This does not fully imply that the *Rhizobium* inoculant does not improve plant growth, since the growth parameters were only recorded at an early stage at which the plants had not attained complex morphology.

Generally, plants treated with hyacinth compost+effective microbes (H+EM) seemed to have low *A. fabae* populations in the vegetative and the flowering stage. Biofertilizers with mixtures of microbes including *Rhizobium* have been found to offer better protection against aphids in beans (El-Wakeil and El-Sebai, 2009). At the vegetative stage, *A. fabae* populations were low in *Rhizobium* inoculated plants than in the respective controls when supplied with DAP or water hyacinth compost only. The low *A. fabae* populations in *Rhizobium* treated plants may be attributed to induced resistance, which has been linked with suppression of the insect pest in faba bean and the non-legume squash plants (El-Wakeil and El-Sebai, 2009; Martinuz et al., 2012). However, in plants with hyacinth compost+cattle manure culture (H+CMC) at the vegetative stage, *A. fabae* populations were higher in *Rhizobium* treatments. This was also the case at the flowering stage in plants grown with farmyard manure, DAP and those without fertilizer. High aphid populations have also been reported in soybean inoculated with commercial *Rhizobium* inoculant, which has been linked to improved host nutritive suitability (Dean et al., 2009). The actual mechanism involved in the increase and decrease of *A. fabae* populations on beans inoculated with *Rhizobium* under the various soil fertility conditions requires in-depth investigation as the related information is scarce.

Interestingly, the number of root nodules and the occurrence of anthracnose infection seemed to follow the trend in the *A. fabae* population, whereby *Rhizobium* inoculated plants had more nodules and higher anthracnose incidences. Aphids have been found to induce *Rhizobium* nodulation in the leguminous crop *Medicago truncata* (Heath and Lau, 2011). In the case of anthracnose disease, wounds inflicted by *A. fabae* stylets and their excreted honey dew may have enhanced *C. lindemuthianum* infection, which was severe on *Rhizobium* inoculated plants due to higher *A. fabae* populations. This explanation is however disputable, because the tiny wounds inflicted by aphid stylets rarely permit the entry of fungal pathogen inocula that are relatively large in size (Mitchell, 2004). Furthermore, feeding by aphids has been found to cause induced resistance reactions that may limit plant colonization by *Colletotrichum* spp. (Russo et al., 1997). It is therefore possible that improved nitrogen content in bean plants due to *Rhizobium* nodulation favours both *Colletotrichum* and *A. fabae* infestations. This is because *Colletotrichum* has high affinity for tissues with high nitrogen content in beans and other plants (Nam et al., 2006; Tavernier et al., 2007; Lobato et al., 2009).

Bean plants grown with water hyacinth composts had high number of flowers, particularly those that received water hyacinth compost+effective microbes (H+EM) in the first trial, and all formulations of water hyacinth compost in the second trial, when compared to plants without fertilizer. Furthermore, plants with water hyacinth composts had more harvested pods in the second trial. However, in the first trial, pod production was poor in bean plants treated with water hyacinth compost enhanced with either cattle manure culture (H+CMC) or molasses (H+Mol). DAP fertilizer had a positive effect on flower and pod production, particularly in the first season; as phosphorus in DAP promotes flower and pod production in legumes (Lauer and Blevins, 1989). By contrast, bean plants inoculated with *Rhizobium* had lower number of pods than the controls in the second trial.

The average seed weight per plant in beans inoculated with *Rhizobium* was higher than for the controls in the first trial. This was also numerically reflected in the bean yields per unit area; partly confirming the potential of *Rhizobium* in promoting bean production (Dilworth et al., 2008). In contrast, average seed weight per plant in *Rhizobium* treatments was lower than for the controls in the second trial, which could be linked to the fewer pods they produced. *Rhizobium* may have burdened the plants under the relatively stressful conditions of the second trial. Some strains of *Rhizobium* have been found to lower the fitness of the host legumes due to unfavourable resource allocation trade-offs (Friesen, 2012; De Mita, 2012). The effects of *Rhizobium* inoculants on seed weight per plant seemed not influenced by the water hyacinth composts, farmyard manure, and DAP, as there was no interaction between the treatments.

The average seed weight per plant in the first trial was higher in DAP than in all the water hyacinth composts. Conversely in the first trial, the average seed weight per plant was even higher in plants without fertilizer than in the ones treated with water hyacinth composts enhanced with cattle manure culture and with molasses. In the second trial, average seed weight per plant in farmyard manure and the three formulations of water hyacinth compost was higher than for plants without fertilizer and those with DAP. These plants grew under relatively low rainfall. Therefore, apart from nutrients, other factors including enhanced moisture retention by organic matter in the manures may have contributed to their improved growth (Kimetu et al., 2008).

Yield per unit area in plants treated with all the water hyacinth composts and farmyard manure were higher than those for DAP in both trials. This was opposite of the average seed weight per plant, which was higher in DAP. The immediate explanation for decreased yields in DAP treatment is the low percentage germination that resulted in fewer harvested plants per unit area. The benefit of DAP fertilizer on the growth of the few germinated seedlings becomes elaborate as the toxicity subsides (Kabir et al., 2010), which results in a low population density of plants that yield heavy seeds. In general, plant growth and yields were better in the first trial that had better rainfall than in the second trial.

The application of synthetic fertilizer (DAP) could be disadvantageous as yields per unit area were lower than for plants without fertilizer; while the plants took longer to mature. The benefit of water hyacinth composts on beans in the study location was quite not evident, because yields from plants that were grown with all the composts were similar to those grown without fertilizer. However, continued use of the water hyacinth composts may have long-term benefits in nutrient deficient soil, as has been found with other organic soil amendments (Kimetu et al., 2008). The water hyacinth composts have alkaline pH, which makes it an ideal component for acidic soils such as those of the study location. The fact that water hyacinth compost formulations such as H+CMC and H+EM promoted nodulation in bean plants inoculated with commercial *Rhizobium* inoculant indicates compatible interaction that could enhance nitrogen fixation. The other benefit of water hyacinth compost with effective microbes (H+EM) is the capacity to lower aphid infestations. Therefore, compatibility between water hyacinth compost and *Rhizobium* depends on compost formulations and bacterial strain, with variable consequences on infestations by *A. fabae* and *C. lindemuthianum*. In conclusion, the commercial *Rhizobium* inoculant is predominantly compatible with water hyacinth compost formulations containing effective microbes and cattle manure culture, which could enhance tolerance of bean plants to aphids and possibly to anthracnose disease. These two water hyacinth compost formulations need further investigation for their potential in enhancing food production and alleviating the water hyacinth problem in the Lake Victoria basin.

Acknowledgements

This work was conducted under the sponsorship of the Lake Victoria Research (VicRes) Initiative, a regional collaborative research programme of the Inter-University Council for East Africa (IUCEA). Research funds were provided by the Government of Sweden through the Swedish International Development Cooperation Agency (Sida), under the framework of the Lake Victoria Development Partnership (LVDP) Programme. Thanks to our field assistant Fredrick Wandera Majimbo for his efforts that led to this publication.

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