Applied Soil Ecology 128 (2018) 54–60

Contents lists available at ScienceDirect

Applied Soil Ecology

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

Is there a need for *Bradyrhizobium yuanmingense* and *B. japonicum* reinoculation in subsequent cropping seasons under smallholder farmers' conditions?

Jacob Ulzen[a], Robert Clement Abaidoo[b,d,*], Cargele Masso[c], Emmanuel Degraft Johnson Owusu-Ansah[e], Nana Ewusi-Mensah[a]

[a] Department of Crop and Soil Sciences, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana
[b] Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana
[c] International Institute of Tropical Agriculture (IITA), Nairobi, Kenya
[d] International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria
[e] Department of Mathematics, College of Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

ARTICLE INFO

Keywords:
Bradyrhizobium yuanmingense
Hyperbolic function
B. japonicum
Survival rate

ABSTRACT

Reliable information on the persistence of rhizobium in soil in the absence of host between growing periods is important in deciding whether inoculation on the same plot in subsequent seasons is necessary. This study determined the survival of introduced rhizobium strains and predominant factors that influence the declining rates of their populations. *Bradyrhizobium yuanmingense* (BR 3267) and *B. japonicum* (USDA 110) were manually incorporated into soils at four different locations (Kpalga, Tanina, Tunayilli and Busa) in northern Ghana at 2.5×10^8 ($\log_{10}$ 8.4) and 2.5×10^7 ($\log_{10}$ 7.4) cells g^{-1} peat, respectively, per 6 m^2. The populations of surviving cells were estimated at 0, 21, 42, 81, 142 and 296 days using the Most Probable Number (MPN) count technique. Several decline functions were applied to the data with hyperbolic regression function emerging as the option that provides the best fit for *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 at all locations. There was no significant difference in the declining rates between the different locations; however, there were differences in the declining rates for the sampling times. At 296 days, the numbers of surviving cells of *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 were $\log_{10}$ 1.9 and $\log_{10}$ 1.7, respectively. Native rhizobium population and soil moisture were the predominant factors that affected the survival of the introduced strains. It is evident from the studies that these strains can survive in sufficient numbers at least within a year; therefore, re-inoculation may not be necessary for a following season especially when using *B. yuanmingense* strain BR 3267.

1. Introduction

The need to re-inoculate legumes depends on resident rhizobia population, and more importantly the ability of the introduced rhizobia to survive in the absence of the host plant (Triplett et al., 1993). The survival rate is affected by many abiotic and biotic factors but the predominant ones are soil moisture, rainfall, soil temperature and native rhizobia population (Slattery et al., 2004). A desirable strain must be saprophytically competent in sufficient numbers after the growing period of the host plant. This is usually expected of the introduced strains, which must overcome the abiotic stress of their new environment to infect the host legumes (Vachot-Griffin and Thies, 2005). Native rhizobia are well adapted to local conditions and are widely perceived to have competitive advantage over introduced strains resulting in poor colonization and establishment of the latter. Therefore, introduced populations must be in numbers large enough to overcome the competitive advantage of the indigenous populations.

Most of the persistence studies in sub Saharan Africa have focused on greenhouse assessment of previously inoculated fields (Sanginga et al., 1996; Zengeni et al., 2006). Oves et al. (2017) demonstrated the survival of *Ensifer adharens* in the presence of heavy metals under laboratory conditions. However, it is known that conclusions from the works conducted in greenhouse or laboratory conditions do not always reflect strain performance in the field environment (Pitkajarvi et al., 2003). In parallel, few studies have addressed the persistence of introduced strains in the field (e.g., Woomer et al., 1992; Duodu et al.,

* Corresponding author at: Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana.
E-mail address: rcabaidoo@knust.edu.gh (R.C. Abaidoo).

https://doi.org/10.1016/j.apsoil.2018.04.003
Received 15 February 2018; Received in revised form 27 March 2018; Accepted 4 April 2018
Available online 10 April 2018
0929-1393/

Table 1
Physical and chemical properties of the soils at the study locations.

Soil parameters	Locations			
	Kpalga	Tunayilli	Tanina	Busa
pH(1:2.5) (H_2O)	6.13 ± 0.25a[†]	6.3 ± 0.1a	6.0 ± 0.06a	6.0 ± 0.04a
Total N (%)	0.43 ± 0.02a	0.52 ± 0.022a	0.33 ± 0.012a	0.33 ± 0.003a
Available P (mg kg^{-1})	1.69 ± 0.23a	1.53 ± 0.22a	2.04 ± 0.025a	1.20 ± 0.18a
Exchangeable K (cmol (+) kg^{-1})	1.21 ± 0.09a	1.06 ± 0.1a	1.06 ± 0.021a	1.11 ± 0.05a
Organic C (%)	0.42 ± 0.02b	0.74 ± 0.05a	0.28 ± 0.01b	0.49 ± 0.01ab
Exchangeable Ca (cmol (+) kg^{-1})	3.15 ± 0.11a	4.41 ± 0.65a	2.66 ± 0.01a	2.93 ± 0.09a
Exchangeable Mg (cmol(+) kg^{-1})	0.38 ± 0.02a	0.60 ± 0.52b	0.62 ± 0.015b	0.62 ± 0.08b
Sand (%)	64.42 ± 1.50b	69.05 ± 7.04a	68.92 ± 0.02a	68.52 ± 1.60a
Silt (%)	27.74 ± 1.54a	24.08 ± 0.96b	12.88 ± 0.02c	24.64 ± 1.64ab
Clay (%)	7.84 ± 1.54b	5.84 ± 6.08c	18.2 ± 0.15a	6.84 ± 0.04bc
Texture	Sandy loam	Sandy loam	Sandy loam	Sandy loam

[†] Represent standard deviation of the means. Figures between columns with the same letters are not significant at 5% probability.

2005; Crozat et al., 1982; Corman et al., 1987; Narożna et al., 2015). For example, Woomer et al. (1992) predicted the persistence of introduced rhizobium in the field under varying climatic factors while Crozat et al. (1982) and Corman et al. (1987) studied the survival kinetics of rhizobium without considering the effects of the prevailing climatic conditions.

In recent times, there have been renewed interests in finding suitable inoculant strains for cowpea production in sub-Saharan Africa (SSA) since recent evidence suggests that the potential exists for improving biological nitrogen fixation in cowpea and groundnut cropping systems. The inoculant strain *B. yuanmingense* (BR 3267) from Brazil has shown a huge potential in increasing grain yields of cowpea in Ghana. Boddey et al. (2016) and Ulzen et al. (2016) reported of significant increases in grain yield of cowpea in response to inoculation with *B. yuanmingense* (BR 3267). Consequently, *B. yuanmingense* (BR 3267) has been recommended for inoculant production for smallholder farmers in Ghana. However, little is known about the persistence of the strain under smallholder farm conditions; an attribute of the strain needed for the decision on whether or not repeated inoculation would be needed in subsequent cropping seasons. The absence of such baseline data on the effects of environmental factors on the survival of introduced rhizobia in soils has made it difficult to predict the fate of introduced strains. The study hypothesized that the introduced strains have the same saprophytic competence as the native strains and therefore can survive the harsh environmental conditions in the Northern Ghana. The study specifically evaluated the saprophytic competence of *B yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 and determined the major environmental factors (rainfall, soil moisture, temperature, relative humidity, sunshine and indigenous rhizobium population) affecting the survival rates in the study area. Such determinations will help rhizobiologists and farmers to make informed decision on the frequency of re-inoculation in subsequent seasons.

2. Materials and methods

2.1. Site characteristics

The experiments were set up in four different sites namely Kpalga (latitude 09°26′.447′ N and longitude 000°57′.575′ W with an elevation of 167 m above sea level), Taunayilli (latitude 09°20′.398′ N and longitude 000°59′.154′ W with an elevation of 177 m above sea level) in the Northern region of Ghana; and Tanina (latitude 09°53.126′ N and longitude 002°27.480′ W with an elevation of 353 m above sea level) and Busa (09°59.186′ N and longitude 002°20.370′ W with an elevation of 345 m above sea level) located in the Upper West region of Ghana (Suppl Fig. S1). The soils of the study locations are Acrisols (Kpalga and Tunayilli) and Lixisols (Tanina and Busa) (IUSS, 2006). The study sites have a unimodal rainfall distribution pattern with an average annual rainfall of 1000 – 1200 mm and mean temperature between 26 and 30 °C with little variation throughout the year. The fields had no known history of rhizobia inoculation and had previously been planted with sorghum.

2.2. Experimental setup

Each field was ploughed and harrowed to a depth of 15 cm. Twenty-five grams of *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 as peat-based inocula were manually introduced into an area measuring 2 m × 3 m as separate treatments. The *B. yuanmingense* and *B. japonicum* contained 2.5×10^8 ($\log_{10}$ 8.4) and 2.5×10^7 ($\log_{10}$ 7.4) cells g^{-1} peat, respectively. Each inoculum was manually incorporated into the soil using a hoe that was pre- sterilized with 95% ethanol. Proximate analysis of the carrier materials of *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 was carried out following the procedures of (AOAC, 1990). Measures were taken to ensure that there was no legume on the plots that could have influenced the persistence of the strains throughout the study period.

2.3. Soil sampling

Soil samples were collected from each site at 0, 21, 42, 81, 142 and 296 days after incorporation of the peat based inoculant using an auger (Eijkamp, Netherland). Five core soil samples were thoroughly mixed and composite samples taken for enumeration of rhizobium population. The auger was surface-sterilized with 95% ethanol between sites. The samples were kept in refrigerator at 4 °C before the cell count. Prior to the introduction of the strains, composite soil samples were collected and analyzed at the Kwame Nkrumah University of Science and Technology (KNUST) Soil Science Laboratory following standard laboratory procedures for particle size (hydrometer method), soil pH (1:2.5 soil to H_2O), organic carbon (Walkley-Black), total nitrogen (Kjeldahl method), available soil phosphorus (Bray No. 1 solution) and exchangeable potassium (ammonium acetate (NH4OAc) extract) (Table 1). Calcium and magnesium were determined in 1.0 M ammonium acetate (NH4OAc) extract.

2.4. Soil moisture measurement

Soil moisture was measured with a Time Domain Reflectometer (TDR) (Trase system 6050X1 Santa Barbara California 93105 USA) at each sampling time. The 15 cm probe of the TDR was inserted into the soil at five different spots, to measure the moisture, and the average recorded.

2.5. Weather data

Weather data such as rainfall, soil temperature, relative humidity and sunshine hours over the period of the experiment for each site were downloaded from www.awhere.com.

2.6. Rhizobial enumeration

The enumeration of rhizobia in soils and inoculants were carried out using the most probable number count method (Vincent, 1970) where cowpea and soybean grown in plastic growth pouches (Mega International, USA) were inoculated with serial dilutions of soils from the experimental sites and inoculants (Woomer et al., 1997). Uniform clean cowpea and soybean seeds of good viability were surface-sterilized with 95% alcohol for 10 s and 3% hydrogen peroxide for 3 min and rinsed in at least six changes of sterilized distilled water as described by (Somasegaran and Hoben, 2012). Ten steps, ten-fold dilutions and six steps, fivefold dilutions were prepared for the peat-based inoculants and the soil samples respectively. Irrigation with Broughton and Dilworth N free nutrient solution (Broughton and Dilworth, 1970) was done as and when necessary. Nodulation was assessed after 28 days based on the presence or absence of root nodules. Rhizobia population estimates were assigned using MPNES software (Woomer et al., 1990).

2.7. Bradyrhizobium strains used in this study

The *B. yuanmingense* strain BR 3267 is a cowpea elite strain imported from Brazil (EMBRAPA, Brazil) as slant culture while *B. japonicum* strain USDA 110, specifically for soybean, was imported from (MEA Ltd, Kenya) as peat-based Biofix inoculant.

2.8. Inoculant preparation

The *B. yuanmingense* strain BR3267 was sub-cultured on yeast mannitol agar (YMA) incubated at 28 °C for 7 days. Broth cultures of the strain were prepared in yeast extract mannitol broth and placed in an orbital incubator at a temperature of 28 °C and 125 rpm until the late logarithm growth phrase. Peat imported from International Institute of Tropical Agriculture (IITA), Nigeria was bagged (50 g of peat per bag) and gamma radiated at Ghana Atomic Energy Commission (GAEC). Using a 20 ml sterile syringe with 18-gauge needle, 50 ml of the *B. yuanmingense* strain BR 3267 broth culture was introduced into the 50 g peat aseptically under the laminar flow cabinet as described by (Somasegaran and Hoben, 2012). The needle hole was sealed and the peat-based inoculum labelled, accordingly. The bag was gently massaged until the peat absorbed the inoculum. The freshly prepared inoculant was incubated 28 °C for two weeks to cure (Somasegaran and Hoben, 2012). Direct cell count by the drop plate method was done to estimate the number of cells (colony forming units) per gram of peat.

2.9. Data analysis and fitting of models

The MPN data was log transformed to minimize the variation associated with the enumeration technique before fitting the models. The decline rates from the various locations were pooled together because there was no significant difference between them (Table S1) and analysed using ANOVA function in XLSTAT version 19.7. *Bradyrhizobium* population (log_{10}) were counted over time and fitted into various decline functions using non-linear regression functions such as hyperbolic, exponential, logistics, Gompertz and exponential decline. The best-fit model was chosen based on the correlation coefficient (r) and the Akaike information criterion corrected (AICC) values (Leggett et al., 2017; Owusu-Ansah et al., 2017). The AICC value describes the amount of information that is lost in fitting the model. The model with least information lost is considered the best-fit model. Once the general non-linear regression function was selected, individual environmental factors (soil moisture, native rhizobia population, daily rainfall, soil temperature, relative humidity and solar radiation) were regressed singularly against the introduced *Bradyrhizobia* population (log_{10}) using curveExpert Professional software version 2.5.1 (Hyams, 2016). Furthermore, all the environmental factors were combined and regressed against the introduced strains using the multivariate non-linear regression function in XLSTAT version 19.7. Boxplots were constructed for each location using Microsoft Excel 2016 to compare the survival rates of the introduced strains in the different locations. T – Test was used to compare the carrier materials used to formulate the inoculant.

3. Results

The physical and chemical properties of the study locations are presented in Table 1. The pH values ranged from 6.0 to 6.3. The study locations had medium levels of nitrogen but very low levels of organic C and P. There was significant differences in organic carbon, exchangeable Mg, sand, silt and clay contents between the locations. However, these did not elicit significant differences in the decline rate of the introduced strains in the different locations as indicated in Table SI.

A sharp numerical significant (p = .0001) decline of *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 after 21 days of introduction into the field was observed (Table 2). Thereafter, the numbers declined at a slower rate for both strains. The decline rates from day 21 to 142 days were not significantly different from each other. The persistence of *B. japonicum* strain USDA 110 remained constant from day 21 to day 142 (Table 2). At the end of day 296, *B. yuanmingense* strain BR 3267 treatment plots had population of log_{10} 1.9 rhizobia cell g^{-1} soil whereas that of *B. japonicum* strain USDA 110 was log_{10}1.7 rhizobia cell g^{-1} soil.

The box plot shows the distribution of the *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA at the various locations (Figs. 1a and 1b). The highest point of the boxplot reflects the initial number of strains introduced and the lowest point reflect the remaining number of strains at the end of the experiment. There was slight variation between the boxes for each location but such variations were not significant (Figs. 1a and 1b). Therefore, the decline in population of the introduced strains *B. yuanmingense* strain BR3267 and *B. japonicum* strain USDA 110 revealed that in general the strains did not behave differently in the different locations (Figs. 1a and 1b).

Among the several regression functions that were applied to the decline rates of *B. yuanmingense* strain BR3267 and *B. japonicum* strain USDA 110, hyperbolic function was identified as the option that provided the best fit based on the AICC value (Table 3). Native rhizobia population, soil moisture and rainfall mostly influenced the persistence of *B. yuanmingense* strain BR 3267 over time (Table 4). Assuming that all other environmental factors remain constant, native rhizobia population, soil moisture and rainfall, accounted for 98, 96 and 0.45%, respectively of the variations in the observed decline of *B. yuanmingense* strain BR 3267 (Table 4). Similarly, soil moisture, native rhizobia

Table 2
Persistence of *Bradyrhizobium* spp. after 296 days of introduction.

Day following release	*B. yuanmingense* (BR 3267)	*B. japonicum* (USDA 110)
	Rhizobial cells g^{-1}soil	
0	8.4 ± 0.0a†	7.4 ± 0.0a†
21	2.5 ± 0.03b	2.4 ± 0.12b
42	2.3 ± 0.05c	2.2 ± 0.11b
81	2.2 ± 0.05c	1.9 ± 0.11b
142	2.1 ± 0.07c	1.9 ± 0.10b
296	1.9 ± 0.06d	1.7 ± 0.07c
P-value	*< .0001*	*< .0001*

† Within column, figures followed by the same letters are not significantly different from one another at 5% probability.

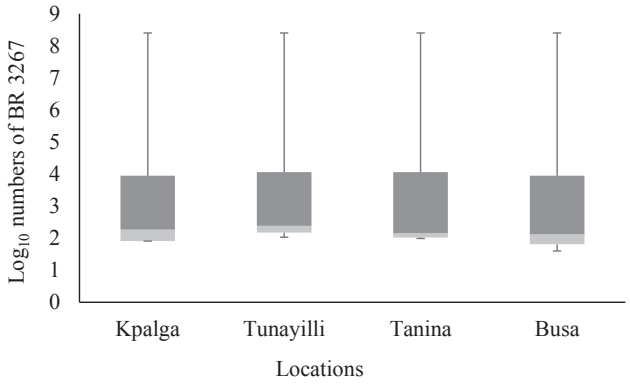

Fig. 1a. Boxplot distribution of *B. yuanmingense* strain BR 3267.

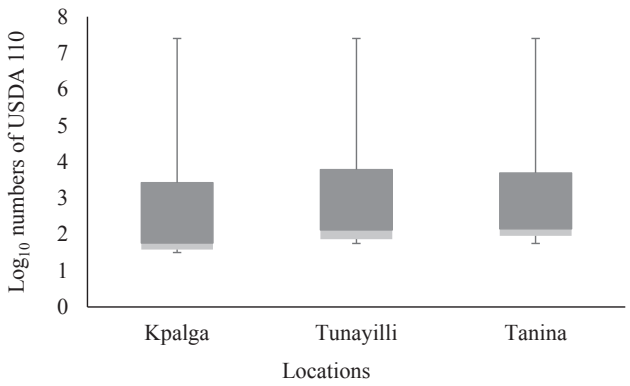

Fig. 1b. Boxplot distribution of *B. japonicum* strain USDA 110.

population and solar radiation were identified as the most influential factors affecting the persistence of *B. japonicum* strain USDA 110. Similarly, soil moisture, native rhizobia and solar radiation accounted for 98, 0.81 and 0.48%, respectively of the variations in the observed decline of *B. japonicum* strain USDA 110 (Table 4). Assuming all other environmental factors remain constant, the prevailing relative humidity at the study location had no effect on the persistence of *B. yuanmingense* strain BR 3267 and *B. yuanmingense* strain BR 3267 (Table 4).

Temperature and soil moisture were inversely proportional to the survival rates of the introduced strains as indicated by the surface response curve (Fig. 2). Predictions based on the hyperbolic regression function indicate that *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 can persist in sufficient numbers up to 3 years assuming the current environmental conditions remain fairly the same (Fig. 3). To evaluate the precision of the prediction, the predicted decline rates were regressed against the observed decline rate (Fig. 4). The regression revealed strong relationship ($r^2 = 0.98$) for *B. yuanmingense* strain BR 3267 and ($r^2 = 0.96$) for *B. japonicum* strain USDA 110, respectively (Fig. 4).

The multivariate non-linear regression analysis (Table 5) shows the variations in magnitude and direction of the effect of environmental factors on the strains. For example, rainfall had negative effect on persistence of the strains whereas soil moisture and soil temperature effects were positive irrespective of the strain. The effect of native rhizobia population was negative on *B. yuanmingense* strain BR 3267 and positive on *B. japonicum* strain USDA 110. The effects of solar radiation and relative humidity on *B. yuanmingense* strain BR 3267 were positive but negative on *B. japonicum* strain USDA 110. Given that, the correlation coefficients were 0.98 and 0.96 for *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110, respectively, 96 and 92% of the dynamics of *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110, respectively in the soils were explained by the environmental factors.

The proximate analyses of the carrier materials for the two strains are illustrated in Table 6. Except for the percentage fat, fibre and protein contents, there was no significant differences in the other properties of peat and filter mud.

4. Discussion

This study determined the persistence of introduced strains under smallholder farmers' condition and the predominant factors that affect their performance. The results show that the *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 survived with considerable number of cells ranging from $\log_{10}$ 1.7 to 1.9. Native rhizobia population, soil moisture, daily rainfall and solar radiation were the predominant factors that influenced the strains persistence. Predictions based on the hyperbolic function suggested that the strains could persist for 3 years with the assumptions that little changes would occur with the prevailing environmental factors.

A study on saprophytic competence of rhizobium is of great biological importance as it helps in predicting the nitrogen fixing ability of the legume-rhizobium symbiosis (Woomer, 1990). In addition, for a strain to be selected for inoculant production, it must be able to colonize the soil rapidly and adapt to the prevailing environmental conditions (Brockwell et al., 1995). Mostly, the poor establishment of introduced rhizobium strain is due to the inability of the strain to persist between growing seasons. Our results show that there was an initial rapid decline in the survival rates of the two strains during the first 21 days of introduction but the decline rate remained constant, thereafter. There are few studies on rhizobia survival; however, the reports from such studies suggest that survival rates remain constant after initial decline in numbers due to predation by protozoa (Heijnen and Van

Table 3
Fitting of regression models to the survival rates of introduced *Bradyrhizobium* spp.

Strain	Type of function	Equation	Coefficients				Standard error	r	AICC
			q_o	a	b	c			
BR3267	Hyperbolic	$y = q_o(1 + bx/a)^{(-1/b)}$	8.40	0.009	10.21		0.031	0.99	−38.04
	Logistics	$y = a/(1 + be^{-cx})$		2.09	−0.75	0.068	0.15	0.99	−19.36
	Exponential decline	$y = q_o\exp^{-x/a}$	7.88	30.58			1.79	0.77	7.60
	Gompertz relation	$y = a\exp^{-e^{b-cr}}$		3.23	−45.04	1.2×10^7	3.28	0.00	17.84
USDA 110	Hyperbolic	$y = q_o(1 + bx/a)^{(-1/b)}$	7.40	0.03	7.60		0.06	0.99	−29.06
	Logistics	$y = a/(1 + be^{-cx})$		1.86	−0.75	0.051	0.14	0.99	−19.34
	Exponential decline	$y = q_o\exp^{-x/a}$	6.82	35.62			1.56	0.77	5.89
	Gompertz relation	$y = a\exp^{-e^{b-cr}}$		2.98	−39.54	1.7×10^7	2.85	0.00	16.42

AICC = Akaike Information Criterion Corrected.

Table 4
Fitting of hyperbolic regression to parameters that influence the survival rates of *Bradyrhizobium* spp.

Strain	Parameters		Coefficients		Standard error	r	AICC
		q_o	a	b			
BR3267	Soil moisture	1.72	−102.73	5.06	0.37	0.98	−8.12
	Native rhizobia population	1.58	−10.46	5.51	0.37	0.99	−8.14
	Temperature	5.56	245.32	−5.86	3.10	0.58	17.44
	Relative Humidity	3.23	3.8×10^7	2.0×10^7	0.00	0.00	18.08
	Solar energy	4.0 x10^4	74.58	0.32	2.59	0.61	15.24
	Daily rainfall	5.45	0.002	10.36	2.41	0.67	14.40
USDA 110	Soil moisture	1.85	−290.23	12.90	0.32	0.99	−9.78
	Native rhizobia population	1.56	−12.87	7.14	2.15	0.90	13.04
	Temperature	2.8×10^6	0.87	0.14	2.70	0.59	15.74
	Relative Humidity	2.92	2.1×10^7	2.1×10^7	2.85	0.00	16.42
	Solar energy	5.5×10^4	84.12	0.27	2.06	0.69	12.51
	Daily rainfall	4.0	0.0069	11.24	2.41	0.54	14.37

AICC = Akaike Information Criterion Corrected.

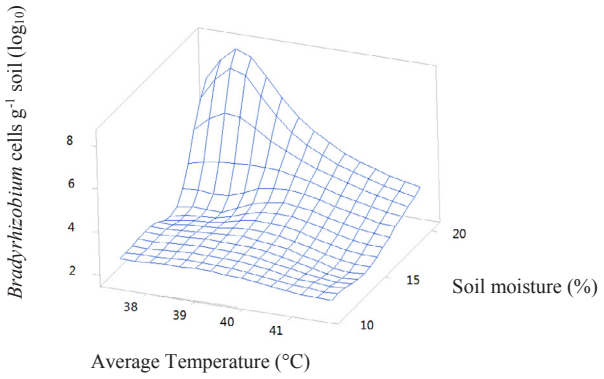

Fig. 2. The relationship between temperature, soil moisture and introduced *Bradyrhizobium* strains.

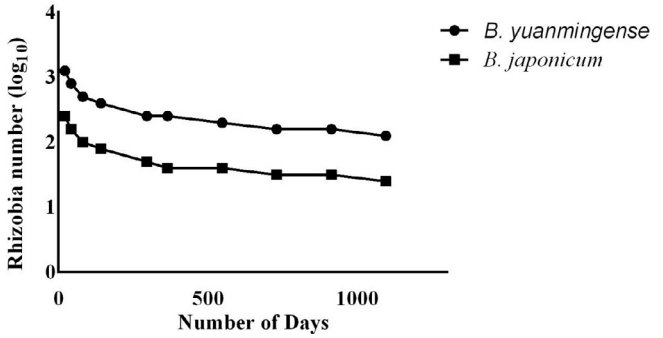

Fig. 3. Predicted number of survived *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 for 3 years (1095 days).

Veen, 1991; Woomer et al., 1992; Hirsch and Spokes, 1994; Hirsch, 1996; Pitkajarvi et al., 2003). Similar trends have been reported for other bacterial such as *Escherichia coli*, *Listeria monocytogenes*, *Salmonella dublin* after predation and parasitism by other organisms (Brennan et al., 2014).

The successful establishment of introduced rhizobia strains depends largely on the population size of rhizobia in the soil (Slattery et al., 2004). The likelihood of survival of introduced strains is very low in the absence of host legume when native rhizobia population is high. This is probably due to the disadvantage placed on introduced strains by the native rhizobia in competition for carbon. Although, Woomer (1990) indicated that indigenous rhizobia by definition are adapted to the stresses in their environment, observations from the most probable

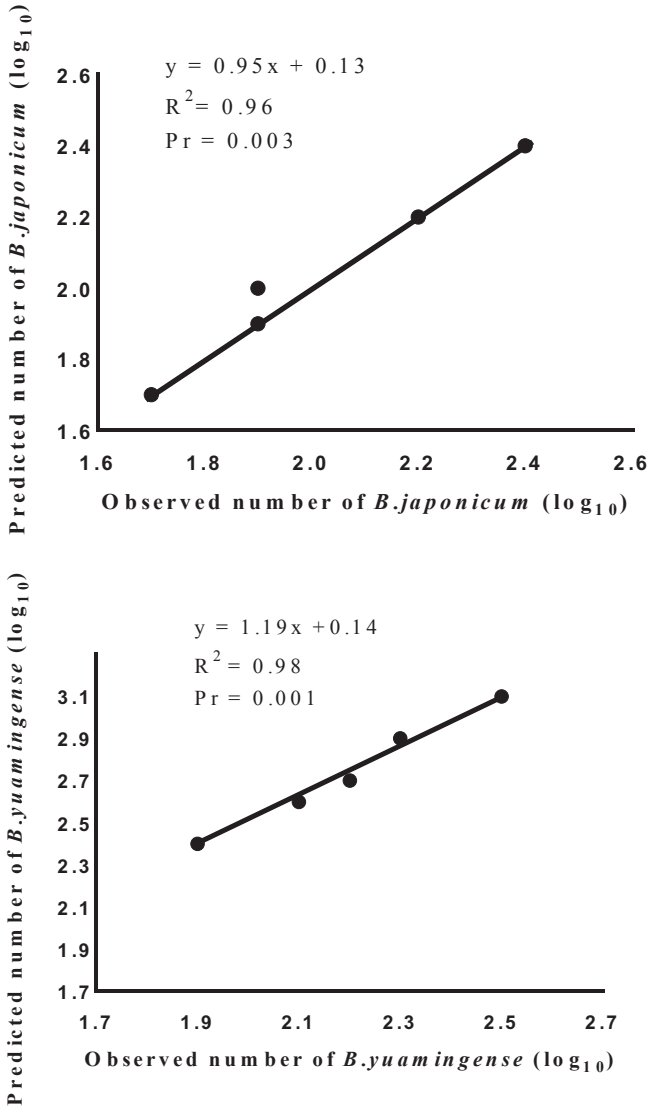

Fig. 4. Relationship between predicted and observed decline number of *Bradyrhizobium* spp.

number counts from this study suggest that both indigenous and introduced strains suffer the same fate in terms of reduction in numbers. Therefore, the ability of introduced strains to withstand stress and persist with higher population than the indigenous strains makes it

Table 5
Multivariate non-linear regression of the factors affecting rhizobia survival.

Parameters/coefficient	Strain	
	B. yuanmingense (BR 3267)	*B. japonicum* (USDA 110)
Intercept	−16.12	−53.38
Native rhizobia population	−7.17	8.09
Soil moisture	1.42	1.05
Rainfall	−0.88	−0.01
Soil temperature	0.149	0.80
Relative humidity	0.024	−0.019
Solar radiation	0.000	−0.002
r	0.98	0.96

Table 6
Properties of the carrier materials for USDA 110 (filter mud) and BR 3267 (Peat)

Parameters	Strain			
	B. yuanmingense (BR 3267)	*B. japonicum* (USDA 110)	*P-value*	Confidence interval
Fat (%)	0.33 ± 0.006[†]	0.79 ± 0.0064	.001	0.44–0.48
Fiber (%)	10.79 ± 0.58	7.43 ± 0.53	.024	0.82–5.83
Ash (%)	30.66 ± 0.58	29.59 ± 0.56	.281	−1.53–3.68
Moisture (%)	42.00 ± 0.58	37.20 ± 1.2	.09	−1.60–9.87
Protein (%)	8.25 ± 0.65	5.15 ± 0.49	.012	1.02–5.17
Carbohydrate (%)	9.50 ± 0.37	10.62 ± 0.42	.12	−0.41–2.49

[†] Represents standard error of the mean.

more desirable for selection for inoculant production.

Different environmental and climatic conditions are said to influence the saprophytic competence and population dynamics of introduced strains (Pitkajarvi et al., 2003). Evidence from this study suggests that soil moisture, temperature, solar energy and daily rainfall influence the dynamics of the introduced strains with varying significance. The most prominent factor was found to be soil moisture and this is probably due to the significant effect soil water content can have on cell viability (Rattray et al., 1992). Beringer and Kay (1993) also reported that soil moisture is the obvious factor that affects rhizobia population levels in the soil. Hungria and Vargas (2000) also highlighted the effect of soil moisture on rhizobia survival and growth in the soil. Our results therefore support these earlier findings, as soil moisture seemed to have directly influenced the survival of the introduced strains (Tables 4 and 5).

Our results also show that the population sizes of the introduced strains decreased as the average temperature increased and the soil moisture decreased. This can be explained by the fact that as the temperature increases the available moisture evaporates and the soil atmosphere dries up and becomes detrimental to the survival of the introduced rhizobium. The temperature effect accounted for less than or equal to 35% of the variations observed for decline rates at all the locations. In the tropics, high temperatures are likely to cause decreases in the survival rate of rhizobia because it does not form heat-resistant spores and cannot survive extreme soil temperatures (Hirsch, 1996; Hungria and Vargas, 2000). Tolerance to temperature is strain dependent; but some rhizobia are capable of surviving at a temperature of 35–40 °C (Michiels et al., 1994) and 28–47 °C (Mpepereki et al., 1996). In this study, the highest temperature recorded was 41 °C and for most part, it was either below or up to 37 °C.

Many researchers have predicted the survival of introduced strains using either Mistcherlich equation (Woomer et al., 1992), logistic function (Crozat et al., 1982) or Gomptz function (Corman et al., 1987) but the data of this study was best described largely by hyperbolic functions. It is worth noting that the above researchers studied the persistence of introduced strains for more than a year. However, after the decline rate reached equilibrium, there were less mortalities and therefore depending on when equilibrium is attained, the subsequent years may not have so much effect on the surviving strains. Therefore, in general, the length of this study did not affect the model selection. Rather, the prevailing environmental variables might have had a stronger influenced on the kind of model, hence the differences in the models derived for this and their work.

The purpose of this study was not to compare the survival rates of *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110. Our results show that the observed differences could be explained by the differential sensitivity among strains to environmental conditions. In addition, the behavior of bacteria within soil could be species and strain specific (Brennan et al., 2014). McLoughlin et al. (1990) observed variation in the saprophytic competence of two *B. japonicum* strains CPAC 15 and CPAC 7. The observed variation was attributed to the intrinsic abilities of the strains to survive. Barthelemy-Delaux et al. (2014) affirmed that variation exists among the same species of rhizobia in their response to stressful conditions. The variation among the properties of the carrier materials for the two strains (Table 6) could not have significantly accounted for the variation in the survival of *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110. It could therefore be suggested that the inherent ability of the strains to persist cannot be overlooked. Although, we could not confirm the identity of the strains used in this work, using molecular tools due to challenges in equipment, we have quantitatively determined the persistence of the introduced strains in reference to the indigenous rhizobia in comparison with the uninoculated plots using the MPN technique as had been done by other researchers (e.g., Woomer et al., 1992; Barthelemy-Delaux et al., 2014).

The multivariate non-linear regression revealed that soil moisture had positive effect with a higher magnitude on the survival of the strains in this study. It implies that soil moisture up to 27% as observed in the study favor persistence of rhizobia. Daily rainfall had negative effect implying that very low rainfall such as 0.7 mm per day observed in this study could be detrimental to the survival of rhizobia. The highest daily rainfall recorded in this study was 12.7 mm. The indigenous rhizobia populations at the introduction sites of *B. yuanmingense* strain BR 3267 were relatively higher than that of *B. japonicum* strain USDA 110 which could have increased competition. This may explain negative effect on *B. yuanmingense* strain BR 3267. The higher R-value obtained is probably due to the higher number of independent variables. Woomer et al. (1992) reported an R-value of 0.74 using Mistcherlich decline function with mean annual rainfall and water holding capacity as predictors. This may indicate that the higher the number of environmental factors evaluated the better prediction of the persistence of introduced strains in soils.

Based on the results of the study, cowpea and soybean may not require re-inoculation a year after inoculation based on the number of the inoculum strain that survived, and on an assumption that little variation in native rhizobia population and soil moisture will occur during the period. The numbers will certainly increase in the presence of host and favorable conditions such as adequate soil moisture and availability of carbon source during the planting season. Hirsch (1996) reported that *R. legumnosarium* and *Sinorhizobium meliloti* populations can increase up to four-folds in the presence of their host. Although, effectiveness test was not carried out, during the MPN assay in N-free plant culture medium, the leaves of the cowpea and soybean plants were dark green, compared to the yellowish colouration associated with control treatments, indicating that the strains had sustained their effectiveness.

The selection of rhizobia depends on its effectiveness and saprophytic competence. The persistence of *B. japonicum* strain USDA 110 is well known (Woomer et al., 1992; Narożna et al., 2015) but not that of *B. yuanmingense* strain BR 3267. Boddey et al. (2016) and Ulzen et al. (2016) have reported on the effectiveness of the strains used in this work. The results suggest that the potential saprophytic competence of

B. yuanmingense strain BR 3267 has been confirmed by this work since the number of the surviving cells did not differ significantly from one location to the other. This also suggests that its introduction into wider areas is not likely to be affected dramatically by the varying environmental and climatic conditions and can therefore be recommended for legume-rhizobium dissemination programs.

5. Conclusion

This study has shown that *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 can persist under smallholder farmer conditions in sufficient numbers at least a year after first inoculation confirming the hypothesis that introduced strains have the same saprophytic competence than indigenous rhizobia. Soil moisture and native rhizobia populations were the major determinants of the survival rates of the introduced strains in this study. This implies that in areas where rhizobia inoculants are scarce due to varied reasons, farmers may not have to worry about re-inoculation for a year.

Acknowledgements

Kwame Nkrumah University of Science and Technology (KNUST) funded the research activities through a sub-agreement with the International Institute of Tropical Agriculture (IITA) under the COMPRO-II project implemented by IITA and funded by Bill and Melinda Gates Foundation – United States. The authors appreciate the technical assistance of Ayamah Azumah.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2018.04.003.

References

AOAC, 1990. Official methods of analysis. 15th ed. Association of Official Analytical Chemists Washington, DC, USA.

Barthelemy-Delaux, C., Marburger, D., Delaux, P.-M., Conley, S., Ané, J.-M., 2014. Effect of drought on *Bradyrhizobium japonicum* populations in Midwest soils. Plant Soil 382, 165–173.

Beringer, J., Kay, H., 1993. Monitoring the survival of *Bradyrhizobium japonicum* released into Italian soils. In: BRIDGE/BIOTECH Final Sectorial Meeting on Biosafety and First Sectorial Meeting on Microbial Ecology, Granada, pp. 113.

Boddey, R., Fosu, M., Atakora, W.K., Miranda, C.H., Boddey, L.H., Guimarees, A.P., Ahiabor, B.D., 2016. Cowpea (*Vigna Unguiculata*) crops in Africa can respond to inoculation with rhizobium. Exp. Agric. 1–10.

Brennan, F.P., Moynihan, E., Griffiths, B.S., Hillier, S., Owen, J., Pendlowski, H., Avery, L.M., 2014. Clay mineral type effect on bacterial enteropathogen survival in soil. Sci. Total Environ. 468, 302–305.

Brockwell, J., Bottomley, P.J., Thies, J.E., 1995. Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. Plant Soil 174, 143–180.

Broughton, W.J., Dilworth, M.J., 1970. Methods in legume-rhizobium technology: plant nutrient solutions. Handbook Rhizobia 245–249.

Corman, A., Crozat, Y., Cleyet-Marel, J., 1987. Modelling of survival kinetics of some *Bradyrhizobium japonicum* strains in soils. Biol. Fertility Soils 4, 79–84.

Crozat, Y., Cleyet-Marel, J., Giraud, J., Obaton, M., 1982. Survival rates of *Rhizobium japonicum* populations introduced into different soils. Soil Biol. Biochem. 14, 401–405.

Duodu, S., Bhuvaneswari, T., Gudmundsson, J., Svenning, M.M., 2005. Symbiotic and saprophytic survival of three unmarked *Rhizobium leguminosarum* biovar *trifolii* strains introduced into the field. Environ. Microbiol. 7, 1049–1058.

Heijnen, C., Van Veen, J., 1991. A determination of protective microhabitats for bacteria introduced into soil. FEMS Microbiol. Lett. 85, 73–80.

Hirsch, P.R., 1996. Population dynamics of indigenous and genetically modified rhizobia in the field. New Phytol. 133, 159–171.

Hirsch, P.R., Spokes, J.D., 1994. Survival and dispersion of genetically modified rhizobia in the field and genetic interactions with native strains. FEMS Microbiol. Ecol. 15, 147–159.

Hungria, M., Vargas, M.A., 2000. Environmental factors affecting N 2 fixation in grain legumes in the tropics, with an emphasis on Brazil. Field Crops Res. 65, 151–164.

Hyams, D,G., 2016. CurveExpert Professional; a comprehensive data analysis software systems for windows, mac and linues.

IUSS, W., 2006. World reference base for soil resources 2006. World Soil Resources Reports 103.

Leggett, M., Diaz-Zorita, M., Koivunen, M., Bowman, R., Pesek, R., Stevenson, C., Leister, T., 2017. Soybean Response to Inoculation with *Bradyrhizobium japonicum* in the United States and Argentina. Agron. J. 109, 1031–1038.

McLoughlin, T., Alt, S., Merlo, P., 1990. Persistence of introduced *Bradyrhizobium japonicum* strains in forming nodules in subsequent years after inoculation in Wisconsin soils. Can. J. Microbiol 36, 794–800.

Michiels, J., Verreth, C., Vanderleyden, J., 1994. Effects of temperature stress on bean-nodulating *Rhizobium* strains. Appl. Environ. Microbiol. 60, 1206–1212.

Mpepereki, S., Wollum, A., Makonese, F., 1996. Diversity in symbiotic specificity of cowpea rhizobia indigenous to Zimbabwean soils. Plant Soil 186, 167–171.

Narożna, D., Pudełko, K., Króliczak, J., Golińska, B., Sugawara, M., Mądrzak, C.J., Sadowsky, M.J., 2015. Survival and competitiveness of *Bradyrhizobium japonicum* strains 20 years after introduction into field locations in Poland. Appl. Environ. Microbiol. 81, 5552–5559.

Oves, M., Khan, M.S., Qari, H.A., 2017. *Ensifer adhaerens* for heavy metal bioaccumulation, biosorption, and phosphate solubilization under metal stress condition. J Taiwan Inst Chem Eng 80, 540–552.

Owusu-Ansah, E., Sampson, A., Amponsah, S.K., Abaidoo, R.C., Dalsgaard, A., Hald, T., 2017. Probabilistic quantitative microbial risk assessment model of norovirus from wastewater irrigated vegetables in Ghana using genome copies and fecal indicator ratio conversion for estimating exposure dose. Sci. Total Environ 601, 1712.

Pitkajarvi, J., Rasanen, L.A., Langenskiold, J., Wallenius, K., Niemi, M., Lindstrom, K., 2003. Persistence, population dynamics and competitiveness for nodulation of marker gene-tagged *Rhizobium galegae* strains in field lysimeters in the boreal climatic zone. FEMS Microbiol. Ecol. 46, 91–104.

Rattray, E.A., Prosser, J., Glover, L., Killham, K., 1992. Matric potential in relation to survival and activity of a genetically modified microbial inoculum in soil. Soil Biol. Biochem. 24, 421–425.

Sanginga, N., Abaidoo, R., Dashiell, K., Carsky, R., Okogun, A., 1996. Persistence and effectiveness of rhizobia nodulating promiscuous soybeans in moist savanna zones of Nigeria. Appl. Soil Ecol. 3, 215–224.

Slattery, J., Pearce, D., Slattery, W., 2004. Effects of resident rhizobial communities and soil type on the effective nodulation of pulse legumes. Soil Biol. Biochem. 36, 1339–1346.

Somasegaran, P., Hoben, H.J., 2012. Handbook for Rhizobia: Methods in Legume-Rhizobium Technology. Springer Science & Business Media.

Triplett, E., Albrecht, K., Oplinger, E., 1993. Crop rotation effects on populations of *Bradyrhizobium japonicum* and Rhizobium meliloti. Soil Biol. Biochem. 25, 781–784.

Ulzen, J., Abaidoo, R.C., Mensah, N.E., Masso, C., AbdelGadir, A.H., 2016. Bradyrhizobium inoculants enhance grain yields of soybean and cowpea in Northern Ghana. Front. Plant. Sci. 7, 1770.

Vachot-Griffin, A., Thies, J., 2005. Fingerprinting the Australian rhizobial inoculant mother cultures using refined PCR protocols yields beneficial inoculant management applications. Anim. Prod. Sci. 45, 141–150.

Vincent, J.M., 1970. A manual for the practical study of the root-nodule bacteria. A manual for the practical study of the root-nodule bacteria.

Woomer, P., Asano, W., Bohlool, B., 1992. Predicting the persistence of introduced *Bradyrhizobium japonicum* in tropical soils. Biol. Nitrogen Fixation Sustainability Trop. Agric. 235–243.

Woomer, P., Bennett, J., Yost, R., 1990. Overcoming the inflexibility of most-probable-number procedures. Agron. J. 82, 349–353.

Woomer, P., Karanja, N., Mekki, E., Mwakalombe, B., Tembo, H., Nkwiine, C., Silver, M., Nyika, M., Ndakidemi, P., Msumali, G., 1997. Indigenous populations of rhizobia, legume response to inoculation and farmer awareness of inoculants in East and Southern Africa.

Woomer, P.L., 1990. Predicting the abundance of indigenous and the persistence of introduced rhizobia in tropical soils.

Zengeni, R., Mpepereki, S., Giller, K.E., 2006. Manure and soil properties affect survival and persistence of soyabean nodulating rhizobia in smallholder soils of Zimbabwe. Appl. Soil Ecol. 32, 232–242.