academicJournals

Vol. 6(11), pp. 154-160, December 2014
DOI: 10.5897/JEN2014. 0114
Article Number:5E287EC48740
ISSN 2006-9855

http://www.academicjournals.org/JEN

Journal of Entomology and Nematology

Full Length Research Paper

Effect of infection by *Metarhizium anisopliae* isolate ICIPE 51 on developmental stage, fecundity and intrinsic rate of increase of *Rhopalosiphum padi* and *Metopolophium dirhodum*

Patrick Murerwa[1]*, Peter Futi Arama[2], Alice Wanjiku Kamau[1] and Nguya Kalemba Maniania[3]

[1]Crops, Horticulture and Soils Department, Faculty of Agriculture, Egerton University, Nakuru, Kenya.
[2]Department of Crop Protection, Faculty of Agriculture, Rongo University College, Rongo, Kenya.
[3]Arthropod Pathology Unit, International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya.

Received 12 August 2014; Accepted 17 October 2014

This study assesses the pathogenicity of *Metarhizium anisopliae* isolate ICIPE 51 against different nymphal instars and adults of *Rhopalosiphum padi* and *Metopolophium dirhodum* and investigates effects of fungal infection on fecundity and intrinsic rate of aphid increase. To obtain different developmental stages, adult aphids were inoculated onto fresh leaf discs, reproducing parthenogenetically. Rearing was carried out to ensure different developmental stages were obtained at the same time so that treatments could be performed concomitantly. Concentrations of 1.0 x 10^6, 3.0 x 10^6 and 1.0 x 10^7 conidia/ml were used for each developmental stage. Mortality was recorded daily for 10 days. For fecundity, treated aphids were transferred to a leaf in an assay cell, one aphid per cell and observed for 7 days. New born nymphs were removed after counting. Five to seven day adults were significantly more susceptible than nymphs of other developmental stages. No significant difference in susceptibility was observed within each stage in the first three days. Thereafter, susceptibility increased steadily to maximum levels of 71 and 57% for five to seven day old adults and 0 - 2 day old nymphs, respectively. *M. dirhodum* was significantly less fecund than *R. padi* at all concentrations. Fecundity and intrinsic rate of increase among both aphid species declined progressively over time. Thus, maximum fecundity of 3 and 3.5 nymphs/aphid among *M. dirhodum* and *R. padi* respectively was recorded during the first day as compared to less than 1 nymphs/aphid/day in each species from the sixth day. These results indicate that susceptibility of *R. padi* and *M. dirhodum* to entomopathogenic fungal control increases with aphid maturity and that both species are significantly more fecund in early adulthood, suggesting the stage as ideal for biopesticide management intervention.

Key words: *Metarhizium anisopliae*, *Metopolophium dirhodum, Rhopalosiphum padi,* fecundity, intrinsic rate of increase.

INTRODUCTION

Bird-cherry oat aphid, *Rhopalosiphum padi* (Linnaeus) and Rose Grain aphid, *Metopolophium dirhodum* (Walker) pose serious threat to bread wheat growers in Kenya. Both nymphs and adults suck plant sap and

cause serious damage right from the seedling to maturity stage. In addition, the most damage is caused by transmission of a number of viruses, especially *Barley yellow dwarf virus* (BYDV), for which the two species are the most important vectors (Riedell et al., 2003; A. Wangai, National Agricultural Laboratories, Kenya, personal communication).

A number of synthetic chemical insecticides have been used to reduce populations to below damage threshold level. However, large reproductive rates and wide range of host plants make aphids difficult to control (Borer et al., 2009). Moreover, concern about the hazardous effect of synthetic chemical insecticides on the environment and humans has prompted the search for more effective and safe control strategies (Sezen et al., 2004; Muratoglu et al., 2011). Entomopathogenic fungi (EPF) which have been reported to be pathogenic against a wide range of insect pest species including aphids (Purwar and Sachan, 2005) are among the strategies being considered. However, insect susceptibility to fungal infection is affected by a number of factors, such as the properties of the pathogen population, the host population as well as environmental conditions (Inglis et al., 2001). Among the host factors, host species, host age, the developmental stage and sex have been reported to affect host susceptibility to EPF.

Cereal-infesting aphids are multivoltine pests and individuals in all developmental stages are usually present on an infested wheat crop (Helmut and Richard, 2007). An understanding of the susceptibility of different developmental stages to fungal infection is important for the development of management tactics and will enable the optimization of the impact of biological control agents (Butt et al., 2001). A pathogen that is able to cause infection to more than one developmental stage of its host would be preferable to the one that is only pathogenic to specific stages, especially when the host insect has a high reproductive potential.

Entomopathogenic fungi have also been reported to affect fecundity and fertility in many arthropods, which may have implications for the population dynamics of the host (Quesada-Moraga et al., 2004). The possible reduction of reproductive potential of *M. dirhodum* and *R. padi* adults that are fungally challenged during oviposition may contribute to the overall efficacy of the treatment.

Results from previous screen house experiments identified *M. anisopliae* (Metschnikoff) Sorokin (Ascomycota: Hypocreales) isolate ICIPE51 as a potential candidate for management of *R. padi* and *M. dirhodum*. The present study therefore investigates the effects of infection by *M. anisopliae* isolate 51 on different developmental stages of *R. padi* and *M. dirhodum* as well as the effects of fungal infection on fecundity and intrinsic rate of natural increase of both aphids.

MATERIALS AND METHODS

Aphid rearing

M. dirhodum and R. padi were reared on wheat plants, *Triticum aestivum*, variety Mbuni in ventilated Plexiglas cages (60 x 35 x 70 cm) at temperatures between 24-28°C, 60-70% relative humidity (RH) and a photoperiod of 12:12 h (L:D) in a rearing room at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya. The initial culture originated from aphids collected from Njoro town (0° 23'S and 35° 35'E), Kenya, in 2008. To obtain the different developmental stages for the experiments, adult aphids were collected from the aphid culture and put on fresh leaf discs placed on wet cotton wool in Petri dishes. The inoculated aphids reproduced parthenogenetically. Newly-emerged (one-day old) first-instar nymphs were transferred to new leaf discs and thereafter leaf discs were changed every four days. The rearing was carried out in such a way that different developmental stages were obtained at the same time so that treatments could be performed concomitantly.

Fungal pathogen

M. anisopliae isolate ICIPE 51 was used in the study. It was sourced from the ICIPE's Arthropod Germplasm Centre and was selected because of its virulence against *M. dirhodum* and *R. padi.* The fungus was grown for 21 days on Sabouraud dextrose agar (SDA) plates at 26 ± 2°C. Conidia were harvested by scrapping the surface using a sterile rubber. Inocula were suspended in 10 ml sterile distilled water containing 0.05% Triton X-100 in universal bottles containing glass beads. Conidial suspensions were vortexed for 5 min to produce a homogenous suspension. Spore concentrations were determined using a haemocytometer. Viability of conidia was determined before each bioassay by spread-plating 0.1 mL of conidial suspension titrated at 3.0×10^6 conidia/mL on SDA plates. Sterile microscopic cover slip was placed on each plate and plates were incubated at 26 ± 2°C and examined after 15 h. Percentage germination was determined from 100-spore counts. Each plate was replicated four times. Over 94% of conidia germinated in all the tests.

Inoculation of developmental stages

Nymphs aged 0-2 days, three to four days and adults (five to seven days old) were used in the bioassays. Both sides of fresh wheat leaves were sprayed with 10 mL of conidial suspension using Burgerjon's spray tower and allowed to dry for 20 min. Aphids were then transferred to the leaf discs in Petri dishes (90 mm diameter) using a camel hair brush. Concentrations of 1.0×10^6, 3.0×10^6 and 1.0×10^7 conidia/mL were used for each developmental stage. Control lots were treated with sterile distilled water containing 0.05% Triton X-100. Test-aphids were exposed to treated wheat leaf discs for 4 days, after which treated discs were removed and replaced with fresh and untreated leaf discs. Aphids were maintained in an incubator at 26 ± 2°C and 70-80% RH. Mortality was recorded daily for 10 days. Dead aphids were transferred to Petri dishes lined with moist filter paper to allow the growth of the fungus on the surface of the cadavers. Mycosis was confirmed by microscopic examination. Treatments consisted of 20 aphids each replicated five times and repeated twice.

*Corresponding author. E-mail: patrickmurerwa@gmail.com. Tel: +254-728-851685.

Table 1. Mean percent mortality of different nymphal instars and adults of *R. padi* and *M. dirhodum* treated with *M. anisopliae* isolate ICIPE 51.

Stage	Mean mortality (%)			
	M. dirhodum		*R. padi*	
	Treatment	Control	Treatment	Control
0-2 day-old nymphs	18.5[c]	0.3[b]	15.2[c]	0.5[b]
3 and 4 day-old nymphs	21.8[b]	0.8[b]	20.0[b]	0.6[b]
5-7 days old adults	31.1[a]	2.4[a]	25.6[a]	2.0[a]

Means within a column followed by the same letter are not significantly different at α = 0.05.

For fecundity bioassays, five replicates of three conidial concentrations (1.0 x 10^6, 3.0 x 10^6 and 1.0 x 10^7 conidia/mL) were inoculated to groups each containing about 30 apterous adult aphids. This total included extra aphids to ensure that each treatment would have 20 live aphids after being treated with conidial suspension. Treated aphids were transferred to a leaf in an assay cell, one aphid per cell. Each assay cell consisted of a 60-mm transparent plastic Petri dish containing a 5-cm length of wheat leaf from a greenhouse-grown plant (2-3 week old) with the ends contacting bands of water-soaked, sterile cotton. The assay cells were maintained in the ventilated Plexiglas cages. The cotton wool in the Petri dishes was saturated daily with water and every three to five days aphids were transferred to new leaf disks. New born nymphs were removed after counting. The treated aphids were observed daily for seven days to record mortality and fecundity. The experiment was repeated twice.

Statistical analysis

Percentage mortality was normalized through angular transformation after correcting for natural mortality (Abbott, 1925). Mortality rates were separated across treatments using the ANOVA procedure of SAS (SAS Institute, 2003). Mean values were separated using LSD at 0.05 level.

Differences in fecundity and intrinsic rate of increase were tested by analysis of variance (Anova). The intrinsic rate of natural increase (rm) was calculated using the following formula as described by Wyatt and White (1977):

$$rm = \frac{0.74\ (\ln Md)}{d}$$

Where, Md is the number of nymphs produced over a period of time equal to that of the entire pre-reproductive period (d). This formula gives a good estimate of population growth rates in aphids (Dixon et al., 1993).

RESULTS

Susceptibility of different *M. dirhodum* and *R. padi* developmental stages to *M. anisopliae* isolate ICIPE 51

In the viability test, more than 94% of spores germinated. Control mortalities for 0-2 day-old nymphs, 3 and 4 day-old nymphs and 5-7 days old adults in both aphid species ranged between 0.3 - 0.5, 0.6 - 0.8 and 2.0 and 2.4%, respectively after 9 days post treatment. Table 1 shows the mortality caused by *M. anisopliae* isolate ICIPE 51 at different developmental stages among the two aphid species. There were significant differences among both aphid species observed in mortalities of all nymphal instars and adults ($P < 0.05$). Three and four day-old nymphs were significantly more susceptible than 0-2 day-old nymphs. The five to seven day old adults were the most susceptible stage with 31 and 25% mortality against *M. dirhodum* and *R. padi* respectively as compared to 18 and 15% for *M. dirhodum* and *R. padi* respectively registered among 0-2 day-old nymphs.

There were differences in aphid mortality among all stages with increasing concentration of *M. anisopliae* isolate ICIPE 51 and these differences were statistically significant (*P < 0.05*). The lowest mortalities for *M. dirhodum* and *R. padi* was 19 and 16%, respectively recorded at 1 x 10^6 spores mL^{-1} among the 0-2 day-old nymphs while the highest mortalities for *M. dirhodum* and *R. padi* was 51 and 44% respectively registered at 1 x 10^7 spores mL^{-1} among the 5-7 days old adults. Percent mortality of different nymphal instars of *M. dirhodum* and *R. padi* treated with different concentrations of *M. anisopliae* isolate ICIPE 51 is shown in Table 2.

M. anisopliae isolate ICIPE 51 was able to infect 3 and 4 day-old nymphs and 5-7 days old adults 48 h after treatment whereas 0-2 day-old nymphs recorded mortality after 72 h. 5-7 days old adults were the most susceptible taking between 6 - 7 days to register 50% mortality as compared to the 0-2 day-old nymphs which took the longest time of between 8 - 9 days. At the end of experiment, the lowest mortality of 57% and highest mortality of 71% were observed among the 0-2 day-old nymphs and 5-7 days old adults, respectively (Table 3)

Dose and time effects of *M. anisopliae* isolate ICIPE 51 infection on the fecundity and intrinsic rate of increase of *R. padi* and *M. dirhodum*

Dose effect

Table 4 shows that the maximum fecundity in *M. dirhodum* and *R. padi* was 1.8 and 2.0 nymphs per aphid,

Table 2. Mean percent mortality of different nymphal instars and adults of aphids treated with different concentrations of *M. anisopliae* isolate ICIPE 51.

Stage	Mean mortality (%)							
	Control		Dose (Conidia/mL)					
			1×10^6		3×10^6		1×10^7	
	M. dirhodum	*R. padi*	*M. dirhodum*	*R. padi*	*M. dirhodum*	*R. padi*	*M. dirhodum*	*R. padi*
0-2 day-old nymphs	0.3^c	0.5^b	19.2^c	16.2^c	25.3^c	20.4^c	29.3^c	23.6^c
3 and 4 day-old nymphs	0.8^b	0.6^b	20.7^b	23.3^b	28.7^b	25.5^b	36.9^b	30.7^b
5-7 days old adults	2.4^a	2.0^a	31.4^a	24.3^a	39.2^a	31.7^a	51.4^a	44.4^a

Means within a column followed by the same letter are not significantly different at α = 0.05.

Table 3. Effect of time on mean percent mortality of different nymphal instars and adults of *R. padi* and *M. dirhodum* treated with *M. anisopliae* isolate ICIPE 51.

Days after treatment	Mean mortality (%)			
	Control	0-2 day-old nymphs	3 and 4 day-old nymphs	5-7 days old adults
0	0.0	0.0	0.0	0.0
1	0.0	0.0	0.0	0.0
2	0.0	0.0	0.3	1.4
3	0.0	0.8	2.5	7.0
4	0.0	3.6	7.8	15.5
5	0.2	10.3	16.1	27.3
6	1.0	20.3	26.9	41.5
7	1.8	31.6	39.8	54.9
8	3.5	44.6	52.9	65.0
9	4.5	57.4	62.9	71.0
LSD			1.4	
CV (%)			24.3	

Table 4. Effect of different doses of *M. anisopliae* isolate ICIPE 51 on fecundity and intrinsic rate of increase of treated *M. dirhodum* and *R. padi*.

Treatment	Fecundity		Intrinsic rate of increase (rm), %	
	M. dirhodum	*R. padi*	*M. dirhodum*	*R. padi*
Control	1.8^a	2.0^a	0.49^a	0.54^a
1×10^6	1.8^a	2.0^a	0.49^a	0.55^a
3×10^6	1.6^b	1.7^b	0.47^a	0.48^b
1×10^7	1.2^c	1.4^c	0.40^b	0.47^b

Means within a column followed by the same letter are not significantly different at α = 0.05.

respectively, as observed at the lowest concentration of 1×10^6 spores mL^{-1}. Both aphids species were significantly less fecund at 1×10^7 mL^{-1}, registering 1.2 and 1.4 nymphs per aphid for *M. dirhodum* and *R. padi*, respectively. There was no significant difference in fecundity among both aphid species between the control and 1×10^6 spores mL^{-1} treatments. *M. dirhodum* was significantly less fecund than *R. padi* at all tested concentrations. The intrinsic rate of natural increase (rm) was different among the aphid species as well as among the treatments (*P < 0.05*).

The rm value was the highest at 1×10^6 spores mL^{-1} (0.49 and 0.55 nymphs per aphid day^{-1} for *M. dirhodum* and *R. padi* respectively) as compared to the lowest value of rm at 1×10^7 spores mL^{-1} (0.40 and 0.47 nymphs per aphid d^{-1} for *M. dirhodum* and *R. padi* respectively).

Table 5. Effect of time on fecundity and intrinsic rate of increase of *M. dirhodum* and *R. padi* infected with *M. anisopliae* isolate ICIPE 51.

Days after treatment	Fecundity				Intrinsic Rate of Increase (rm), %			
	M. dirhodum		*R. padi*		*M. dirhodum*		*R. padi*	
	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
1	3.0	3.3	3.5	3.7	0.82	0.87	0.91	0.96
2	3.1	3.5	3.2	3.7	0.67	0.71	0.69	0.74
3	2.6	2.7	2.8	3.0	0.53	0.56	0.55	0.58
4	1.5	1.9	1.8	2.1	0.43	0.45	0.45	0.46
5	0.7	0.9	1.0	1.2	0.30	0.33	0.36	0.40
6	0.3	0.5	0.3	0.5	0.23	0.28	0.30	0.33
7	0.1	0.1	0.1	0.2	0.20	0.25	0.26	0.29
LSD	0.1				0.02			
CV	19.9				10.8			

Time effect

There was a general progressive decline in fecundity over time in both aphid species (Table 5). Fecundity in the first 2 days among both species was more than 3 nymphs/aphid. Thereafter, fecundity at 4 and 7 days post treatment reduced significantly and respectively to 1.5 and 0.1 nymphs/aphid and 1.8 and 0.1 nymphs/aphid for *M. dirhodum* and *R. padi,* respectively.

The highest intrinsic rate of increase (rm) was recorded during the first day (0.82 and 0.91 nymphs/aphid/day for *M. dirhodum* and *R. padi* respectively) while the lowest (0.20 and 0.26 nymphs/aphid/day for *M. dirhodum* and *R. padi,* respectively) was recorded on the seventh day.

DISCUSSION

Numerous studies indicate that aphids are susceptible to infection by diverse species of entomopathogenic fungi including *M. anisopliae* (Ibrahim et al., 2011; Shan and Feng, 2010). This study revealed that *M. anisopliae* isolate ICIPE 51 had pathogenic effects against *R. padi* and *M. dirhodum* although the latter was more susceptible with significant differences in mortality observed in all nymphal instars and adults. Susceptibility among both aphid species increased progressively with aphid age, 5-7 days old adults recording significantly higher mortalities than immature stages.

There are scant registers of the effects of *M. anisopliae* on developmental stages of either *R. padi* or *M. dirhodum*. However, it is possible to make comparisons with other insects. The higher susceptibility of adult aphids than immature 0-4 day old nymphs recorded in our study agrees with observation of Lopes and Alves (2011) that demonstrated adults of *Blattella germanica* (L.) (Blattodea: Blattellidae) were more susceptible to *M. anisopliae* infection than nymphs. Likewise, according to Romaña and Fargues (1992), the older larvae of *Melolontha melolontha* (L.) (Coleoptera: Scarabaeidae) were more susceptible to *Beauveria brongniartii* than the younger larval instars. Similar results have been reported by Ridsill-Smith and Annells (1997) who observed higher infection rate by *Neozygites floridana* in field-collected adults of *Tetranychus urticae* and *Halotydeus destructor* (Tucker) (Acarina, Penthaleidae) than in immature stages. In contrast, Haji et al. (2008) reported that fifth instar nymphs of Sunn pest were more susceptible to *B. bassiana* than adults. The foregoing reinforces an earlier observation by Ferron (1985) that relative susceptibility of different development stages of a host depends on the host species and on the fungal isolate. Ekesi and Maniania (2000) reported moulting to be an important factor in arthropod resistance to fungal infection, especially in arthropods with short ecdysis intervals. If the host is in an immature stage, molting could reduce the effectiveness of the fungal entomopathogen, in part owing to the shedding of conidia attached to the molted cuticle (Luz et al., 2003).

In our studies, germinated and ungerminated conidia were observed on the exuviae of *R. padi* and *M. dirhodum* following infection with *M. anisopliae*. It is probable the fungal inoculum was shed off with the exuvium following ecdysis leading to differential susceptibility observed in different nymphal stages and specifically the apparent decreased susceptibility of the immature aphid stages. The enhanced susceptibility of 5-7 days old aphids could as well be possibly attributed to the observed increased mobility of the mature adults across leaf surfaces as compared to the less active immature stages thereby increasing chances of contact of the relatively larger adult aphids with multiple fungal inocula.

Mortality in all life stages was dose-dependent, with the highest mortality occurring at 10^7 conidia/mL. Comparable results were reported on *T. urticae* with *B. bassiana* (Saenz-de-Cabezirigaray et al., 2003). Similar dose-mortality responses on different developmental stages

have also been reported on many other arthropod pests (Feng et al., 1985; Ekesi and Maniania, 2000). According to our results, high doses and long periods (time) are required for *M. anisopliae* isolate ICIPE 51 to cause satisfactory levels of mortality.

This study showed that both *R. padi* and *M. dirhodum* infected by *M. anisopliae* sustained an increase in reproductive output in response to early stages of infection followed by a reduction 5 days post inoculation. In contrast, other studies have suggested that pea aphid, *Acyrthosiphon pisum* aphids infected by *P. neoaphidis* initially registered fast and sustained decline in fecundity (Baverstock et al., 2006). Studies assessing the alarm response of pea aphids infected with either *P. neoaphidis* or *B. bassiana* support the hypothesis that host-specific fungi like *M. anisopliae* modify the behavior of the host whereas more generalist fungi do not (Roy et al., 2005). Pathogen and host fitness are directly dependent on the number of viable offspring produced and it is predicted that both will be adopting strategies to maximize reproductive output. Many studies have demonstrated that a reduction in host fecundity can increase pathogen fitness as host resources such as energy are used by the pathogen for conidia production rather than by the host for reproductive output (Xu and Feng, 2002). In our study, the increase in aphid fecundity may thus have been a result of the host diverting resources to reproduction as a defense strategy to increase fitness and possibly ensure that part of their reproductive potential is realized. This may also benefit the pathogen through ensuring the continuation of a susceptible host population (Blanford and Thomas, 2001). The subsequent reduction in fecundity may be the outcome of an incidental process in which the indiscriminate invasion of host tissues and production of secondary metabolites interferes with nymph production. These hypotheses, however, require further exploration.

M. anisopliae isolate ICIPE 51 infection led to significant reduction of the host aphid's progeny in both species. Low levels of inocula (10^6 conidia/mL) of the entomopathogen appeared to have no significant effect on aphids' fecundity and intrinsic rate of increase. Baverstock et al. (2006) observed that infection of the pea aphid, *Acyrthosiphon pisum* by either *P. neoaphidis* or *B. bassiana* reduced the number of nymphs produced within 24 h of inoculation and over the entire infection period as compared to uninfected aphids. However, infection for 24 or 72 h did not alter the intrinsic rate of increase of the host aphid. Similar results to our study were observed in the reproductive output of *Tutta absoluta* (Pires et al., 2008) and *Diuraphis noxia* (Wang and Knudsen, 1993) using *M. anisopliae* and *B. bassiana*, respectively. Other studies that have shown comparable results on this topic include that on *Cylas puncticollis* (Ondiaka et al., 2008), *Anoplophora glabripennis* (Hajek et al, 2008) and *Megalurothrips sjostedti* (Ekesi and Maniania, 2000).

Conclusions and recommendations

M. anisopliae isolate ICIPE 51 demonstrated pathogenicity against *R. padi* and *M. dirhodum* under controlled laboratory conditions. Virulence for all stages was dose-dependent and mortality increased with time. Low doses of the isolate appeared not to affect pre-lethal reproductive effects, such as fecundity and intrinsic rate of increase. Both aphid species were significantly more fecund in their early adulthood, suggesting the stage as ideal for biopesticide management intervention. These results showed that *M. anisopliae* isolate ICIPE 51 could be a viable alternative for control of *R. padi* and *M. dirhodum* in bread wheat.

On the other hand, it should be considered that the laboratory and greenhouse bioassays were conducted under optimal conditions for fungal growth (e.g., high humidity and constant temperatures and photoperiods), which are obviously very different from environmental conditions that would be encountered in the field (Butt and Goettel, 2000). Hence, additional research at field conditions to further evaluate and consolidate findings regarding biopesticide potential *M. anisopliae* isolate ICIPE 51 would be necessary.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to International Centre of Insect Physiology and Ecology (*icipe*) for providing them with all the isolates used in the study and allowing the usage of their facilities. We also wish to thank Ms E.O. Ouna of the Arthropod Pathology Unit (APU), *icipe* for technical support and Mr. J. Kamundia of KARI, Njoro for help assistance with statistical analysis. Special thanks go to linguistics and communication expert Dr. Ndambuki J. for assistance in stylistic editorial improvement of manuscript.

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