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Journal

Biocontrol Science and Technology, 26(2)

ISSN

0958-3157

Authors

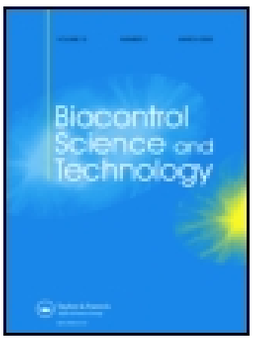
Bayissa, Wakuma
Ekesi, Sunday
Mohamed, Samira A
et al.

Publication Date

2016-02-01

DOI

10.1080/09583157.2015.1099148



Biocontrol Science and Technology

ISSN: 0958-3157 (Print) 1360-0478 (Online) Journal homepage: http://www.tandfonline.com/loi/cbst20

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To cite this article: Wakuma Bayissa, Sunday Ekesi, Samira A. Mohamed, Godwin P. Kaaya, John M. Wagacha, Rachid Hanna & Nguya K. Maniania (2015): Interactions among vegetable infesting aphids, the fungal pathogen Metarhizium anisopliae (Ascomycota: Hypocreales) and the predatory coccinellid Cheilomenes lunata (Coleoptera: Coccinellidae), Biocontrol Science and Technology, DOI: 10.1080/09583157.2015.1099148

To link to this article: http://dx.doi.org/10.1080/09583157.2015.1099148

Accepted online: 28 Sep 2015.

Publisher: Taylor & Francis

Journal: *Biocontrol Science and Technology*

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Interactions among vegetable infesting aphids, the fungal pathogen *Metarhizium anisopliae* (Ascomycota: Hypocreales) and the predatory coccinellid *Cheilomenes lunata* (Coleoptera: Coccinellidae)

Wakuma Bayissa[a,b], Sunday Ekesi[a,]*, Samira A. Mohamed[a,] , Godwin P. Kaaya[b], John M. Wagacha[b] , Rachid Hanna[c] and Nguya K. Maniania[a]

[a]*Plant Health Division, International Centre of Insect Physiology and Ecology (icipe); Nairobi, Kenya;* [b]*School of Biological Sciences, University of Nairobi, Nairobi, Kenya;* [c]*International Institute of Tropical Agriculture (IITA), Yaoundé, Cameroon*

(Received 28 July, 2015; Returned 27 August; Accepted 20 September, 2015)

*Corresponding author. Email:sekesi@icipe.org

Abstract

Entomopathogenic fungi are among biocontrol agents being considered for the control of aphids on a variety of crops. Predatory coccinellids, although generalist, are also among important natural enemies that must be conserved for aphid management. Laboratory studies were carried out to investigate interaction between three vegetable infesting aphids, *Metarhizium anisopliae* isolate ICIPE 62 and the coccinellid predator *Cheilomenes lunata*. At concentration of 1×10^8 conidial ml^{-1}, the fungus was found to cause mortality of 7.5% to *C. lunata*, compared to 2.5% mortality in the control at 10 days post-treatment. Female adult *C. lunata* to which fungus-infected aphids were offered as prey never accepted them as food source in non-choice bioassays. However, live and dead non-infected aphids were fed upon. In choice bioassay, a total of 1-3 out of 24 infected non-sporulating aphids per species (average of 0.1- 0.4 aphids per arena) were consumed by 48 h starved *C. lunata* within a period of 60 min, but avoided sporulating cadavers. Foraging adult *C. lunata* enhanced the spread of conidia of *M. anisopliae* from infected cadavers to fourth instars *A. gossypii* feeding on okra (0.8-15.0% mortality), *B. brassicae* (3.3-15.0% mortality) and *L. pseudobrassicae* (0.8-14.2% mortality) on kale plants. Results of this study demonstrate compatibility between *M. anisopliae* and *C. lunata*, and could provide a sustainable strategy for effective management of aphids on crucifers and okra cropping systems.

Keywords: entomopathogenic fungus; coccinellid; aphids; starvation; spread

1. Introduction

Crucifers (e.g. cabbage and kale) and okra crops are among the most widely grown vegetables in sub-Saharan Africa (SSA) at large and small-scale farm levels (Smith & Eyzaguirre, 2007; Shackleton, Pasquini, & Drescher, 2009; Ojiewo, Tenkouano, & Yang, 2010). Cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae) and turnip aphids, *Lipaphis pseudobrassicae* Davis (Hemiptera: Aphididae) are considered as the most important pests causing yield losses of up to 100% on kale and cabbage. In addition to these two aphid species, the cotton/melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) is also regarded as an economically important pest on several vegetable crops including okra in tropical Africa (Sithanantham et al., 1997; Nderitu, Kasina, Kimenju, & Malenge, 2008; Sæthre, Godonou, Hofsvang, Tepa-Yotto, & James, 2011). Vegetable growers in Africa commonly rely on frequent use of synthetic chemical insecticides such as pyrethroids, organo-phosphates and carbamates to control arthropod pests including aphids (Ntow, Gijzen, Kelderman, & Drechsel, 2006; Williamson, Ball, & Pretty, 2008; de Bon et al., 2014). Resistance development by these pests has also been documented. For instance, *A. gossypii* has developed resistance to several pyrethroid and organophosphate insecticides (Carletto, Martin, Vanlerberghe-Masutti, & Br´evault, 2010; Koo, An, Park, & Ju-Il Kim, 2014). Therefore, exploring non-chemical alternatives for controlling aphids is crucial.

Entomopathogenic fungi (EPF) are being used as alternative control agents against many insect pests either as a stand alone control agents or as a component of integrated pest management programmes. Some of them have already been registered

for aphid control in Europe and North America (de Faria & Wraight, 2007; Kabaluk, Svircev, Goettel, & Woo, 2010; Jandricic, Filotas, Sanderson, & Wraight, 2014). However, there are no reports of a commercial biopesticide product registered for aphid control in SSA. Recently, we screened the virulence of five isolates of *Metarhizium anisopliae* (Metsch.) Sorok. and three of *Beauveria bassiana* (Bals.) Vuill. against apterous adults of *B. brassicae*, *L. pseudobrassicae* and *A. gossypii*, and determined their thermotolerance and conidial yield as a prerequisite for strain selection against the target pest. The study identified *M. anisopliae* ICIPE 62 as highly pathogenic to the three aphid species causing mortality of 83-97% on the 3 aphid species, after 7 d post-inoculation (Bayissa et al., 2015). *Cheilomenes lunata* Fabricius (Coleoptera: Coccinellidae) has been documented in many parts of Africa as an important biocontrol agent of different aphids species (Brown, 1972; Ofuya, 1995; Adisu & Freier, 2003; Woin, Volkmar, & Ghogomu, 2006). In Kenya, it has been observed preying on *B. brassicae* and *A. gossypii* on crucifers and okra plants (S. Ekesi, unpublished data). Despite their role as a biocontrol agent for aphids, coccinellids are also known to be susceptible to EPF such as *Hesperomyces virescens* Thaxter and *Beauveria bassiana* (Balsamo) Vuillemin - in fact *B. bassiana*, is widely documented as major mortality factors of overwintering coccinellids (Iperti, 1966; Ceryngier & Hodek, 1996; Barron & Wilson, 1998; Ormond, Thomas, Pell, & Roy, 2006); but there are no similar records for other Hypocreales. As biopesticides and natural enemies (parasitoids and predators) become available and attractive for incorporation into integrated pest management programs (IPM), it is important to understand their interactive roles. Roy and Cottrell (2008) opined that there is considerably less information on interaction between coccinellids and naturally-occurring fungal pathogens and recommended the need for future research to address the profound absence of knowledge.

Several studies have demonstrated the existence of asymmetric and symmetric intra-guild predation between entomopathogens and insects (Roy & Pell, 2000). For example, *C. septempunctata*, *Coccinella septempunctata brucki* Mulsant (Coleoptera: Coccinellidae) and *H. axyridis* consume aphids at a late stage of *Pandora neoaphidis* (Remaudière and Hennebert) Humber infection (Pell, Pluke, Clark, Kenward, & Alderson., 1997; Roy, Clark, & Alderson 1998; Roy, Pell, & Alderson, 2003; Roy et al., 2008). It was concluded that these coccinellids have the potential to negatively impact on *P. neoaphidis* dynamics but in all cases a preference was shown for uninfected aphids even though this preference was only marginal for *H. axyridis* (Roy et al., 2008). Host and non-host dispersal are important for pathogen transmission within an agroecosystem. Roy, Pell, Clark, & Alderson (1998) and Wells et al. (2011) demonstrated the impact of coccinellid foraging on *P. neoaphidis* transmission within a plant. Similar studies among the Hypocrelean fungi are needed to improve our understanding of insect-host-pathogen dynamics (Roy and Cottrell, 2008).

Metarhizium anisopliae is considered to have minimal risks to non-target organisms (Zimmermann, 2007). However, since it has been reported on more than 200 insect hosts (Veen, 1968), there is the need to assess their interactions and compatibility with non-target organism if they have to be used together within the context of aphid IPM programs (Jaronski et al., 1998). This study was therefore carried out to (1) assess the pathogenicity of *M. anisopliae* isolate ICIPE 62 against adult *C. lunata*, (2) evaluate the effects of fungal infection on the predation behavior of *C. lunata* on *M. anisopliae*-infected *A. gossypii, B. brassicae* and *L. pseudobrassicae,* and (3) assess the likelihood of *C. lunta* to vector fungal conidia to susceptible aphids during foraging behavior on host plants.

2. Material and methods

2.1. Aphid culture

The stock cultures of aphids (*B. brassicae, L. pseudobrassicae* and *A. gossypii)* originated from field populations infesting cabbage, kale and okra in vegetable production sites of Nyeri, Kenya (0°21'10.69"S 37°5'14.35"E, 1878 meters above sea level (m.a.s.l)), and Nguruman, Kenya (1°48'22.1"S 36°03'41.2"E, 746 m.a.s.l). The cabbage aphids, *B. brassicae* and turnip aphids, *L. pseudobrassicae* were reared on kale (*Brassica oleracea* L. var. 1000-headed) and cotton/melon aphids, *A. gossypii* on okra (*Abelmoschus esculentus* (L.) Moench var. Pusa sawani) in the insectary at 27-28°C, photoperiod of 12: 12 L: D and 45-50% RH. In order to obtain insects of uniform aged groups, apterous adults of each species were removed from the insectary colony and introduced on fresh kale or okra leaves in Petri dishes (90 mm) to larviposit for 24 h. The offspring that were produced were reared synchronously until they reached the desired stage and thereafter used for the bioassays.

2.2. Coccinellid colony

The initial cohort (n > 50) of *C. lunata* originated from organic-grown kale or okra plants infested with aphids at the *icipe'*s Thomas R. Odhiambo campus, Mbita Point (0°34'S 34°10'E; 1170 m.a.s.l). The insect was identified using the reference collection materials at *icipe'*s Biosystematics Unit and the National Museums of Kenya. The adult coccinellids were maintained on aphid-infested kale or okra seedlings at *icipe'*s insectary in a mosquito-net cage (40 × 40 × 63 cm) at 27-28°C, photoperiod of 12:12 L: D and 45-50% RH. As with other similar studies (Roy, Pell, Clark, & Alderson, 1998), coccinellids are generally difficult to rear. In our study, attempt was made to establish

laboratory colony of *C. lunata*, however there was high mortality of the immature stages. Therefore, field collected adults of *C. lunata* were used for this study. In the laboratory, the field collected adult female *C. lunata* were kept individually in transparent 90-mm Petri dish with approximately 2 mm ventilation hole on the lid and fed on aphids (*B. brassicae*, *L. pseudobrassicae* or *A. gossypii*) for at least two weeks prior to being used for the treatments. Only female *C. lunata* were used in the study.

2.3. Fungal culture

The *M. anisopliae* isolate ICIPE 62 was obtained from the *icipe*'s Arthropod Germplasm Centre and maintained on Sabouraud dextrose agar (SDA) at 26 ± 2°C in complete darkness. This isolate originated from soil sample collected at Matete, Kinshasa (D.R. Congo) in 1990 and was selected for its virulence against *B. brassiace*, *L. pseudobrassicae* and *A. gossypii* (Bayissa et al., 2015). Conidia were harvested from 2-3 week old sporulating cultures by scraping using a sterile spatula and suspended in 10 ml sterile distilled water containing 0.05% Triton X-100 in universal bottles containing glass beads (3 mm). The suspension was vortexed for 5 minutes at 700 revolutions per minutes (rpm) to break the conidial clumps and ensure a homogeneous suspension. Conidia were quantified using a haemocytometer under a light microscope and thereafter used for viability test and various bioassays.

For viability test, 0.1 ml of a of 3×10^6 conidia ml^{-1} suspension was spread-plated on SDA using sterile glass rod and three sterile microscope cover slips were placed randomly on the surface of each inoculated plate. The plates were sealed with Parafilm M and incubated at 26 ± 2°C under complete darkness. Conidia germination was assessed after 18 h by counting 100 conidia under each coverslip using a light microscope (× 400 magnifications).

2.4. Inoculation of insects

2.4.1. *Coccinellid*

A set of 20 *C. lunata* adults were transferred into Petri dishes (100 diam. × 15 mm height) and chilled on ice for approximately 5 min to immobilize them. They were then sprayed with 10 ml of aqueous suspension of *M. anisopliae* isolate ICIPE 62 at a concentration of 1×10^8 conidia ml^{-1} using Burgerjon's spray tower (Burgerjon, 1956). Air atomizing nozzle with a valve providing a constant airflow under 4 bar pressure fitted to the tower resulting to suspension deposit of approximately 3.9×10^7 conidia cm^{-2}. Rotating plate fitted underneath of the tower also ensure even distribution of the suspension on the surface of the insects. Another set of 20 *C. lunata* was sprayed with sterile distilled water containing 0.05% Triton X-100 and served as a control. Test-insects were then transferred separately to transparent plastic box (120 ×140 × 40 mm) lined with moistened Whatman filter paper to maintain humidity for a period of 24 h at 26 ± 2°C. Each dish contained aphids infested kale or okra as a source of food for the coccinellids. After 24 h, coccinellids were transferred to a mosquito-net cage (180 × 180 × 180 mm) and then fed on healthy aphids throughout the experimental period. Mortality was recorded daily for 10 days post-treatment. Dead insects were kept individually in a Petri dish lined with sterile moistened Whatman filter paper to initiate mycosis. Mortality due to *M. anisopliae* was confirmed through microscopic examination of hyphae and spores on the surface of the cadaver. The experiment was replicated four times.

2.4.2. Aphids

Two-day old apterous adult aphids of each species were transferred on to fresh leaf disks (ca. 80 mm diameter) of kale (for *B. brassicae* and *L. pseudobrassicae*) and okra

(for *A. gossypii*) in a 90-mm Petri dish. Insects were allowed to settle on the leaves for about 20 min and were then sprayed with 10 ml of aqueous suspension of *M. anisopliae* isolate ICIPE 62 at a concentration of 1×10^8 conidia ml^{-1} corresponding to a surface coverage rate of 3.9×10^7 conidia cm^{-2} using Burgerjon's spray tower as described above. Leaf discs containing aphids were allowed to dry for 5 min, after which aphids were transferred to fresh unsprayed surface-sterilized leaf disks (ca. 100 mm diameter) in 120 ×140 × 40 mm plastic box lined with moistened filter paper. Test-insects were incubated at 26 ± 2°C. Dead insects were transferred into 90-mm Petri dish lined with sterile moistened filter paper to allow for development of mycosis on the surface of cadavers. Three- and 5-day old non-sporulating and sporulating cadavers, respectively, were used in the experiment on the effect of starvation period and prey type (fungus-infected aphids) on *C. lunata* predation. In the control, insects were sprayed with distilled water containing 0.05% Triton X-100 and served as source of live or dead insects. Non-infected aphids were killed by freezing at -18°C for 24 h.

2.5. Effect of starvation period and prey type (fungus-infected aphids) on C. lunata predation

The effect of starvation and prey type on predation of *C. lunata* was investigated in non-choice and choice experiments. *Cheilomenes lunata* adult females were starved for 0, 24 and 48 h and were transferred individually into 90-mm Petri dish with ventilated lid containing aphids prey types. In non-choice experiments, individual coccinellids were offered the following prey types: (1) six dead non fungus-infected aphids, (2) six live non fungus-infected aphids, (3) six dead non-sporulating aphids and (4) six sporulating aphids. In choice experiments, two sets of bioassays were done using the method described by Roy, Pell, Clark, & Alderson (1998). Individual *C. lunata* were offered the following prey types: (1) three sporulating and three dead non fungus-infected

aphids and (2) three dead non-sporulating and three dead non fungus-infected aphids. Each prey item was placed in 90-mm Petri dish lined with moistened filter paper. For the choice assay, prey items (infected and non-infected aphid cadavers) were placed at random positions within a dish. The Petri dishes were then covered with ventilated lids to provide aeration for the coccinellids. Each treatment was replicated eight times. All the experiments were done in the laboratory at 27 - 28°C and 45 - 50% relative humidity (RH). The number of prey items entirely consumed was recorded after 60 min and 180 min for non-choice and 60, 120 and 180 min for choice experiments.

2.6. Effect of C. lunata foraging on transmission of fungal infection to healthy aphids on kale and okra plants

Kale and okra were planted in 10 × 15 × 20 cm plastic pots by direct seeding. When plants were approximately 20 cm high, they were thinned to one plant/pot, and transferred to a mosquito-netting cage (40 × 40 × 63 cm). Thereafter, the kale plants were infested with 30 healthy fourth-instar *B. brassicae* or *L. pseudobrassicae*, and the okra was infested with *A. gossypii*. Test-insects were obtained from the insectary under production conditions as previously described. Aphids were allowed to settle on their respective host plants and thereafter, 5-day old profusely sporulating cadaver(s) of the respective aphid species were placed on the plants at the following densities: (1) One cadaver, (2) 5 cadavers, (3) 10 cadavers and (4) no cadaver (control). One adult *C. lunata* per plant was then introduced in to each cage and allowed to forage on the prey. The same setup was used for the control group except that no beetle was introduced. After 5 h, the coccinellids were removed from each cage and all surviving aphids were monitored daily for mortality for 7 days. Each treatment was replicated four times. Dead aphids were placed individually in a Petri dish lined with moistened filter paper. Aphid

cadavers were assessed for mycosis as previously described for coccinellid bioassay.

2.7. Data analysis

All data analyses were performed using R v2.15.1 statistical software package (R Development Core Team, 2011). Percent mortality data of *C. lunata* was checked for normality and homogeneity of variance using Shapiro-Wilk and Bartlett's tests before being subjected to analysis of variance (ANOVA). The data on percentage prey consumed in the non-choice treatments were normalized by arcsine square-root transformation (Gomez & Gomez, 1984) to stabilize the variances before subjecting to repeated measures ANOVA. To assess for the infected and non-infected prey item consumed in the choice treatments, the number of infected (C_i) and non-infected (C_u) prey item were subjected to log transformation after adding 0.05 to allow zero counts (i.e. $r = C_i + 0.05$ and $l = C_u + 0.05$) and then log- ratios (i.e. $\log(l/r)$ computed: where no preference was shown between prey type then $\log(l/r) = 0$, non-infected aphids preferred prey type, $\log(l/r) > 0$, and infected aphids were preferred prey type, $\log(l/r) < 0$ and then repeated measures ANOVA was applied on log-ratio values (Roy et al., 2008). Generalized linear model (i.e. binomial error) was used to assess the effect of cadaver density and coccinellid vectoring role on spread of conidia to aphid population.

3. Results

3.1. Pathogenicity of M. anisopliae against C. lunata

M. anisopliae ICIPE 62 caused mortality of 7.5 ± 0.9% in *C. lunata* after 10 days post-treatment while mortality in the control was of 2.5 ± 0.9% and was significantly different ($F = 6$; df =1,6; $P = 0.049$) with the fungus treatment.

3.2. Effect of starvation period and prey type (fungus-infected aphids) on C. lunata predation

3.2.1. Non-choice test

Regardless of starvation status and prey species, *C. lunata* did not consume fungus-infected aphids at 60 and 180 min observation periods (Figs. 2 and 3). However, they fed on both live and dead non-infected aphids. Among the non-infected aphids, more *A. gossypii* (35.4-95.8%) were consumed by the predator compared to *B. brassicae* (10.4-60.4%) and *L. pseudobrassicae* (10.4-72.9%) across the observation period. Predation was significantly influenced by aphid species ($F = 84.48$; df = 2,252; $P < 0.0001$), prey type ($F = 643.45$; df = 3, 252; $P < 0.0001$) and coccinellid starvation ($F=16.77$; df = 2,252; $P < 0.0001$) over a period of 60 and 180 min observation. Significant interaction was also observed between aphid species and prey type ($F = 29.20$; df = 6,252; $P < 0.0001$) and, prey type and coccinellid starvation time ($F = 5.96$; df = 6,252; $P < 0.0001$) in determining prey consumed by coccinellid. For example, consumption by 48 h-starved *C. lunata* of dead non-infected *A. gossypii* was 70.8 and 83.3% during 1 h and 3 h observation, respectively; which was greater than consumption by non-starved *C. lunata* (35.4 and 72.9%). Overall, consumption by coccinellids increased with increasing starvation period, except for 24 and 48 h starved coccinellids when offered live non-infected *A. gossypii* and dead non-infected *L. pseudobrassicae* after 60 and 180 min, respectively. However, interaction between prey species and coccinellid starvation time ($F = 1.2$; df = 4,252; $P = 0.310$) and among aphid species, prey type and coccinellid starvation ($F =0.98$; df =12,252; $P = 0.465$) had no significant effect on coccinellid consumption over observation periods. Similar trends were also observed when offered with non-infected *B. brassicae* and *L. pseudobrassicae* (Figs. 1 and 2).

3.2.2. Choice test

In the choice bioassay, the mean numbers of non-infected and fungus-infected (sporulating and non-sporulating) aphid species consumed by coccinellids at different starvation time are presented in Table 1. Starved and nonstarved coccinellids were able to differentiate between sprorulating and non-infected aphid cadavers and showed feeding avoidance behavior to fungus-infected cadavers over the period of observation (Table 1). However, 48 h-starved coccinellids attempted to feed on non-sporulating aphid cadavers. For example, 6.3% of non-sporulating cadavers of *A. gossypii* and 2.1% of *B. brassicae* were consumed during the first 60 min observation time by 48 h-starved coccinellids. In the case of *L. pseudobrassicae*, predation (2.1% of non-sporulating cadavers) by 48 h-starved coccinellids occured after 180 min observation time.

Overall, coccinellid foraging behavior was significantly affected by aphid species (F= 19.24; df = 2,126; $P < 0.0001$) and coccinellid starvation status (F= 8.28; df = 2,126; $P < 0.0004$). For example, 48 h-starved *C. lunata* consumed 83.3, 60.4 and 54.2% of dead non-infected *A. gossypii*, *B. brassicae* and *L. pseudobrassicae* respectively, over 3-h observation periods. Moreover, interaction between aphid species and prey type (F= 7.51; df = 2,126; P = 0.001), prey type and coccinellid starvation status (F= 14.79; df = 4,126; $P < 0.0001$) had significant effect on coccinellid foraging behavior over observed times.

The mean differences between infected and non-infected aphids consumed by coccinellid were greater than zero (Figs 3 A, B & C). However, these values, varied with coccinellid starvation status and aphid prey species. For example, the highest values of mean difference (M_D) between fungus-infected and non-infected cadavers is 1.79 and standard error of difference (SE_D) = 0 for *A. gossypii*, and M_D = 1.76, SE_D = 0.02 for *B. brassicae* and M_D = 1.66, SE_D = 0.06 for *L. pseudobrassicae* after 180 min.

Overall, the results showed that non-infected prey were preferred by the coccinellid in all the three aphid species (Figs. 3 A, B & C).

3.3. Effect of C. lunata foraging on transmission of fungal infection to healthy aphids on kale and okra plants

Fungal infection did not occur in all three aphid species used in the control treatments (absence of fungus-infected cadavers) in the presence or absence of coccinellid, and data were therefore excluded from analysis. Exposure of healthy *B. brassicae, L. pseudobrassicae* and *A. gossypii* on their respective host plants to varying densities of fungus-infected cadavers resulted in fungal infection due to the foraging activity of coccinellids (Fig. 4). However, infection varied according to the density of cadavers, with 10 cadavers having the highest infection rate. In the presence of foraging *C. lunata,* infection significantly increased in *A. gossypii* (χ^2 = 3.6; df = 2; $P < 0.0001$) on okra, and *B. brassicae* (χ^2 = 6.4; df = 2; P = 0.002) and *L. psueudobrassicae* (χ^2 = 4.2; df = 2; $P < 0.0001$) on kale plants compared to the absence of coccinellid (Fig. 4). *Metarhizium anisopliae* profusely sporulated on dead aphids of the three species.

4. Discussion

For successful integration of biological control agents into IPM programs, they must be virulent to the target pests and at the same time have minimal negative effect on nontarget organisms (Goettel, Poprawski, Vandenberg, Li, & Roberts, 1990). *Metarhizium anisopliae* isolate ICIPE 62 whose virulence to the three aphid species namely, *A. gossypii, B. brassicae* and *L. pseudobrassicae,* has been demonstrated earlier (Bayissa et al., 2015) and and currently found to be less detrimental to adult *C. lunata* meets these criteria and warrants integration into management programs targeted at the

3 aphid species. In our study, less than 10% mortality of *C. lunata* was observed after 10 d post-infection with the fungus. Our results support the assertion by various workers that despite the wide host range of *M. anisopliae*, certain strains and genotypes are more restricted to particular species of insects (Bidochka & Small, 2005). Furthermore, some isolates of EPF are also more specific under field conditions compared to laboratory studies (Jaronski, Goettel, & Lomer, 2003). For example, Ekesi, Maniania, Ampong-Nyarko, & Onu, (1999) found no adverse effects on nontarget organisms including coccinellids following applications of *M. anisopliae* to control *Megalurothrips sjostedti* (Trybom) in a cowpea agroecosystem. A similar result was also reported in onion cropping systems following weekly spray of *M. anisopliae* to control *Thrips tabaci* L. (Maniania et al., 2003). On the contrary, Ginsberg, Lebrun, Heyer, & Zhioua, (2002) showed that an isolate of *M. anisopliae* was pathogenic to the convergent ladybird beetles, *Hippodamia convergens* Guérin-Méneville in the laboratory. However, in this study, high mortality was observed only over a period of 30 days. The underlying mechanism for the lack of susceptibility of *C. lunata* to infection by *M. anisopliae* in our study is still unclear.

In non-choice test, our results showed that preying of *C. lunata* on *M. anisopliae*-infected aphids were not affected by the interaction effect of prey species, prey type and coccinellid starvation status within 180 min observation period. This would probably reduce the intraguild interactions between the coccinellid and fungal pathogen. However, when *C. lunata* was offered the choice between non-sporulating and dead non-infected aphid prey, few infected non-sporulating aphid cadavers were perceived as a food source by 48-h starved *C. lunata* during the first 60 min. When offered the choice between sporulating and dead non-infected aphid cadavers, the coccinellids did not feed on sporulating aphid cadavers; which suggests the ability of *C.*

lunata to descriminate among different stages of infection of the prey by fungal pathogen as previously observed in *Dicyphus hesperus* Knight (Alma, Gillespie, Roitberg, & Goettel, 2010). Pell & Vandenberg (2002) demonstrated that the convergent ladybird, *H. convergens*, avoided feeding on Russian wheat aphids, *Diuraphis noxia* (Mordavilko) (Hemiptera: Aphididae), infected with *P. fumosoroseus*. Feeding avoidance of *C. lunata* on fungus-infected cadavers observed in the present study is also similar to the one reported with other generalist insect predators: *Coccinella septempunctata* L. (Pell, Pluke, Clark, Kenward, & Alderson, 1997), *Anthocoris nemorum* L. (Meyling & Pell, 2006) and *Orius albidipennis* Reuter (Pourian, Talaei-Hassanloui, Kosari, & Ashouri, 2011).

In the single caged-plant foraging experiments, the presence of adult *C. lunata* enhanced spread of *M. anisopliae* from infected cadavers and increased infection of *A. gossypii* on okra, and *B. brassicae* and *L. pseudobrassicae* on kale and okra. The high mobility of adult coccinellids (Dixon, Hemptinne, & Kindlmann, 1997) might have increased the spread of conidia and subsequent contamination of healthy aphids. Moreover, the escape response to avoid predation in aphids would increase acquisition of conidia through direct contact with the cadaver. The role of *C. lunata* in vectoring inoculum observed in the present study is similar to the one reported with *C. septempunctata* (Pell, Pluke, Clark, Kenward, & Alderson , 1997; Roy, Pell, Clark, & Alderson, 1998; Ekesi, Shah, Clark, & Pell, 2005; Wells et al., 2011). Variation of infection of aphids following exposure to different cadaver density observed in the current study can be explained by high density of conidia received by susceptible host (Vandenberg, Ramos, & Altre, 1998). However, infection was generally low across the three aphid species. The foraging period of coccinellids on conidial dissemination has probably an effect on infection of aphids although it was not investigated in the current

study. As previously suggested by Ekesi, Shah, Clark & Pell (2005), extended time of coccinellid foraging on the plants could increase the infection of aphids. Overall, these behavioural activities should increase secondary uptake of spores from the plant surfaces and enhance infection and subsequent suppression of aphid populations in the agroecosystems.

5. Conclusions

We conclude that *C. lunata* is less susceptible to *M. anisopliae* isolate ICIPE 62 at a concentration that caused high mortality in all the three aphid species tested. In addition, prey preference from *M. anisopliae*-infected to non-infected was not observed as a result of coccinellids starvation status and aphids prey species under prey choice and/or non-choice test. Fungus-infected aphids were not preferred as prey, thus, it is unlikely that coccinellids feeding would reduce the amount of inoculum in order to initiate infection in aphid populations. Rather, the presence of *C. lunata* on host plants enhances transmission and spread of conidia which should be advantageous to the overall aphid suppression strategy. However, further field studies are necessary to generate more information on the interaction among fungal pathogen, coccinellids and aphid pests.

Acknowledgements

This study was jointly supported by funds from the German Ministry of Economic Cooperation and Development (BMZ) through a joint International Institute of Tropical Agriculture (IITA)/icipe/World Vegetable Centre/ Friedrich-Schiller-University of Jena, German Academic Exchange Service/Deutcher Akademischer Austausch Dienst (DAAD) through the African Regional Postgraduate Programme in Insect Science (ARPPIS) of the International Centre of Insect Physiology and Ecology (*icipe).* The

authors are grateful to Dr. Daisy Salifu and Mr. Benedict Orindi for assisting in statistical analysis, and Mr. Gordon Otieno and Oreng Pascal for technical assistance.

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Table 1. Mean (±SE) number of *M. anisopliae* infected and non-infected cadavers consumed by *C. lunata* when given as a choice over a period of 180 min.

Aphid prey Species	Coccinellid starvation Status	Mean number of prey consumed by *C. lunata* within:											
		60 min		120 min		180 min		60 min		120 min		180 min	
		SC	NC	SC	NC	SC	NC	NSC	NC	NSC	NC	NSC	NC
A. gossypii	Non-starved	0.0±0.0	1.6±0.2	0.0±0.0	2.1±0.1	0.0±0.0	2.6±0.2	0.0±0.0	1.9±0.2	0.0±0.0	2.3±0.2	0.0±0.0	2.8±0.2
	24 h starved	0.0±0.0	2.8±0.3	0.0±0.0	3.0±0.0	0.0±0.0	3.0±0	0.0±0.0	1.9±0.2	0.0±0.0	2.3±0.2	0.0±0.0	2.9±0.1
	48 h starved	0.0±0.0	1.0±0.4	0.0±0.0	1.6±0.4	0.0±0.0	1.9±0.4	0.4±0.2	1.8±0.4	0.4±0.2	2.3±0.4	0.4±0.2	2.6±0.2
B. brassicae	Non-starved	0.0±0.0	0.3±0.2	0.0±0.0	0.3±0.1	0.0±0.0	0.4±0.2	0.0±0.0	0.1±0.1	0.0±0.0	0.4±0.3	0.0±0.0	0.9±0.4
	24 h starved	0.0±0.0	0.5±0.2	0.0±0.0	0.9±0.2	0.0±0.0	1.1±0.3	0.0±0.0	2.0±0.3	0.0±0.0	2.1±0.3	0.0±0.0	2.9±0.1
	48 h starved	0.0±0.0	0.6±0.3	0.0±0.0	1.5±0.4	0.0±0.0	1.5±0.4	0.1±0.1	1.8±0.3	0.1±0.1	2.4±0.2	0.1±0.1	2.6±0.2
L. pseudobrassicae	Non-starved	0.0±0.0	1.0±0.2	0.0±0.0	1.3±0.1	0.0±0.0	1.6±0.3	0.0±0.0	1.1±0.2	0.0±0.0	2.0±0.4	0.0±0.0	2.4±0.3
	24 h starved	0.0±0.0	1.5±0.3	0.0±0.0	2.0±0.4	0.0±0.0	2.5±0.3	0.0±0.0	0.5±0.3	0.0±0.0	1.8±0.3	0.0±0.0	1.8±0.3
	48 h starved	0.0±0.0	0.8±0.3	0.0±0.0	2.1±0.4	0.0±0.0	2.3±0.3	0.0±0.0	0.6±0.4	0.0±0.0	1.0±0.4	0.1±0.1	2.0±0.2

Where, SC =sporulating cadavers; NC = non-infected cadavers; NSC = non-sporulating cadavers

Figures caption in the manuscript

Figure 1. Effect of stage of mycosis development in aphid cadavers on the proportion of (A) *A. gossypii* (B) *B. brassicae* and (C) *L. pseudobrassicae* preyed by *Cheilomenes lunata* after 60 min in non-choice test. Where □0 h = Non-starved *C. lunata*; ▣24 h = 24 hours starved *C. lunata*; ■48 h = 48 hours starved *C. lunata.*

Figure 2. Effect of stage of mycosis development in aphid cadavers on the proportion of (A) *A. gossypii* (B) *B. brassicae* and (C) *L. pseudobrassicae* preyed by *Cheilomenes lunata* after 180 min in non-choice test. Where □0 h = Non-starved *C. lunata*; ▣24 h = 24 hours starved *C. lunata*; ■48 h = 48 hours starved *C. lunata.*

Figure 3. Means of the log-ratios of numbers dead non-infected aphids (A) *A. gossypii*, (B) *B. brassicae* and (C) *L. pseudobrassicae* consumed relative to number of infected non-sporulating aphids consumed by non-starved (—■— 0 h), 24 hour starved (—◆— 24 h) and 48 hour starved (—▲— 48 h) *C. lunata* over a period of 180 min.

Figure 4. Transmission of *M. anisopliae* ICIPE 62 from cadavers (1, 5 and 10 per plant) by foraging adult female *C. lunata* to fourth instar of (A) *A. gossypii* on okra, (B) *B. brassicae* and (C) *L. pseudobrassicae* on kale plants. Bar represents S.E. at 95% CI.