**Short Communication**

# Auto Inoculation of the African Invader Fruit Fly; *Bactrocera invadens* Drew, Tsuruta and White (Diptera: Tephritidae) with *Metarhizium anisopliae*, Isolate IC-20

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## Abstract

The potential of auto-inoculation of African invader fly (*Bactrocera invadens*) with *Metarhizium anisopliae* isolate IC-20 was assessed in a field cage experiment after the compatibility of the fungus with methyleugenol, a male fruit fly attractant. Compatibility of the male fruit fly attractant methyleugenol with the *Metarhizium* isolate was tested using two methods; direct contact and exposure to volatiles. Mortality of fruit flies reached 100% in 7-12 days with $LT_{50}$ of 4 days and $LT_{90}$ of 8 daysrecorded in two rounds of experiments. Compatibility tests revealed that methyleugenol reduced spore germination down to 2.7% in 10 days. Dry conidia were evenly spread on velvet material covering the inside of a lynfield trap and left in a field cage in which 500 male fruit flies were released to allow auto inoculation with conidia of *M. anisopliae* to take place and the exposure time was set at 14 hrs. Mean spore uptake of $9.8x10^4$ per individual fly was recorded. Results indicated the potential use of auto-inoculation of fruit flies with *M. anisopliae* for population suppression in the field.

**Key words**: *Metarhizium anisopliae, Bactrocera invadens, auto inoculation, compatibility test*

## Introduction

The African invader fly, *Bactrocera invadens* Drew, Tsuruta & White (Diptera: Tephritidae) is an invasive fruit fly species of Asian origin and has been reported from over 15 countries in Africa (including Ethiopia) (Drew *et al.*, 2005). In Ethiopia, *B. invadens* has been reported from southern and western Ethiopia including Arbaminch, Asossa, Arjo, Bako, Gambella, Gibe, Ghimbi, and Welkitie as an important pest on guava and mango (Ferdu Azerefegne and Difabachew Belay, unpublished data).

It has been reported to attack a number of fruit hosts including lemon, orange, tomato, banana, guava, custard apple (*Annona reticulata*), avocado but mango appears to be its primary host (Ekesi *et al.*, 2006). A range of 30 to 80% direct damage to mango due to *B. invadens* has been reported but depends on the cultivar, locality and season (Ekesi *et al.*, 2006; Rwomushana, 2008; Vayssie'res *et al.*, 2009). Enormous indirect losses have been attributed

to quarantine restrictions and both direct and indirect losses entail wide reaching socio-economic implications for millions of rural and urban populations involved in the mango value chain across Africa (Ekesi *et al.*, 2011).

Control of fruit flies has largely relied on the use of broad spectrum chemical insecticides in bait sprays to control adults and soil treatment to kill larvae and pupae (Dimbi *et al.*, 2003). More recent management techniques most commonly used to suppress adult fruit flies and pupae are combinations of food attractants with killing agents such as GF-120 (Ekesi *et al.*, 2011; Mangan and Moreno 2004; Vayssieres *et al.*, 2009). Other options include male annihilation, sterile insect techniques and biological control (Mau *et al.*, 2007; Vargas *et al.*, 2008). Fruit fly suppression programs targeting pupae, larvae and adults using entomopathogenic fungi as a component are being considered as alternatives throughout the world (Ekesi *et al.*, 2002, 2003,2005, 2007; Lacey and Shapiro, 2007; Dimbi *et al.*, 2009). A contemporary strategy in crop protection involves the use of entomopathogenic fungi (Lacey *et al.*, 1994, Vega *et al.*, 1995; Migiro *et al.*, 2010). This strategy employs dissemination of the entomopathogen among target populations through the use of contamination devices baited with attractants that inoculate the pest with the pathogen and allow its escape to the non-infected individuals for possible horizontal transmission (Vega *et al.*, 1995).

The major challenge in using entomopathogenic fungi to control dipteran flies has been their application in the field (Migiro *et al.*, 2010). However, Dimbi *et al.*, (2003) developed and tested auto-inoculation devices for contamination of three fruit fly species namely; *Ceratitis capitata*, *C. cosyra* and *C. rosa* var. *fasciventris* with *Metarhizium anisoplea* isolate IC 20 with success indicating the possibility of other fruit fly species suppression using entomopathogenic fungi. The objective of this study was therefore to test the possibility of auto-inoculation of the African invader fly with the entomopathogenic fungi *M. anisopliae* isolate IC 20 using a contaminated lynfield trap.

# Materials and Methods

## Source of fruit flies

Adult fruit flies were obtained from a laboratory colonies maintained at the insectaries of the international Center of Insect Physiology and Ecology (ICIPE) Nairobi, Kenya. The colonies were reared on sugar and yeast hydrolysate based artificial diet. Five to ten days old flies were used for the experiments.

## Source and handling of the fungus

The isolate (*Metarhizium anisopliae* , IC 20) used for the experiments was obtained from ICIPE culture collections. The isolate was cultured on Sabouraud Dextrose Agar (SDA) medium in Petri dishes and incubated at $26^0$C in an incubator. Three weeks later, conidia were harvested by scrapping the surface of the media using sterile spatula. To determine the viability of the harvested conidia, a spore suspension of $3X10^6$ was prepared and 0.1ml was spread plated on SDA medium in three replicate plates (Petri dishes). After 14 hrs of incubation, at least100 spores were counted from each of the plates to determine percent germination using microscopy at 40x magnification. The mean percent germination was found to be 93%. The isolate was then mass produced on rice substrate using autoclavable polyethylene bags by inoculating 3 days old blastospores produced in liquid medium. The substrate was first sterilized by autoclaving for 1hr at 121 $^0$C before being inoculated. The inoculated substrate was incubated at 20-26 $^0$C temperature and 40-70% relative humidity for three weeks. Conidia were harvested using a sieve with a mesh size of 295 µm after allowing the substrate to dry for 5 days at room temperature. Viability was tested as described above immediately before use and was confirmed by germination test to be 92.9%.

## Test of compatibility the fungus with methyleugenol

The compatibility of methyleugenol (ME), a fruit fly attractant used in the experiment, with the spores of the fungal isolate was tested using two methods. In the first case direct contact of spores with ME was tested. Three velvet coated lynfield trap auto-inoculators were sprinkled with 5 ml of ME and allowed to dry for about 3 hrs in a laminar flow cabinet. One lynfield trap was sprinkled with 5ml sterile distilled water and served as control. Dry conidia (1g of spores) of *M. anisopliae* isolate IC-20 containing 1x $10^9$conidia/g was applied to the velvet material of the trap. The traps were exposed outdoors under tree shade. Viability of the spores was tested by scraping spores from the traps after 24, 48, 72hrs and 10 days using standard germination test as described above.

In the second case, compatibility of methyleugenol volatiles with spores of the fungal isolate was tested. Four velvet coated lynfield traps were coated with spores as described above, and labeled 1, 2, 3, and 4. Four Methyleugenol wicks were exposed outdoors to allow volatilization of the attractant for 24, 48, 72 and 96 hrs before being inserted and hang into the lynfield traps labeled 1, 2, 3, and 4 above respectively. For each of the cases lynfield traps containing spores and cotton wicks without ME served as controls. Viability of spores was tested using standard germination tests as described above after 24, 48, 72, and 96hrs, for each of the cases (treatments).

## Field cage assessment

A field cage assessment was conducted to determine auto-inoculation of populations of fruit flies by the fungal spores, quantify spore uptake and assess fly mortality. Five hundred male *B. invadens* were released into a 3m x 2m x 3m field cage in which velvet covered lynfield traps coated with the fungus and containing methyleugenol wick was hanging. After 14hr of exposure, 60 flies were randomly aspirated into 3 perplex cages with 20 flies per cage and taken to bioassay room to monitor mortality on daily basis for 12 days. To confirm mortality due to mycosis, dead insects were surface sterilized using 70% alcohol and rinsed with sterile distilled water before placed in Petri-dish containing dump sterilized filter paper. Mycosis was then confirmed using microscopy. Flies were fed with sugar and yeast hydrolysate-based artificial diet. The experiment was repeated after a week.

## Estimation of spore uptake

In a similar way as described above, 20 flies were randomly aspirated from the same cage and taken to the laboratory for quantification of conidial uptake from the lynfield traps. Each of the flies was transferred into 2ml cryogenic tubes containing 1ml of sterile 0.05% Triton - X- 100 solution and vortex shook for 3 minutes to dislodge conidia. The number of spores picked by individual flies was quantified by counting spores using an improved Neuber – heamocytometer.

## Statistical analysis

Percentage mortality of flies was adjusted for control mortality using Abbot's formula. Probit analysis method for correlation data was used to determine $LT_{50}$ and $LT_{90}$ values using SPSS statistical package version 17.

# Results and discussion

## Compatibility tests

Compatibility test using direct contact of the attractant (methyleugenol) and spores (table1) showed that the germination of spores after 24 hrs was 78.01% compared to the control which had a germination of 81.25 %. Forty-eight hours later, germination of the spores in direct contact with attractant decreased to 74.62 % while the control did not change much (81%). However, after 10 days the germination of the spores was only 2.7% in the treated traps while the control showed 73.02% germination indicating that the longer the spores are exposed to the attractant the higher the loss of viability. In contrast, when the spores were exposed to volatiles in the compatibility test using methyleugenol wicks, the germination steadily increased from 82% for 24 hrs outdoor exposure of the wicks to about 90% for 72 hrs

outdoor exposure (table 1), before dropping to 83% after 96 hrs of outdoor exposure. Some semiochemicals are known to negatively affect growth parameters of *M. anisopliae*. For example Nana *et al.* (2011) reported that attraction-aggregation-attachment pheromone (AAAP) significantly inhibited all the growth parameters (germination, radial growth, conidial yield) of *M. anisopliae*. The results of this experiment suggest that direct contact of methyleugenol with spores of *M. anisopliae* on auto-inoculation devices such as the lynfield trap for attraction and infection of fruit flies is not advisable and must be avoided since it kills the spores. It also indicated that use of methyleugenol wicks would be better choice for use with lynfield traps for attract and infect strategy. As a result, exposing the methyleugenol wicks outdoors for 72 hrs before insertion to the spore coated lynfield traps was chosen and used for the cage experiments.

Table 1. Percent germination of spores of *M. anisopliae* isolate IC-20 exposed to methyleugenol directly or to volatiles from wicks at different exposure times

| | % germination of *M. anisopliae* exposed to methyleugenol | | | |
|---|---|---|---|---|
| Exposure or *keeping time | Volatile wicks | Control | Direct contact | control |
| 24hrs | 92.9 | 82.21 | 78.1 | 81.25 |
| 48hrs | 87.5 | 83.26 | 74.62 | 81 |
| 72hrs | 93.13 | 90.32 | 67.3 | 80.4 |
| 96hrs | 87.95 | 83.03 | - | - |
| 10days | - | - | 2.7 | 73.02 |

* Outdoors keeping time of methyleugenol wicks before insertion into the lynfield traps

## Field cage assessment

Figure 1 below shows the cumulative mortality of *B. invadens* inoculated with IC 20 isolate of *M. anisopliae* . The mean cumulative mortality of sampled flies reached 100% in 12 days in the first round of experiment with an $LT_{50}$ value of 4 days and $LT_{90}$ of 8 days (Table 2). In the second round of the experiment, too, mean mortality reached 100% in 8 days (Figure 1) with $LT_{50}$ value of 4 days and $LT_{90}$ value of 8.52 days (Table 3) which is slightly slower than the first round of the experiments. Mortality did not exceed 27% and 15% in 12 days in control cages of the first and second round experiments respectively. In a similar cage experiment using an auto-dissemination device containing cheese cloth, Dimbi *et al* (2003) reported 87.5 % mortality with an $LT_{90}$ of 4.2 days for the fruit fly *Ceratitis capitat* and 90% mortality with $LT_{90}$ of 4.1 days for *C. rosa var. fasciventris*. Up to 100% mortality was also observed on leaf miner flies captured from fungus treated cages (Migiro *et al.*, 2010). These findings demonstrated that auto-inoculation and infection of adults of fruit flies with spores of different isolates of *M. anisopliae* is possible using auto-dissemination devices.

Several auto- dissemination devices have been developed for entomopathogens, whereby insects are used to vector inoculations among con-specifics in the environment after they have been attracted and acquired the pathogen (Vega *et al.*, 2007). Manipulated dissemination of *M. anisopliae* by insects using auto-inoculation devices has also been demonstrated for the Japanes beetle *Popillia japonica* (Klein and Lacey, 1999), tse tse flies *Glossia fuscipes fuscipes* (Manania *et al.*, 2006) and pea leaf miners *Liriomyza huinoresis* (Migiro *et al.*, 2010).

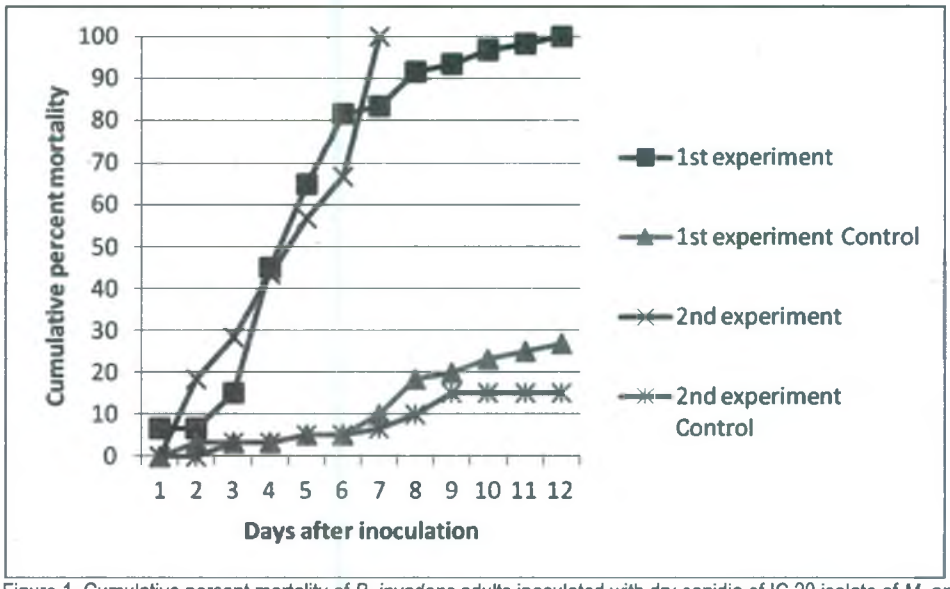


Figure 1. Cumulative percent mortality of *B. invadens* adults inoculated with dry conidia of IC 20 isolate of *M. anisopliae* in lynfied trapsafter 14 hrs of exposure in a field cage.

**Table 2. LT50 and LT90 days with 95% confidence limits in two rounds of experiment**

| | Trial | 95% confidence limit | | *$X^2$ | df | P | Intercept |
|---|---|---|---|---|---|---|---|
| | | Lower | Upper | | | | |
| **LT50 after 4 days** | 1 | 3.15 | 4.85 | 20.1 | 10 | 0.028 | -2.61 |
| | 2 | 3.43 | 4.65 | 7.76 | 5 | 0.17 | -2.35 |
| **LT 90 after 8 days** | 1 | 6.52 | 11.13 | 20.1 | 10 | 0.028 | -2.61 |
| | 2 | 6.81 | 12.78 | 7.76 | 5 | 0.17 | -2.35 |

*Pearson's Chi-square goodness of fit test on the probit model ($\alpha = 0.05$)

## Estimation of spore uptake

Spore uptake by individual flies from the lynfield trap contaminated with spores of *M. anisopoliae* isolate IC 20 reached up to $3x10^5$ conidia per individual fly. However, the mean spore up take calculated from 20 flies was found to be $9.8 \times 10^4$ spores per individual fly. In a similar study conducted on three other species of fruit flies (*Ceratitis capitata, C. rosa* and *C. cosyra* ), Dimbi *et al.* (2003) found that the number of conidia picked up by individual flies ranged from $4.1 \times 10^5$ to $1.0 \times 10^6$. These results suggest that *B. invadens* pick up more spores than the other fruit fly species. Migiro *et a.l* (2010) also showed that adult leaf miner flies *Liriomyza huinoresis* picked up $4.1x10^5$ and $39.6 \times 10^5$ conidia of *M. anisopliae* after one day and 5 days of exposure to the auto dissemination device in a cage, respectively.

The technique of Auto-inoculation of insects with entomopathogenic fungi is an important environment friendly component of integrated pest management. It is also a cost effective control strategy as only 80 – 100 g of conidia per ha are required on a weekly basis compared to 2- 3 kg of spores that is required for inundative treatment of 1 ha of a crop the cost of which is about US $210 ( Ekesi *et al.*, 2007).

From the experiments it can be concluded that auto-inoculation of *B. invadens* with *M. anisopliae* using dry spore impregnated lynfied traps has a promising potential to be used as attract and infect strategy for biological control of the pest in an integrated pest management system. It is also possible to conclude that methyleugenol has a strong inhibitory effect on conidial germination of *M. anisopliae* and must be used very carefully and preferably by pre-

exposing the methyleugenol wicks out doors for 72 hours before using them in the auto-dissemination device. Since methyleugenol is a male specific attractant, its use with auto-dissemination devices is limited to keeping the male population low so that mating either does not occur or is reduced to low level. It would be reasonably recommended to test fruit fly attractants which are not sex specific alongside IC-20 isolate of *M. anisopliae* used in this experiments for better control or suppression of fruit flies in the field. Finally, since the experiments were conducted only under laboratory and field cage conditions, open field experiments are recommended to confirm the consistency of the results under field condition.

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