Research Article

Spatial separation of semiochemical Lurem-TR and entomopathogenic fungi to enhance their compatibility and infectivity in an autoinoculation system for thrips management

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Abstract

BACKGROUND: The effect of spatial separation of the semiochemical Lurem-TR, which has been found to inhibit conidia of entomopathogenic fungi when put together, on the persistence of conidia of *Metarhizium brunneum* and *M. anisopliae* was evaluated in the greenhouse and field in order to develop an autodissemination strategy for the management of *Megalurothrips sjostedti* on cowpea crop. Influence of spatial separation of the semiochemical on thrips attraction and conidial acquisition by thrips from the autoinoculation device was also investigated in the field.

RESULTS: Persistence of conidia of *M. brunneum* and *M. anisopliae* increased with distance of separation of Lurem-TR. Direct exposure of fungus without separation from Lurem-TR recorded the lowest conidial germination as compared with the other treatments. Attraction of thrips to the device also varied significantly according to distance between device and semiochemical, with a higher number of thrips attracted when Lurem-TR was placed in a container below the device and at 10 cm distance. There was no significant difference in conidial acquisition between spatial separation treatments of conidia and Lurem-TR. Attraction of other insect pests to the device did not significantly vary between treatments. Positive correlations were found between conidial acquisition and thrips attraction.

CONCLUSION: This study suggests that spatial separation of fungal conidia from Lurem-TR in an autoinoculation device could provide a low-cost strategy for effective management of thrips in grain legume cropping systems.
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1 INTRODUCTION

Grain legumes are among the key economical crops widely grown in Eastern and Western Africa as important sources of food and animal fodder.[1,2] In Kenya, the annual bean production is estimated at 577 674 Mt.[3] However, the production of grain legumes is compromised by a complex of insect pests such as the legume pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Pyralidae), bean stem maggots, *Ophiomyia* spp. (Diptera: Agromyzidae), aphids (Hemiptera: Aphididae) and thrips (Thysanoptera: Thripidae).[4] Among the thrips, the bean flower thrips (BFT), *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae), is considered to be the most important pest attacking the reproductive structures of grain legumes.[5] Damage by BFT includes early flower blemishes, abscission and necrosis, with yield losses ranging from 20 to 100%.[6]

Thrips are difficult to control owing to their cryptic flower-dwelling behaviour and their minute size.[7] Chemical control is the most widely adopted management strategy by farmers, who often resort to using obsolete or banned chemical pesticides, with detrimental consequences to human, environmental and animal

health.[8] The introduction of stringent regulations by European importing countries such as the maximum residue limit (MRL) has led to several crop rejections and economic losses. In addition, thrips have developed resistance to most of the chemical insecticides, and hence the need to explore other control strategies, including biological control.[9–12]

Entomopathogenic fungi (EPF) are among the most promising alternatives to synthetic chemical pesticides that are being explored.[13–15] Fungal-based biopesticides for control of thrips

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are commercially available and include *Metarhizium anisopliae* (Metschnikoff) Sorokin ICIPE 69, marketed as Campaign® by RealIPM in Kenya. The most common application technique of EPF is through inundative spray.[16] However, EPF conidia applied on foliage have short persistence owing to environmental factors such as UV light, temperature and rain.[17−20] For instance, Ekesi *et al.*,[14] reported persistence of *M. anisopliae* conidia for 3–4 days on cowpea leaves. Such short persistence in the field requires frequent applications of EPF at short intervals, resulting in higher inoculum requirement and high costs. Another application technique referred to as autodissemination or autoinoculation, consisting of attracting insects to an autoinoculator where they are infected with a pathogen before returning to the environment to disseminate the pathogen to conspecifics, is also being considered.[21] This approach has already been tested against *Frankliniella occidentalis* Pergande on French bean[22] and is based on the combined use of visual cues (blue colour), semiochemical attractant Lurem-TR and the entomopathogenic fungus *M. anisopliae*. However, Lurem-TR was found to have a negative effect on conidial germination and infectivity of *M. anisopliae* in the field.[22] The introduction of Lurem-TR in a dessicator containing a culture of *M. anisopliae* resulted in complete inhibition of its germination after 48 h, confirming field results (Niassy S, personal observation). In order to improve the performance of autodissemination devices for thrips management, we explored the effect of distance separation of Lurem-TR from fungal conidia on the persistence of *M. brunneum* in the greenhouse and *M. anisopliae* under field conditions. We also evaluated the influence on thrips attraction and conidial acquisition in various distance separation treatments under field conditions.

2 MATERIALS AND METHODS

2.1 Study site

The study was conducted in the greenhouse at Plant Research International, Wageningen, The Netherlands (51.986, 5.663; 13 m above sea level) ($T = 20\,°C$, L16:D8 photoperiod), and in the field in Kamiti, Kiambu County, Kenya (1.191S, 36.883E; 1640 m above sea level) and at ICIPE, Nairobi (1.221S, 36.896E; 1616 m above sea level). In the greenhouse, the experiments were conducted to assess the effect of Lurem-TR on the persistence of *M. brunneum*, while experiments in the field were conducted to assess the effect of Lurem-TR on the persistence of *M. anisopliae* strain ICIPE 69, the attraction of thrips and other insects and conidial acquisition by thrips. Experiments were carried out during the dry season of May–August 2013. Average temperatures and relative humidity of 20.8 °C and 74.2%, respectively, were recorded in the experimental field.

2.2 Entomopathogenic fungi

Conidia of *M. brunneum* were obtained from the commercial product BIO1020 (strain Met52) (Bayer CropScience, The Netherlands). They were cultured on Sabouraud dextrose agar medium (SDA) at 25–27 °C, pH = 5.6 ± 0.2.[23] Conidia were harvested from the plate and suspended in 0.01% Triton X-100, and conidial concentration was determined using a haemocytometer (Fuchs-Rosenthal 0.2 mm). A spore suspension of approximately 10^9 conidia mL^{-1} was prepared and stored for 2 days at 5 °C until use in the experiment. *M. anisopliae* isolate ICIPE 69 is commercially available and marketed as Campaign® by RealIPM in Kenya. Conidia of *M. anisopliae* were mass produced on long-rice substrate in Milner bags

(60 cm long by 35 cm wide). Rice was autoclaved for 1 h at 121 °C and inoculated with a three-day-old culture of blastospores.[24] The rice containing fungal spores was then allowed to dry for 5 days at room temperature. Conidia were harvested by sifting the substrate through a 295 μm mesh sieve and stored for 2–5 days at 5 °C until use. Conidial viability was determined before any experiment by spread plating 0.1 mL of the suspension (3×10^6 conidia mL^{-1}) on SDA plates. Sterile microscope cover slips were placed on each plate. Plates were then incubated at 24–28 °C, 12:12 L:D photoperiod, and examined after 16–20 h. Percentage germination was determined by counting the number of germ tubes formed among 100 random conidia for each plate at 400× under a light microscope.[25] Conidial germination was approximately 90% and was considered to be acceptable.

2.3 Semiochemical

Lurem-TR, a commercial semiochemical whose active ingredient is methyl-isonicotinate, previously reported to be effective in monitoring thrips populations, was used in this study.[26] It was obtained from Pherobank (Wageningen, The Netherlands).

2.4 Effect of spatial separation of Lurem-TR on the persistence of conidia of *M. brunneum* in the greenhouse

Four 9 cm petri dishes without cover were placed at 0, 5, 10 and 20 cm, corresponding to treatments P0, P5, P10 and P20 respectively, on a rack with platforms connected to a stick in such a way that all platforms/petri dishes were vertically under each other (Fig. 1). Lurem-TR was placed above the top petri dish (P0). Petri dishes contained water agar (1.5% w/w), on which eight cover slips of 10 mm diameter (0.79 cm^2) previously atomised with a spore suspension of *M. brunneum* were placed. Atomisation was done by spraying 4 mL of conidial suspension (approximately equivalent to 600 L ha^{-1}) of *M. brunneum* on eight glass cover slips placed on petri dishes without water agar at a pressure of 7.5 bar using
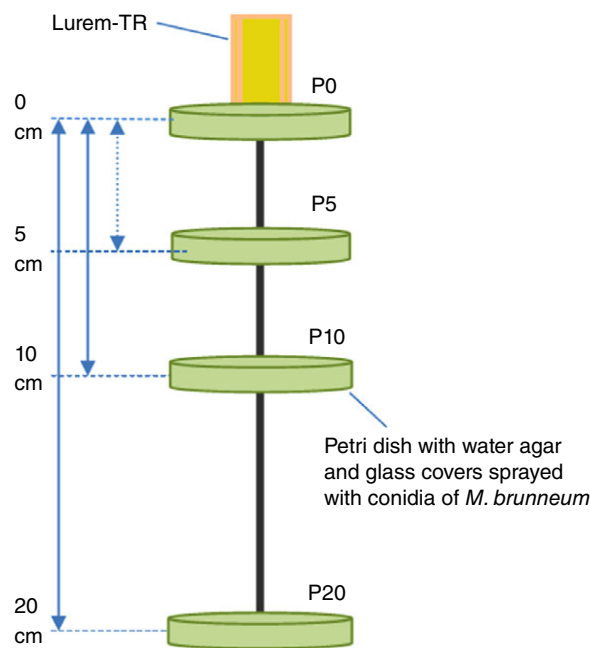

Figure 1. Experimental design for the evaluation of the effect of distance separation of Lurem-TR on *M. brunneum* conidial persistence in the greenhouse.

a Potter precision laboratory spray tower (Burkard Manufacturing Co Ltd, Rickmansworth, UK). Petri dishes were allowed to dry for 20–30 min, after which cover slips were transferred to the petri dishes containing water agar and then placed in the rack. The treated petri dishes were exposed to Lurem-TR for 24 h. As a control, a petri dish was atomised with conidial suspension as described above and allowed to dry, and conidial germination was determined immediately. All treatments were replicated 2 times and repeated 4 times.

To determine the maximum effect of Lurem-TR on inhibition of conidial germination, in addition to the four treatments described above, petri dishes were prepared as detailed above and placed in closed boxes (diameter 10 cm, height 10 cm) with or without Lurem-TR. After 24 h, the spore germination was determined. The persistence of conidia was determined after a period of 24 h for all the treatments, including the control. Conidial viability was determined according to an adapted method of Faria et al.[27] Each cover slip with conidia was removed from the petri dish, placed in a 10 mL Greiner tube containing 1 mL of 0.01% Triton X-100 water solution and vortexed for 20 s to dislodge conidia. From each Greiner tube, three samples (10 μL each) were pipetted separately on one glass slide covered with a thin layer of SDA and incubated in a closed container on humidified filter paper in the dark for 24 h at 25 °C. Percentage germination was determined by pipetting one droplet of lactophenol on each sample after 24 h, covering it with a cover slip and counting the number of germinating and non-germinating conidia (minimum count was 200 spores droplet^{-1}).

2.5 Field experiment with autoinoculation device

The effect of spatial separation of Lurem-TR on the persistence of *M. anisopliae*, attraction of BFT and other insects and conidial acquisition by thrips was evaluated in field experiments. Cowpea, *Vigna unguiculata* L. Walp var. *Ken-Kunde1*, was planted in 10 m^2 plots with an inter- and intrarow spacing of 10 and 45 cm respectively. No fertilisers, organic matter or synthetic chemical insecticides were applied during the experimental period.

The autoinoculation device used in the present study and the procedure for the inoculation device were as described by Niassy

et al.[22] Briefly, a Lynfield trap (11 cm diameter × 10 cm height) was perforated with six entry/exit holes (2 × 3 cm) near the top and bottom of the bottle at alternate positions. A velvet (8 × 8.5 cm) and a blue netting (3.5 × 11 cm) were wrapped around a smaller inner cylindrical bottle (5.2 cm diameter × 6 cm height) that was then hung in the trap. The semiochemical dispenser used to lure thrips was placed in different positions (see Fig. 2). Approximately 2–3 g of dry conidia was spread evenly on the velvet cloth of the autoinoculation device. Blue netting was then wrapped around the velvet cloth containing spores and tightened with two office pins. The device was then hung at canopy level (35 cm).

The following treatments were used in the field with the autoinoculation device: T_1 – direct exposure of fungal conidia to Lurem-TR; T_2 – conidia separated from Lurem-TR placed inside a small container fixed just below the device, hereafter also referred to as 0 cm; T_3 – conidia separated from Lurem-TR at 10 cm above the device; T_4 – conidia separated from Lurem-TR at 20 cm above the device; T_5 – control (device without Lurem-TR) (Fig. 2). Treatments were laid out in a complete randomised block design with three blocks as replicates. The blocks and treatments were separated by a distance of at least 15 m to avoid interferences between treatments and within blocks. Each of the five treatments was deployed in a single plot, so there were five plots, and these were repeated 3 times. For conidial viability, five treatments replicated 4 times were used, giving a total of 20 experimental units. In the experiments on thrips conidial acquisition and attraction, five treatments were repeated 3 times (15 experimental plots in total).

Experiments were conducted during the peak flowering stage of the crop, which corresponds to the period of peak infestation of the crop by thrips, necessitating control measures. The crop was planted on 14 June 2013, and experiments were run from July to August 2013. The flowering stage occurred from 24 July 2013 to 7 August 2013, while the podding stage started from 7 August 2013 up to the harvest.

2.5.1 Persistence of conidia of M. anisopliae

The persistence of conidia of *M. anisopliae* was evaluated for a period of 2 weeks after the onset of the experiment in the field. At 3 day intervals, samples of conidia were collected from
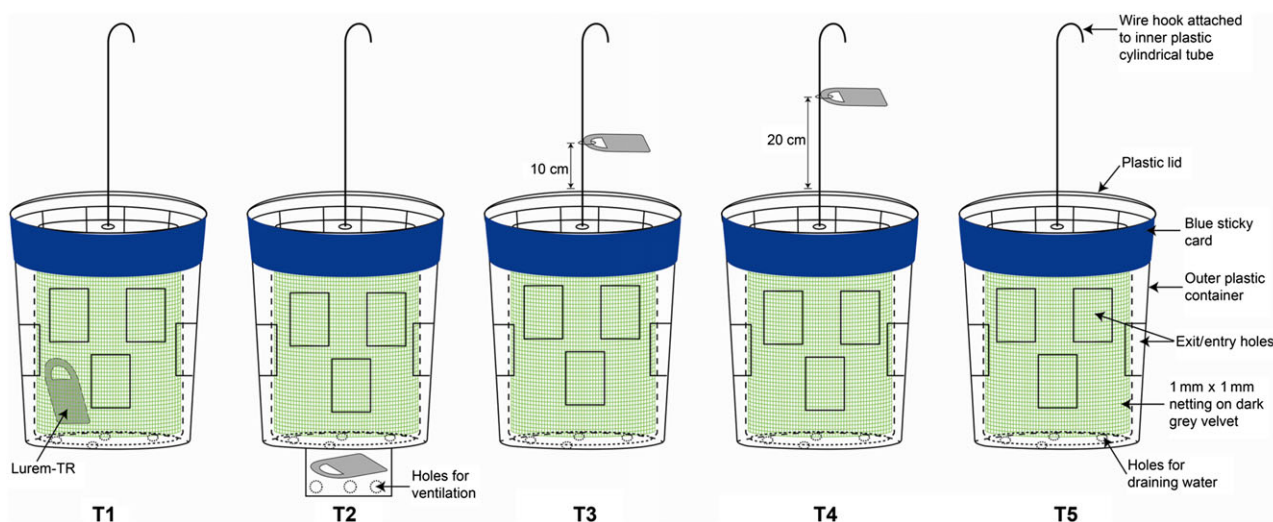

Figure 2. Description of the spatial separation of Lurem-TR for evaluation of *M. anisopliae* conidial persistence in an autoinoculation device in the field. Treatments: T_1 – direct exposure of conidia to Lurem-TR; T_2 – conidia separated from Lurem-TR placed inside a small container fixed just below the device; T_3 – conidia separated from Lurem-TR at 10 cm above the device; T_4 – conidia separated from Lurem-TR at 20 cm above the device; T_5 – control, device without Lurem-TR.

the autoinoculation devices from the five treatments with a moist cotton bud. The end of the cotton bud was cut, suspended in 10 mL of 0.05% (w/v) Triton X-100 and vortexed for 1 min to dislodge conidia. A sample of 100 μL was spread plated on SDA plates and incubated for 16 h at 25 ± 2 °C and L12:D12 photoperiod. Germination of conidia was determined as described above.

2.5.2 Attraction of M. sjostedti and other pests

A blue sticky patch (5 cm × 10 cm) was fixed to the side of the autoinoculation device with or without Lurem-TR bait to determine the number of insects, including *M. sjostedti*, visiting the device. The sticky cards were replaced every 3 days. Kerosene was used to dissolve the glue on the sticky cards, and insects were removed with a fine brush. Thrips specimens were then cleared, mounted on slides and identified as described in the literature.[28,29] The number of thrips and other insect pests such as leafminers and bean stem maggots were recorded.

2.5.3 Conidial acquisition by M. sjostedti

To assess the amount of conidia acquired by a single thrips visiting the autoinoculation device, 5–10 cowpea plants from a distance of 2 m around the autoinoculation device were randomly sampled using the whole-plant tapping technique.[30] The latter consists of tapping plants on a white barber tray (25 × 45 cm), where the tray is held underneath the selected plant, while the plant is tapped gently (five taps) using the palm of the hand. In each treatment, five bean plants were sampled around the autoinoculation device (1–2 m radius), and 20 insects were collected in a separate 10 mL glass tube using an aspirator. Tubes were labelled and stored in the fridge to immobilise insects, which were thereafter transferred individually into 2 mL cryogenic tubes containing 1 mL of sterile 0.05% Triton X-100. The tube was vortexed for 2–3 min to dislodge conidia from the insect, and the concentration of conidia was determined using a Neubauer haemocytometer.

2.6 Statistical analysis

In the greenhouse experiment, differences in the germination rate of conidia of *M. brunneum* between treatments were assessed by linear logistic regression analysis of the observed counts of germinated spores over the total number of spores examined for the replicate. The data Y were treated as pseudobinomial data, taking the variance to be proportional to binomial variance, i.e. $var(Y) = \sigma^2 np(1 - p)$, where p ($0 < p < 1$) denotes the expected germination rate Y/n of germinated spores Y, n stands for the number of spores examined from a replicate and σ^2 denotes the dispersion parameter. A linear logistic model with main effects of batch and treatment has been used to describe the relationship between the expected germination rate p and effects of batch and treatment. The model reads as follows:

$$ln\left(\frac{p}{1-p}\right) = constant + batch + treatment$$

Estimates for the dispersion parameter σ^2, main effects and F-tests for the main effects were obtained from fitting the model using the generalised linear model procedure in GenStat.[31] The dispersion parameter σ^2 was estimated from Pearson's chi-square statistic. Apart from F-tests for main effects, differences between batches and treatments were assessed by t-tests on all pairwise differences of fitted means on the logistic scale. Data shown

are back-transformed data from the analysis and present the predicted germination rates.

For field experiments, repeated-measures ANOVA was used to analyse *M. anisopliae* conidial viability, conidial acquisition, BFT and other insect counts. BFT and other insect counts were log transformed prior to repeated-measures ANOVA to normalise the data and stabilise variance between treatments. Means were separated using Tukey's HSD test at $\alpha = 0.05$. A linear regression model was used to study the relationship between distance of Lurem-TR and device separation and *M. sjostedti* attraction. Pearson correlation was used to analyse the association between distance of separation and conidial counts. The repeated-measures ANOVA was analysed using R 3.0.1.[32]

3 RESULTS

3.1 Effect of spatial separation of Lurem-TR position on conidial persistence

In the greenhouse, the distance from which Lurem-TR was placed away from conidia had a significant effect on the viability of conidia of *M. brunneum* both over treatments ($F_{6,52} = 19.4$, $P < 0.001$) and over times of observation ($F_{3,52} = 41.6$, $P < 0.001$). The lowest conidial germination (0.6%) was observed when conidia were in the presence of Lurem-TR inside the closed box (Lmax), followed by Lurem-TR in immediate proximity (0 cm) of the conidia (13.8%) in the open air in the greenhouse. However, there was no significant difference in conidial viability when Lurem-TR was placed at a distance of 5, 10 or 20 cm in the open air in the greenhouse, conidial germination being 29.0, 37.4 and 32.8% respectively. The control treatment in the open air (33.1% germination) was also only significantly different from the 0 cm distance treatment and not from the other distance treatments. In the control treatment (Lmin), where conidia in a closed box were not exposed to Lurem-TR, conidial viability was the highest (49.8% conidial germination) and significantly different from all other treatments (Fig. 3).

In the field, the separation distance of Lurem-TR and *M. anisopliae* had a significant effect on overall viability of conidia ($F_{4,12} = 24.0$, $P < 0.0001$) (Table 1A). The lowest conidial germination (39%) was obtained when conidia were in direct contact with Lurem-TR, placed within the autoinoculation device. However, there was no significant difference in conidial viability when Lurem-TR was not in direct contact with *M. anisopliae*, e.g. 0, 10 and

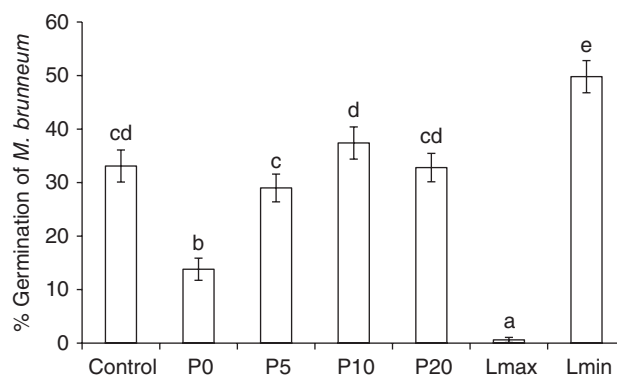**Figure 3.** Effect of spatial separation of Lurem-TR from *M. brunneum* (Met52) on conidial germination. Treatments: L0, L5, L10 and L20 are respectively petri dishes with conidia directly exposed, 5 cm above, 10 cm above and 20 cm above Lurem-TR. Lmin and Lmax represent the minimum and the maximum effect of Lurem-TR on inhibition of spore germination when placed in closed boxes with or without Lurem-TR.

Table 1. Repeated-measures ANOVA table for the response variable: *M. anisopliae* conidial viability (A) and acquisition (B) in autoinoculation devices as affected by spatial separation of Lurem-TR position and *M. anisopliae*

(A) Conidial viability

Source of variation	df	Sum of squares	Mean square	*F*-value	*P*-value
Between plot					
Block	3	306	102	6.90	0.006
Treatment	4	1417	354	23.95	<0.0001
Residuals	12	178	15		
Within plot					
Time	3	5715	1905	39.99	<0.0001
Time × treatment	12	186	16	0.33	0.981
Residuals	45	2144	48		

(B) Conidial acquisition

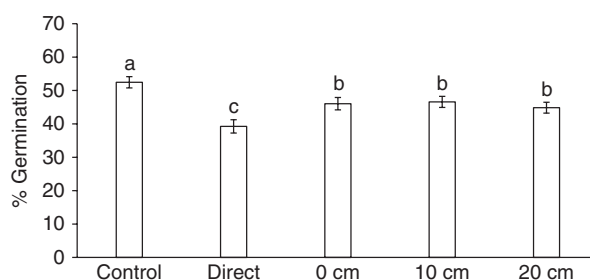| Source of variation	df	Sum of squares	Mean square	*F*-value	*P*-value
Between plot					
Block	2	10.19	5.09	11.02	0.005
Treatment	4	4.20	1.05	2.27	0.150
Residuals	8	3.70	0.46		
Within plot					
Time	4	6.49	1.62	15.73	<0.0001
Time × treatment	16	1.06	0.07	0.64	0.828
Residuals	40	4.13	0.10		

Figure 4. Effect of spatial separation of Lurem-TR on conidial viability of *M. anisopliae* in autoinoculation devices. Bars denote means ± one standard error at $P = 0.05$ (Tukey's HSD). Means (± SE) of three replicates of five autoinoculation devices. Treatments: T_1 – direct exposure of conidia to Lurem-TR; T_2 – conidia separated from Lurem-TR placed inside a small container fixed below the device; T_3 – conidia separated from Lurem-TR at 10 cm above the device; T_4 – conidia separated from Lurem-TR at 20 cm above the device; T_5 – control, device without Lurem-TR.

20 cm away from the autoinoculation device, conidial germination being 46, 47 and 45% respectively. In the control treatment, conidial viability was 52% and significantly different from the other treatments (Fig. 4). Conidial viability decreased significantly over time ($F_{3,45} = 40.0$, $P < 0.0001$) (Table 1A). The treatment with direct exposure had the lowest conidial viability at all observation times, and after 15 days the viability was only 25%, whereas the control recorded the highest viability at all observation times, with 41% viability after 15 days (Table 2). The other treatments were intermediate to direct exposure and control. The differences observed between treatments were consistent over time; therefore, no significant interactions were observed between treatment and exposure time ($F_{12,45} = 0.33$, $P = 0.98$) (Table 1A).

3.2 Effect of spatial separation of Lurem-TR on attraction of *M. sjostedti*
The position of Lurem-TR had a significant effect on thrips attraction ($F_{4,8} = 15.1$, $P < 0.001$ (Table 3A). Thrips were more attracted

to the device when Lurem-TR was placed at 0 and 10 cm distance (Fig. 5). The control treatment recorded the lowest number of thrips (80.2 ± 11.3) and was significantly different from the direct exposure treatment (99.2 ± 16.5) and 20 cm treatment (97.8 ± 11) (Table 4). The mean number of BFT attracted to the device increased over time: 100.0 ± 16.5 at day 3 and 167.8 ± 25.1 at day 15 ($F_{4,40} = 6.3$, $P < 0.0001$), and this did not vary significantly between treatments ($F_{16,40} = 0.73$, $P = 0.75$) (Table 3A).

3.3 Effect of spatial separation of Lurem-TR on conidial acquisition by *M. sjostedti*
The interaction between treatment and time was not significant ($F_{16,40} = 0.64$, $P = 0.83$) (Table 1B).

Overall, there was no significant difference in conidial acquisition by *M. sjostedti* between the different treatments ($F_{4,8} = 2.27$, $P = 0.15$) (Table 1B). However, conidial acquisition increased significantly with time ($F_{4,40} = 15.7$, $P < 0.0001$) (Table 1B), ranging from 0.14×10^5 on day 3 to 0.96×10^5 on day 15 post-treatment (Table 5). The increase rate over time was estimated as 0.069 ± 0.008.

3.4 Effect of spatial separation of Lurem-TR on the attraction of other insects
In addition to the attraction of *M. sjostedti*, other insects such as leafminers, whiteflies and bean stem maggots were also attracted to the device baited with Lurem-TR (Table 6). The attraction did not vary significantly among the treatments ($F_{4,8} = 0.9$, $P = 0.5$) (Table 3B). The mean number of other insects attracted to the device increased over time ($F_{4,40} = 17.3$, $P < 0.0001$) (Table 3B), with day 15 recording the highest number of other insects attracted to the device (62.8 ± 5.8) (Table 6). The interaction between treatments and time was not significant ($F_{16,40} = 0.54$, $P = 0.9$) (Table 3B).

A Pearson correlation test indicated a significant positive correlation between conidial acquisition and *M. sjostedti* attraction

Table 2. Effect of spatial separation of Lurem-TR on the persistence of conidia of *M. anisopliae* in autoinoculation devices over time

Distance of separation	Days after treatment				Mean[a]
	3	6	9	15	
Control	60.6 ± 2.5	58.2 ± 2.8	50.2 ± 1.7	40.8 ± 4.2	52.5 ± 2.5 a
Direct	51.2 ± 3.3	46.1 ± 3.5	35.2 ± 2.9	24.5 ± 4.0	39.3 ± 3.3 c
0 cm	61.4 ± 3.0	57.5 ± 3.3	50.9 ± 2.3	33.8 ± 4.1	50.9 ± 2.2 b
10 cm	61.0 ± 2.0	58.5 ± 3.3	50.0 ± 3.2	39.1 ± 3.6	52.2 ± 2.5 b
20 cm	53.4 ± 2.2	48.5 ± 3.0	52.4 ± 2.8	35.9 ± 3.6	47.6 ± 2.5 b
Mean[b]	57.5 ± 1.3 b	53.8 ± 1.2 b	47.7 ± 1.8 b	34.8 ± 2.2 c	

[a] Means ($\pm$ SE) followed by the same letters within the column are not significantly different according to Tukey's HSD test.
[b] Means ($\pm$ SE) followed by the same letters within the row are not significantly different according to Tukey's HSD test.

Table 3. Repeated-measures ANOVA table for the response variable: *M. sjostedti* attraction (A) and other insect attraction (B) (log-transformed counts) in autoinoculation devices as affected by spatial separation of Lurem-TR position and *M. anisopliae*

| (A) log-transformed thrips counts					
Source of variation	df	Sum of squares	Mean square	*F*-value	*P*-value
Between plot					
Block	2	3.31	1.66	70.94	<0.0001
Treatment	4	1.41	0.35	15.11	0.001
Residuals	8	0.19	0.02		
Within plot					
Time	4	0.57	0.14	6.32	0.000
Time × treatment	16	0.26	0.02	0.73	0.746
Residuals	40	0.90	0.02		
(B) log-transformed other insect counts					
Source of variation	df	Sum of squares	Mean square	*F*-value	*P*-value
Between plot					
Block	2	0.35	0.18	3.13	0.099
Treatment	4	0.20	0.05	0.90	0.507
Residuals	8	0.45	0.06		
Within plot					
Time	4	0.96	0.24	17.25	<0.0001
Time × treatment	16	0.12	0.01	0.54	0.909
Residuals	40	0.56	0.01		
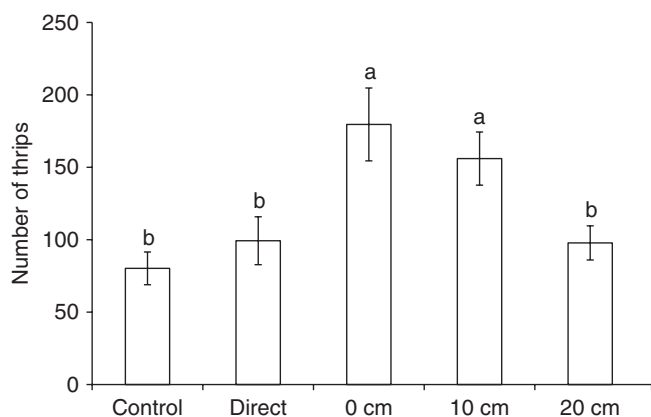
Figure 5. Effect of spatial separation of Lurem-TR and *M. anisopliae* on overall attraction of *M. sjostedti*. Bars denote means $\pm$ one standard error at $P = 0.05$ (Tukey's HSD). Means ($\pm$ SE) of three replicates of five autoinoculation devices. Treatments: T_1 – direct exposure of conidia to Lurem-TR; T_2 – conidia separated from Lurem-TR placed inside a small container fixed below the device; T_3 – conidia separated from Lurem-TR at 10 cm above the device; T_4 – conidia separated from Lurem-TR at 20 cm above the device; T_5 – control, device without Lurem-TR.

($r = 0.77$, $P = 0.0001$). There was also a significant correlation between *M. sjostedti* attraction and other insect attraction ($r = 0.9$, $P = 0.0001$). However, a negative correlation was found between *M. anisopliae* conidial persistence and *M. sjostedti* attraction ($r = -0.7$, $P = 0.0001$), and also between persistence and *M. anisopliae* conidial acquisition ($r = -0.8$, $P < 0.0001$).

4 DISCUSSION

The concept of autoinoculation has been tested against various insect pests and disease vectors.[21,22,33] One of the advantages of the autoinoculation device includes the long persistence of the inoculum, which is protected against environmental factors. For instance, Maniania[33] reported viability of over 60% of conidia of *M. anisopliae* in a contamination device at 31 days post-exposure in field conditions. However, in the present study, only 41% of conidia of *M. anisopliae* remained viable at 15 days post-treatment. This could be explained by the difference in the autoinoculation devices and fungal isolates used in the two studies. Entomopathogenic fungus applied in autoinoculation devices has the potential to suppress insect populations, as reported earlier.[33,34] For instance, Dimbi *et al.*[34] reported mortality

Table 4. Effect of spatial separation of Lurem-TR and *M. anisopliae* on *M. sjostedti* attraction in autoinoculation devices over time

| Distance of separation | Days after treatment | | | | | Mean[a] |
	3	6	9	12	15	
Control	64.7 ± 25.44	97.0 ± 37.4	71.3 ± 24.8	79.7 ± 36.5	88.3 ± 12.9	80.2 ± 11.3 e
Direct	75.3 ± 43.8	104.0 ± 46.8	89.7 ± 66.5	92.3 ± 49.5	134.7 ± 54.3	99.2 ± 16.5 c
0 cm	114.3 ± 34.8	172.3 ± 53.9	168.0 ± 37.1	141.3 ± 34.1	302.0 ± 26.5	179.6 ± 25.2 a
10 cm	141.7 ± 44.9	163.7 ± 56.9	136.7 ± 22.9	118.3 ± 10.6	219.7 ± 30.5	156.0 ± 18.3 b
20 cm	101.7 ± 42.2	123.0 ± 36.7	79.7 ± 24.8	90.3 ± 36.5	94.3 ± 23.9	97.8 ± 11.8 d
Mean[b]	100.0 ± 16.5 e	132.0 ± 19.6 b	109.0 ± 17.9 c	104.0 ± 14.1 d	167.8 ± 25.1 a	

[a] Means ($\pm$ SE) followed by the same letters within the column are not significantly different according to Tukey's HSD test.
[b] Means ($\pm$ SE) followed by the same letters within the row are not significantly different according to Tukey's HSD test.

Table 5. Effect of spatial separation of Lurem-TR position and *M. anisopliae* on conidial acquisition in autoinoculation devices over time

| Distance of separation | Days after treatment | | | | | Mean[a] $\times 10^5$ |
	3	6	9	12	15	
Control	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.7 ± 0.2	0.8 ± 0.3	0.4 ± 0.1 a
Direct	0.1 ± 0.1	0.3 ± 0.2	0.7 ± 0.4	0.7 ± 0.6	0.9 ± 0.6	0.5 ± 0.2 a
0 cm	0.4 ± 0.3	0.6 ± 0.4	1.1 ± 0.6	1.1 ± 0.3	1.7 ± 0.6	1.0 ± 0.2 a
10 cm	0.0 ± 0.0	0.3 ± 0.3	0.3 ± 0.2	0.4 ± 0.5	0.5 ± 0.3	0.3 ± 0.1 a
20 cm	0.1 ± 0.1	0.2 ± 0.2	0.6 ± 0.3	0.9 ± 0.2	0.9 ± 0.5	0.6 ± 0.2 a
Mean[b] $\times 10^5$	0.2 ± 0.1 c	0.3 ± 0.1 bc	0.6 ± 0.2 bc	0.7 ± 0.2 ab	1.0 ± 0.2 a	

[a] Means ($\pm$ SE) followed by the same letters within the column are not significantly different according to Tukey's HSD test.
[b] Means ($\pm$ SE) followed by the same letters within the row are not significantly different according to Tukey's HSD test.

Table 6. Effect of spatial separation of Lurem-TR position and *M. anisopliae* on the attraction of other insects in autoinoculation devices over time

| Distance of separation | Day after treatment | | | | | Mean[a] |
	3	6	9	12	15	
Control	31.7 ± 10.1	41.7 ± 9.0	40.7 ± 9.5	48.7 ± 13.7	58.3 ± 21.3	44.2 ± 5.6 b
Direct	23.0 ± 5.0	34.3 ± 4.5	33.7 ± 8.3	45.0 ± 10.8	56.7 ± 1.2	38.5 ± 3.9 b
0 cm	30.7 ± 3.4	47.3 ± 10.3	48.3 ± 9.1	72.3 ± 6.4	66.3 ± 8.4	52.9 ± 5.0 a
10 cm	42.0 ± 14.2	34.0 ± 3.1	45.3 ± 7.0	65.3 ± 24.4	78.3 ± 19.3	52.9 ± 7.4 a
20 cm	28.7 ± 4.2	35.3 ± 6.8	35.7 ± 9.5	55.7 ± 3.8	54.3 ± 9.7	41.9 ± 3.9 b
Mean[b]	31.0 ± 3.6 e	38.5 ± 3.1 d	40.7 ± 3.4 c	57.4 ± 5.9 b	62.8 ± 5.8 a	

[a] Means ($\pm$ SE) followed by the same letters within the column are not significantly different according to Tukey's HSD test.
[b] Means ($\pm$ SE) followed by the same letters within the row are not significantly different according to Tukey's HSD test.

of between 70 and 93% of fruit flies *Ceratitis rosa* (Karsch) and *C. fasciventris* (Bezzi) (Diptera: Tephritidae) after being attracted to *M. anisopliae*-treated autoinoculators baited with brewer's yeast in a field cage experiment. In another study, 100% mortality was observed among leafminer fly *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) visiting an *M. anisopliae*-treated autoinoculation device.[35] No antifungal effect was observed in either study, although no semiochemical was involved in the second study. The addition of semiochemical in the present study was intended to increase the attraction of thrips and subsequently the infection by fungus. However, direct exposure of conidia of both *M. brunneum* and *M. anisopliae* to Lurem-TR resulted in reduced conidial viability as compared with control treatments, which confirms the antifungal effect of Lurem-TR as reported earlier.[22] Conidial viability increased when the inoculum was separated from Lurem-TR,

indicating that the negative effects of Lurem-TR on conidial viability can be minimised through distance of separation.

More thrips were attracted to the autoinoculation device when Lurem-TR was placed at 0 and 10 cm, which may be attributed to a better diffusion of the semiochemical on account of ventilation.[36–38] Nielsen[38] reported that several extrinsic factors such as airflow and type of dispenser affect the methyl isonicotinate release rate. The higher thrips catches at 0 cm separation could be attributed to the proximity of Lurem-TR to the blue colour as compared with the 10 and 20 cm separation lures.[39] This finding could also explain differences in *M. sjostedti* catches between direct exposure and separation treatments.

The present study has shown that *M. sjostedti* responds to methyl-isonicotinate, which is the active ingredient of Lurem-TR, and confirms a previous report by Muvea *et al.*[40] The positive

correlation observed with thrips attraction could be explained by frequent visits or longer stays of *M. sjostedti* in the device. Methyl-isonicotinate has been reported to stimulate walking and take-off behaviour in *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) female adults.[41] This may explain the finding of Niassy *et al.*,[22] who observed that conidial acquisition by *F. occidentalis* was greater in a Lurem-TR-baited device than in a device without Lurem-TR. Maniania[33] also observed that the time spent by single tsetse flies *Glossina* spp. in the contamination device largely depended on the insect behaviour and varied between 5 and 189 s, and the subsequent number of conidia collected varied between 1.6×10^5 and 40.5×10^5 conidia fly^{-1}.

The effect of conidial acquisition on thrips mortality was not investigated in the present study. However, Niassy *et al.*[22] found that the overall mean mortality of *F. occidentalis* and the mean number of conidia acquired per single thrips were significantly higher in field cages with a semiochemical-baited device at 7 days post-inoculation. Migiro *et al.*[35] reported a positive correlation between conidial acquisition and mortality of leafminer fly *L. huidobrensis*.

Male aggregation and sexual behaviour have been widely documented in thrips,[42] and such behaviours are semiochemically mediated.[43] Classically, male thrips aggregate in numbers to demonstrate courtships (fighting, mounting) to females before mating.[42,44] Such behavioural elements can permit male-to-male or male-to-female conidial transmission during leks. Similar sexual behaviours have also been reported in some fruit fly species,[45,46] resulting in horizontal transmission of *M. anisopliae*, which also affected egg laying and oviposition.[47]

The negative correlation between conidial persistence and *M. sjostedti* attraction and between *M. anisopliae* conidial persistence and conidial acquisition observed in the present study suggest that the proximity of the attractant with colour for attraction needs to be appropriately defined for the success of the lure-and-infect strategy.

In conclusion, the spatial separation of Lurem-TR with fungal conidia could reduce the negative effect of the semiochemical and subsequently enhance fungal persistence in an autoinoculation device. A distance of 0–10 cm away from the conidial source was found to be optimal for thrips attraction in field conditions. In addition to *M. sjostedti*, insect pests such as leafminers, bean stem maggots and whiteflies that are also considered to be important pests of cowpea in Kenya can be attracted to the autoinoculation device, which renders this strategy very saleable for the management of thrips and other insect pests of grain legumes.

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