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Evaluation of optimal light wavelength for the attraction of *spodoptera exigua* as a model insect for mass trapping and control of *Spodoptera frugiperda*

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Abstract

Visual cues have been explored in the past for the management of pests of agronomic importance, including *Spodoptera frugiperda* (J.E. Smith) and *Spodoptera exigua* (Hübner). They are used to monitor, attract and kill, or to suppress the nocturnal behaviour of the insects. The current commercial universal traps, using reflected sunlight, are not effective for nocturnal insects like *Spodoptera* and current light traps may use a suboptimal or unattractive wavelength since the optimal wavelength has not been tested. This research focuses on testing the attractiveness of *Spodoptera exigua* as a model insect of *Spodoptera frugiperda* towards specific light wavelengths. Freshly eclosed moths were tested in the wind tunnel using light emitting diodes (LED) water trap and seven light colours (365, 385, 400, 470, 530, 592 and 650 nm) at the same light intensity (brightness). The roof top light was dimmed to 4.5% of daylight conditions tested on Western flower thrips in a wind tunnel to mimic dusk and UV-A at 3.5% of total dusk light. The number of moths trapped was recorded 30 minutes after the experiment started and at the end of the experiment after 14 hours. The results revealed a significant difference among the light colours. At 30 minutes the UV-A wavelengths (365, 385 and 400 nm) were significantly different from (470, 530, 592 and 650 nm) at p=0.01 whereas at 14 hours they where significantly different at p=0.001. These results show that UV-A has the potential to be utilized in water traps for the control of *Spodoptera exigua* as a model insect of *Spodoptera frugiperda*. However, there is need for validating the results in the field on trapping *S. frugiperda* using UV-A light wavelengths and pheromones.

Keywords: Fall Armyworm (*Spodoptera frugiperda.)*, Beet Armyworm (*Spodoptera exigua*), visual cues, Light wavelengths

1. Introduction

Visual ecology can be defined as the description and analysis of the natural optical environment in terms of the visual system of an animal. It assumes that specialized visual systems are of adaptive advantage to the animal processing them [8]. In insects' colour vision is vital (van Tol and van Dooremalen, (unpublished); [16] when they search for food, mate, oviposition sites or homing [8,13,14].

Many moths especially in the genus *Spodoptera* are nocturnal, meaning they are most active in the night. They have superposition eyes [5,9] which are believed to be sensitive to light and are suitable for life in dim light [4, 9, 13].

Vision in nocturnal insects allows them to orientate during flight and sometimes to locate food. These insects are capable of holding a straight-line course by using celestial patterns of polarized light as compass cue, orienting to constellations of stars and shoreline of a beach at night to enable stability and control over flight and landing. This is achieved by using the pattern of optic flows which involves movement of visual features a cross the retina induced by the animal's own movement [13, 14].

Nocturnal moths use pheromones to detect mates while the visual input is used to navigate obstacles as they follow pheromone plume. Conversely diurnal moths tend to depend on vision for almost all task related to mate detection. Moths have also been reported to rely more on olfaction for selection of oviposition sites than vision [15]. These insects use colour to detect and recognize flowers [15].

According to [10] insects such as moths, beetles and stinkbugs are attracted to artificial light sources. Hawk moths (*Macroglossum stellatarum and Manduca sexta*) prefer blue (440 nm) and yellow (540 nm) lights respectively to lights of other wavelengths [15]. Studies by [6] revealed that *S. frugiperda* and *S. exigua* had peak spectral sensitivity in green and ultraviolet regions but the insects were less attracted to traps with green wavelengths. However, a later study by [1] showed that *S. exigua* has no long wavelengths opsin genes.

Two approaches of visual stimuli have been explored in pest management. The first involves incorporating visual cues in traps for population monitoring or direct control [8, 10, 12]. The second one incorporates the use of visual cues to disrupt pest detection process [8, 10]. Therefore, knowledge of visual properties of moths especially in the genus *Spodoptera* would help to improve the attractiveness of moth traps.

According to van Tol *et al*. (unpublished) and [2], about 10 to 15% of many insect species are caught on traps despite olfactory attraction which means that current traps may not be efficient enough for mass-trapping. Therefore, exploring specific light wavelengths could improve trap efficiency and efficacy. For these reasons, this study aims at identifying the optimal light wavelength for the attraction of *Spodoptera exigua* as a model insect for *Spodoptera frugiperda.* The research opted to use *Spodoptera exigua* instead of

Spodoptera frugiperda due to the fact that *Spodoptera frugiperda* is a quarantine pest in Europe and it could not be used in the experiment.

2. Materials and Methods

2.1 Rearing of *Spodoptera exigua*

The visual cue of *Spodoptera* was tested using freshly eclosed *Spodoptera exigua* moths. Ten days old L4/L5 larvae were put in 2 plastic nasi boxes (12.5 x 17 x 6.2) cm in a climate chamber at 28 °C for three days. After pupation, one day old pupae were kept at 4° C in the Entomology Laboratory where 20 pupae were taken daily and put in plastic nasi boxes on a thin film of vermiculite (2-3) mm from Sigma Aldrich (Cas No: 1318-00-9). Water was then put in a small tube covered with oasis to provide suitable humidity (Fig. 1.) The boxes were covered with paper towels under the lids. Small pores were made on the lids after which they were kept at 25° C. Therefore, 20 freshly eclosed adults were available for the experiment every day.

About 10 to 20 moths were collected from the nasi boxes by sedation using carbon dioxide at around 2.00 pm. They were then put in a cylindrical container (6 cm height and 7 cm diameter) covered with a net at the bottom and parafilm at the top (Fig. 2) which was left in the fridge until 5.00 pm.

2.2 Experiment in the wind tunnel

The experiments began around 5.00 pm in the wind tunnel where 3 hours-starved moths (Fig. 2) were released in the wind tunnel 90cm away from a water trap which was illuminated with LED light of various colours from the bottom as shown (Fig. 4). The water trap (Fig. 3d) is composed of a metal cone with aluminium for light reflection inside and has a height of 10.5cm and diameter of 18 cm. At the bottom a hole in the middle holds the LED bulb (Fig. 3c). A light diffusing glass plate of 18 cm diameter (Fig.3b) was put on the stand, followed by a large sideways black painted Petri dish, which is 5 cm high and a diameter of 19 cm (Fig. 3a). A small amount of Tween 20 obtained from Schuchardt, Germany was added in the Petri dish to break surface tension of the water, needed to drown the landed insects on the water surface. The total height of the trap was 15.5 cm, while the release box was put on a stand 8 cm high (Fig. 4).

A spectralon disc was used to measure the light reflection from the roof light in the wind tunnel. Spectralon reflects all light colours with 99.5% efficiency. Spectral radiance was measured with a spectrophotometer (Specbos 1211 UV (Jeti Techn. Instr. GmbH)) and the values were converted to photons since the photoreceptors in insects can count photons rather than energy [3]. The number of photons of the roof light was 9.59E+16 per sr*nm and the photons for UV-A was 3.33E+15 per sr*nm. The percentage of UV-A = (3.33E +15/9.59E+16) x 100 = 3.5%. While the total light of the roof for the moths relative to the daylight used thrips reflection (van Tol *et al*. (unpublished) was calculated to be (9.59E + 16/2.156+16) x 100 = 4.47% (Fig. 5) and (Fig. 6).

The wind speed was 2 cm/s, while humidity and temperature were 70% and 24°C respectively. The number of the moths in the trap were recorded 30 minutes after the start of the experiment and then at the end of the experiment (after 14 hours). Seven light colours (365, 385, 400, 470, 530, 592 and 650 nm) were tested, and one colour was tested per day. Comparison of the colours was done using the same brightness (3.50E+18 photons/sr*nm) for each tested colour. The experiment was replicated 3 times using Randomized complete block design (RCBD) with days as blocks.

Data was visualized and analysed using r software for statistical analysis R version 3.5.2 (20th December, 2018). Normality test was performed using Shapiro-test to check the distribution of the data for trap catch after 30 minutes and 14 hours. Thereafter, a Levene's test was used to check for homogeneity of the data, followed by a one-way ANOVA.

Fig 1: Pupae in nasi box

Fig 2: Collection and release container

The figures below are Components of the water trap and the set up in the wind tunnel.

Fig 3a: Petri dish, **b:** light diffusing white glass, **c:** stand and bulb, **d:** complete water trap

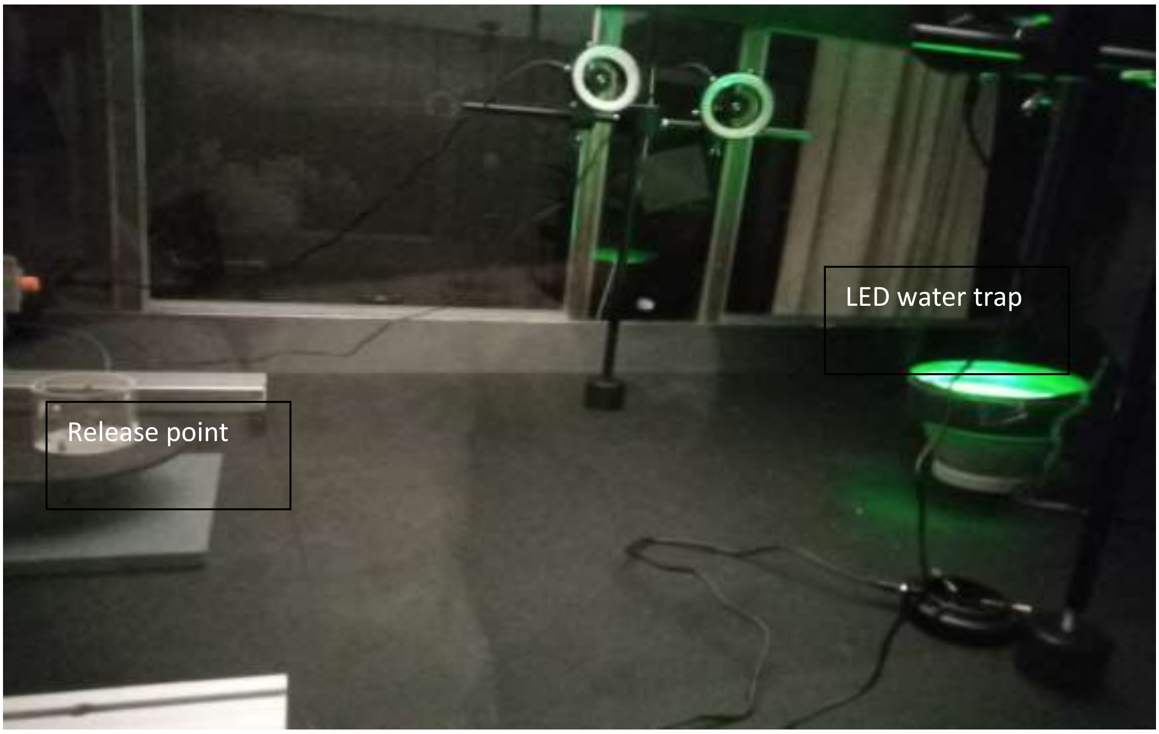

Fig 4: Wind tunnel set up

The figures below are graphs of reflection of the wind tunnel roof light (with spectralon reflection determined) when conducting experiments with thrips (day light condition) and *Spodoptera exigua* (moonlight condition) respectively.

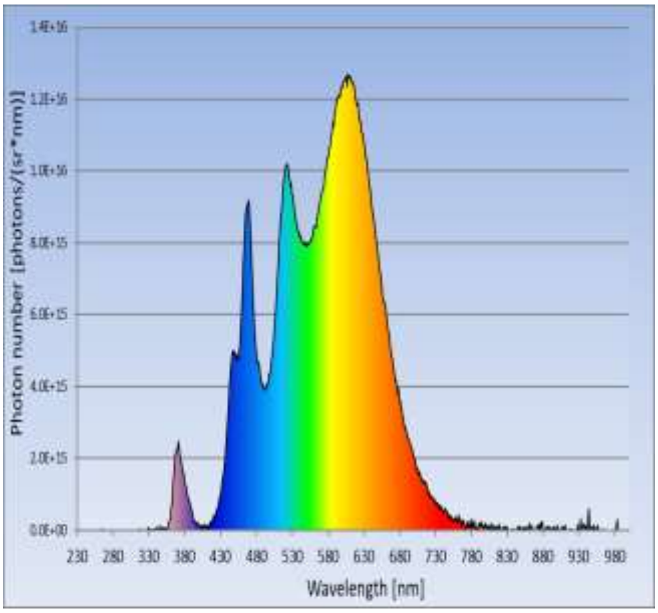

Fig 5: Spectralon rooflight day condition (M1) for thrips

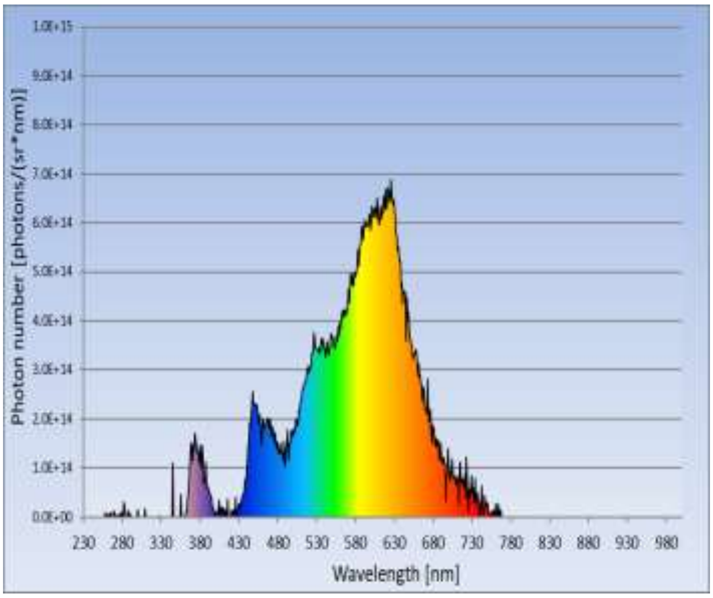

Fig 6: Spectralon moonlight condition (M3) for *Spodoptera exigua*

***Source*:** van Tol (unpublished)

3. Results & Discussion

Results (Fig.7 and Fig. 8) show percentage of attracted moths in the trap after 30 minutes and 14 hours respectively after statistical analysis (N=3).

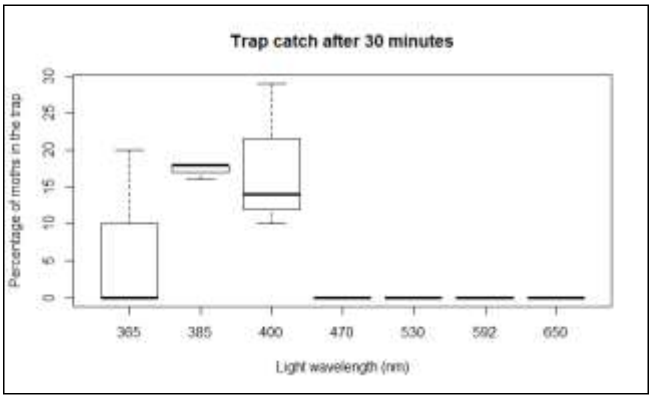

Fig 7: Box plot showing the catch of moths in various traps after 30 minutes

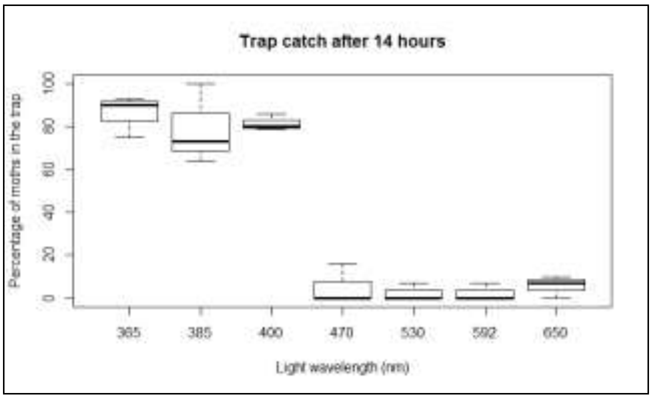

Fig 8: Box plot showing the catch of moths in various traps after 14 hours

3.1 Normality Test

The normality test for the trap catch data after 30 minutes and 14 hours were highly significant (***) p< 0.001. Therefore, null hypothesis was rejected, meaning that data did not come from a normally distributed population.

3.2 Homogeneity Test

Levene's test revealed p values of 0.4373 and 0.6834 for trap catch after 30 minutes and 14 hours respectively. These results show that all population variances were equal.

3.3 One-way ANOVA

Trap catch after 30 minutes

The results showed that the various light wavelengths had significant differences at P=0.00259**<0.01. Mean separations further revealed the significantly different trap light colours at 30 minutes as in (Fig. 9).

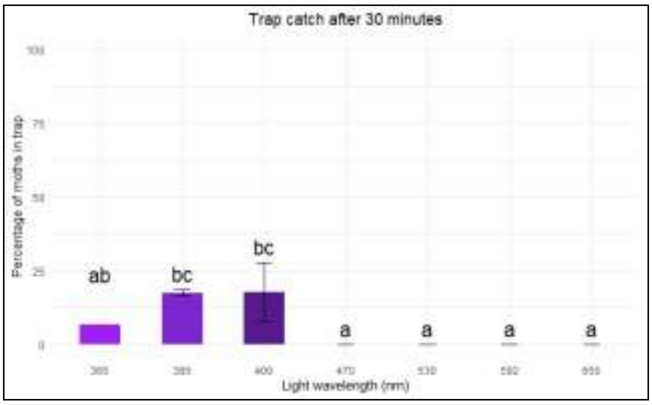

Fig 9: Bar plot of mean percentage of moths caught per light wavelength of a trap. (Means followed by the same letters are not significantly different from each other)

The percentage number of moths trapped after 30 minutes was higher but not significantly different at 365, 385 and 400 nanometer wavelength (Fig 9). The least number was trapped at 470, 530, 592 and 650 nanometer wavelength**.**

Trap catch after 14 hours

The one-way ANOVA test had a significant difference (***) at p<0.001. The mean separations using Tukey HSD method show the results in Fig. 10. The highest comparable percentage numbers (over 75%) of the moths were trapped at 365, 385 and 400 nanometer wavelength after 14 hours compared to the rest of the wavelengths tested (Fig 10).

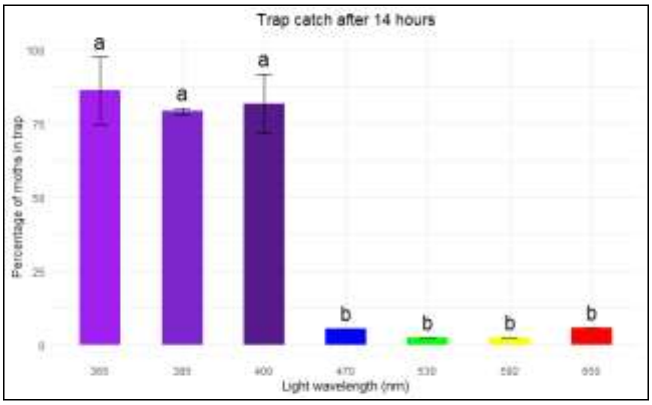

Fig 10: Bar Plot of mean percentage of moths caught per light wavelength of a trap. (Means followed by the same letters are not significantly different from each other)

The results in (Fig. 9) and (Fig. 10) show that UV-A wavelength colours (365 to 400 nm) attract more *Spodoptera exigua* adults. After 30 min these results were still low in contrast to after 14 h. The moths likely require a period of adaptation to take flight which explains the low number after 30 min. Nocturnal and twilight flying insects compensate for dim conditions by integrating light over longer times [11].

The moths were highly attracted to UV-A (365-400 nm) as opposed to other light colours (470,530,592 and 650 nm). These results are in line with reports by [6] that *S. exigua* was attracted to black light (350-360) nm but not to light with longer wavelengths. The results are also consistent with the report by van Tol and van Dooremalen, (unpublished) that insects can only see or respond to a limited number of light wavelengths.

Even though there are reports that noctuids are only motivated to search for visual flower cues in the presence of flower odours [15], this study shows that *S. exigua* could rely on visual cues alone for landing [4]. Postulated that insect's preference for certain distinct colours while searching for food could still be modified by learning. This has however so far only been shown for highly visual orientated insects like bees and bumblebees. The wind tunnel is a small space where insects are within visual range of the traps so further research is needed in the field using the UV-A and odours to see if the insects can still find and recognize the traps.

The study also observed that some moths could fly directly into the trap when provided with UV-A especially 365 nm after a short time. This was an indication that the insect's eye can easily detect light at short wavelengths. Furthermore, [4] also mentioned that superposition eyes are capable of functioning at very low light intensities. This is because their eyes are adapted in such a way that light leaving one lens system is not confined within a single ommatidium, but can reach the rhabdoms of neighboring unit.

It is evident from the results that the moths were not attracted by the lights of longer wavelengths (470, 530, 592, 650 nm). Nonetheless, the results contradict the report by [7] which showed that over 60% of the moths were attracted to green, blue and yellow high-power light emitting diodes (HPLEDs) at 40 lux and 60 minutes exposure time. The difference could be because the moths were tested without roof light in complete darkness. In this case the results could be biased as any light that can be seen by the moths as even not attractive ones but high enough brightness could evoke a signal that will lead to a response. There is a possibility that they used the visual cues for orientation to fly towards the light [13, 14] rather than landing. Moreover, the dorsal eye region of the moth is specialized for the detection of polarized light [14].

The study could not presume that long light wavelengths suppressed nocturnal behaviours of moths such as flying, sucking the juice or mating according to [10]. This is because these parameters were not tested. Nevertheless, flight activities could be observed even when long light wavelengths were provided but they did not land in the traps. For these reasons, more research is required in order to determine whether these lights suppress nocturnal behaviours of the moths or not.

It was also observed that some insects which missed to land directly into the water, landed somewhere on the trap hence they could escape. As a result of this, the study also proposes a lure and infect strategy, where the moths are attracted to UV-A trap and infected with entomopathogens. However, this mechanism has to be tested first to establish if the light can cause damage to the spores or reduce their germination and if the uptake of the spores is high enough to evoke infection and death of the moth before oviposition takes place.

4. Conclusion

According to this study UV-A (365-400) nm have the potential to be incorporated in trapping of *Spodoptera* Spp. Therefore, there is need to validate the results in the field where similar experiments should be conducted using UV-A LED water traps for *S. exigua* and *S. frugiperda*. Since moths rely more strongly on pheromones for mate detection and on odours for finding nectar sources [15] and vision has a much lower distance range of detection by most insects (more cm's than m), it is essential to lure them within the visual range with pheromones or kairomones to make the traps work. We propose a combination of UV-A LED water traps and pheromones in the field to improve the efficiency and efficacy of the traps.

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