Acta Phytopathologica et Entomologica Hungarica 59 (2024) 1, 108–120
DOI: 10.1556/038.2024.00197

AKADÉMIAI KIADÓ

To attract a moth: Wind tunnel and field testing of plant odor and light stimuli and their combination for *Ostrinia nubilalis*

Marianna I. Zhukovskaya[1], Inna V. Grushevaya[2], Alexander A. Miltsen[2], Oksana G. Selitskaya[2], Anna V. Shchenikova[2], Andrei N. Frolov[2] and Miklós Tóth[3]*

[1] Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg, Russia

[2] All-Russian Institute of Plant Protection, Russian Academy of Sciences, St. Petersburg – Pushkin, Russia

[3] Plant Protection Institute, HUN-REN Centre for Agricultural Research, Budapest, Hungary

RESEARCH ARTICLE

Received: September 24, 2023 • Revised manuscript received: February 5, 2024 • Accepted: February 22, 2024
Published online: April 12, 2024

ABSTRACT

The European corn borer, *Ostrinia nubilalis* (Hubner), relies on multimodal sensory information to find food, mates, mating and ovipositional grounds. Successful phytosanitary monitoring demands for the bait for the field traps to obtain the most reliable representation of pest abundance. Attraction to light and blend of key components of host plant odor, was tested both in the laboratory and field conditions. Ultraviolet light, which was the most effective in the wind tunnel experiments, was further tested in the field alone and in combination with bisexual lure. Bisexual lure, being attractive in the lab, as well as in the field, did not improve responses to ultraviolet in both experimental designs. All three baits attracted significantly more females than males in the field. Wind tunnel experiments revealed that ultraviolet elicited the shortest response latencies either alone or paired with the odor bait. The lack of synergistic effect between attractive light and odor stimuli is an important issue for pest monitoring. The possible reasons for the observed lack of synergy are the hierarchy of behavioral responses to different stimuli or the intensities of both stimuli are critically important for attractivity of combined stimulus and differ from separately presented ones.

* Corresponding author. Tel.: +36 1 3918637. E-mail: toth.miklos@atk.hu

KEYWORDS

Ostrinia nubilalis, attraction, bisexual lure, ultraviolet light, combined action

INTRODUCTION

Insects orient themselves in relation to vital conditions and objects by reacting to sensory stimuli such as light, odor, wind direction, humidity, temperature, etc. The European corn borer (ECB), *Ostrinia nubilalis* (Hubner), is a major pest of maize and many other crops and is characterized by high evolutionary plasticity (Lassance, 2010). The monitoring of this insect is traditionally carried out by traps baited with synthetic sex pheromones (Pélozuelo and Frérot, 2007). Traps, baited with lures attracting females or both sexes of ECB, were found to be more effective in predicting the crop damage by the next generation larvae, then traditionally used pheromone traps attracting only males (Maini and Burgio, 1999). Until recently there were many confirmations that attraction of both sexes by light traps provide more reliable monitoring information on pest population and especially beginning of moth egg laying (Palaniswamy et al., 1990; Keszthelyi and Lengyel, 2003; Bereś, 2012).

Adult moths disperse from their overwintering places to present year corn fields following two alternative reproductive tactics: "mating after settlement", when males and females fly to the cornfield of the present year to aggregate in "action sites" for mating (Sappington and Showers, 1983) or "mating before settlement", when females, mated in emergence sites fly to the ovipositional sites unlike males (Zhukovskaya and Frolov, 2022). It is obvious that in the case of the second reproductive tactics we face serious problems when using the sex pheromones for ECB monitoring.

Not so long ago ECB moths were shown to be actively attracted by a bisexual lure, the 1:1 blend of phenylacetaldehyde (PAA) and 2-(4-methoxyphenyl)ethanol (4METH), the host plant emitted odorants (Tóth et al., 2016). Extensive trials in five European countries demonstrated advantage of this attractant over a sex pheromone in ECB monitoring (Tóth et al., 2017).

Migrating animals, including insects use light as a navigational cue. It is well-known, that the directional light sources such as the moon and even Milky Way is used by insects to maintain a straight-line course (Dacke et al., 2013; Warrant and Dacke, 2016). Also, moonlight has varying spectral-intensity characteristics depending on the time and weather conditions, that affects insect navigation and trapping rates (Nowinszky et al., 1979; Yela and Holyoak, 1997). The combination of olfactory and visual stimuli, then, should allow to create the best attracting combination used in the field to monitor corn borer population during the growing season. Some attractant stimuli show strong synergism being applied together, but this is not always true: for example, intermediate walking pathways in response to some simultaneously presented attractive visual and olfactory cues, while the response to other visual stimuli was not altered by the attractive host odor in *Sitophilus zeamais* (Motschulsky), one of the major pests of stored kernels (Arnold et al., 2016).

The present study was aimed to test an assumption that the combination of light stimulus eliciting the best flight-to-light response and bisexual lure has a synergetic or, at least, additive effect on the attraction of ECB in both the laboratory and field conditions.

MATERIAL AND METHODS

Wind tunnel experiments

Insects. Diapausing larvae of ECB were collected from the corn fields in Botanika village of Krasnodar region of Russia (40°47′E, 45°12′N). Upon reactivation, the larvae were placed separately in plastic tubes (diameter 2.5 cm, length 6 cm) at a temperature of 25 ± 2 °C, relative humidity 70 ± 5% and photo mode 16:8 (L:D) in the climatic chamber (MLR-352, Sanyo (Panasonic.). Emerging adults were checked and their tubes were labelled daily. Three-to-five-day old virgin males and females were used for the wind tunnel experiments.

Wind tunnel. Wind tunnel testing was performed as described previously (Zhukovskaya et al., 2023a) under the following laboratory conditions: 0.7 lux of dim red light (650 nm), ambient temperature of 21–23 °C, and relative humidity of 75–80%. The mobile plexiglass tube with a diameter of 400 mm was inserted inside rectangular parallelepiped (150 × 70 × 70 cm) with mesh outlet wall. An airstream (0.2–0.3 m s^{-1}) was created by suction through the mesh. The light source and/or an odor dispenser were placed at the center of the inlet of the plexiglass tube. The platform to release insects was set 800 mm downwind from the odor dispenser.

Stimuli. Bisexual lure contained the mixture of PAA and 4METH, 100 mg of each component (Sigma-Aldrich Kft. Budapest, Hungary. All compounds were >95% pure as stated by the supplier, the preparation of lure was prepared as described earlier (Tóth et al., 2016). For wind tunnel experiments the fraction of a dispenser containing 33 mg of bisexual lure blend was used.

Light stimuli (532, 440 and 365 nm 3w 3535 LEDs), chosen to cover the spectral sensitivity range of ECB eyes (Belušič et al., 2017), were adjusted to the intensity of 2 lux as described earlier (Zhukovskaya et al., 2023a, 2023b). Background red lighting 650 nm producing the illuminance of 0.7 lux was used to observe the behavior of insects. Light intensities were selected according to our previous data obtained for *Ostrinia scapulalis* (Zhukovskaya et al., 2023a).

Description of the LEDs calibration technique. The Yu16 lux meter used by the authors for stimulus calibration also works in the UV range. The LEDs were adjusted as described below:

Equal illumination of the object by LEDs with different emission spectra was achieved as follows (using one LED as an example):

Determine the luminous flux of the LED (for example, 200 Lm).

According to the characteristics of products declared by the manufacturer of LEDs (see Fig. 1) we've determined the direct current through the LED, at which the light flux emitted by it will be equal to 200 Lm. In our case it is 140 mA.

Having switched on the LED (direct current 140 mA) and red light, we took lux meter readings at the object location. As a result, lux meter readings for a certain wavelength of light at a certain distance from the light source emitting a light flux of 200 Lm, plus a constant contribution from the red light were obtained.

We did all of the above for another LED at the same current of 140 mA. As a result, we have got the lux meter readings for certain wavelengths of light at a certain distance from the light source emitting a light flux of 200 Lm, plus a constant contribution from background red light,

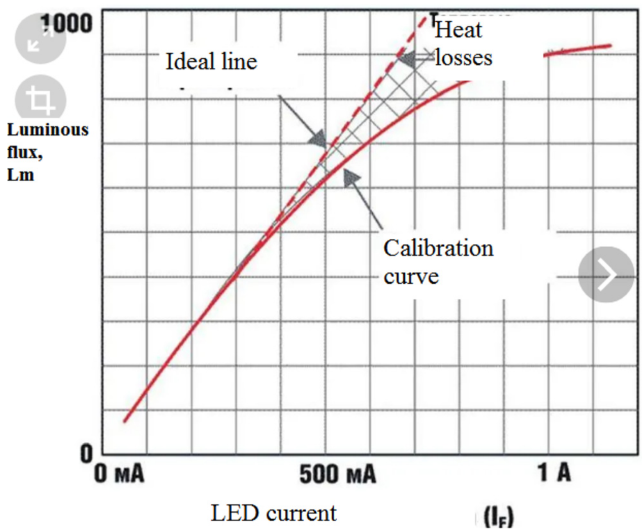

Fig. 1. Description of the LEDs calibration technique

and, since all LEDs are of the same type (type 3535, 3 W, angle of emission 120°) and equally oriented, illumination of the object was the same.

Since the dependence of the LED luminous flux on the current flowing through it (in the area of the values we used) and the sensitivity of photocell F102 is linear, then, having determined the proportionality factor for each LED, we can easily calculate the values of the necessary parameters of the experiment.

Thus, we controlled the light intensity in the experiments by determining the necessary values for each of the light sources.

Experimental protocol. Insects were taken out of climatic chamber at the end of light phase and transferred to the experimental room with temperature of 23–26 °C. Males and females were placed individually in the Petri dishes and were kept before the testing in light-tight compartments in the experimental room at least one hour before the test. Males and females were tested on different days or, in some days, males were tested prior to females to prevent the tunnel contamination with female emitted sex pheromone. The Petri dish with an insect was inserted into the platform and left for a 3 min adaptation period, after that the stimuli were applied and the lid of the dish was gently removed. The Plexiglas tube was glided toward the mesh to make a tight contact with it, creating the laminar airflow inside the tube. The observation period lasted 10 min, after which the insect was taken out of the tunnel. The following parameters of the reaction were recorded: taking flight, direction of the flight (upwind or downwind), source contact, and the time of taking flight (sec). Six series of experiments were performed:

1. Green light (27 females and 34 males);
2. Blue light (28 females and 36 males);
3. UV light (28 females and 24 males);
4. Bisexual lure (45 females and 54 males);

5. UV light+ bisexual lure (43 females and 36 males);
6. Control (55 females and 28 males)

Field trapping

Bucket funnel traps (Epsky et al., 2008) used in the study were modified by installing a light-emitting unit as it was described by the patent RU201632U1 "Light pheromone trap for flying insects". Description of the trap design, its setup and manual for use were published earlier (Frolov et al., 2020). Traps were placed at the level of 2/3 of plant height, as a rule, at the height of the cob attachment and were arranged as follows: 8–10 m in a row, 50 m between rows and were positioned in 30 m from the edge deep into the cornfield. Traps were checked out every day until the first moth was caught, and every 3–4 days later on. The batteries powering the LEDs were exchanged on every trap check. Attractants were exchanged every month.

Field trapping was performed during the summer seasons of 2021 and 2022 near the village Botanika, Krasnodar region, Russian Federation (40°47′E, 45°12′N). There were 4 traps with bisexual lure, 8 traps with UV LEDs, 7 traps with combined baits (UV LEDs+ bisexual lure) and 11 control traps (without both bisexual lure and UV LEDs) which were spatially randomized at the experimental cornfield.

Statistical analysis

The data were tested for normality by Kolmogorov-Smirnov test. Normally distributed data were evaluated by ANOVA. Chi-square test and Fisher exact test were performed using online calculator (http://vassarstats.net/). Benjamini–Hochberg correction for multiple comparisons was used to compare frequencies. The field-trapping data were processed according the method suggested by Roelofs and Cardé (1977), namely, for each trap, the numbers of caught moths were summed over the season. Then, the data were square-root transformed using $\sqrt{x + 0.5}$ equation to normalize distributions and were analyzed by ANOVA.

RESULTS

Wind tunnel experiments

First, the behavioral responses and attractivity of light of different wavelength were evaluated to find the most attractive one (Fig. 2). Males and females did not differ in their responses to any light stimuli as well as control, so, the rest of evaluations were performed regardless of the moth sex. Chi-squared analysis performed for the taking flight frequencies revealed significant differences between all the series (Chi = 37.5; P = 0.000003). The most attractive was found to be UV light, the rate of taking flight differed significantly not only from control group (Chi = 32.2; $P < 0.001$), but also series, in which the green (Chi = 4.7; $P = 0.03$) or blue (Chi = 14.8; $P < 0.001$) light stimuli were presented. Responses to the green light were higher than control (Chi = 11.6; $P < 0.001$); blue light responses did not differ from control, nevertheless, differences between the data obtained for green and blue light were not significantly different. The most of the moths that took flight moved toward the stimulus, *i. e.* upwind, and more than 60% of them reached the source of light; the differences between series were insignificant, including control,

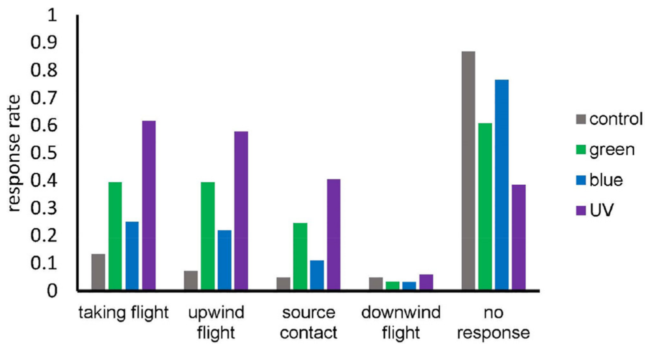

Fig. 2. Responses of *Ostrinia nubilalis* to the light stimuli of different wavelengths in the wind-tunnel

where the light was switched off (Fisher's exact test $P = 0.54$). Neither of stimuli were repellent for the moths, the downwind flight reaction was rare for any treatment.

The latencies of taking flight were the longest for the control group (Fig. 3). All flights to any light were taken faster, then in control (One-way ANOVA, $F_{3/83} = 13.68$; Tukey *post hoc* test, $P < 0.01$) but the shortest latency was found for the UV light (Fig. 3). Latencies for blue and green light stimuli did not differ significantly.

Next, the responses of ECB to bisexual lure and its combination with UV light were evaluated and compared to those to UV light (Fig. 4).

Chi-squared test to compare responding moths (taking flight versus no response) between four treatments (bisexual lure, UV light, combination of bisexual lure and UV light, and control) followed by *post hoc* Fisher exact test (Table 1, Shan and Gerstenberger, 2017). Chi squared test showed high level of significance between series (Table 1). Any experimental treatment caused significantly more flights, than control did (Fig. 4, Table 1). UV light alone or in combination with bisexual lure was more attractive than bisexual lure alone. We did not find any additive effect, not to mention synergy for complex signal for either stage of response (Fig. 4).

The latent period for the taking flight reaction was the longest for the control, the shortest for the UV light stimulus, bisexual lure and combined stimulus had intermediate latencies, the

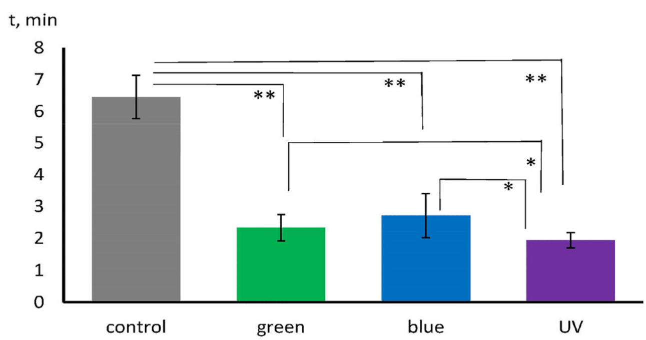

Fig. 3. Latencies of taking flight reaction of *Ostrinia nubilalis* in the wind-tunnel in response to light stimuli of different wavelengths. * – $P < 0.05$; ** – $P < 0.01$ (Tukey HSD *post hoc* test)

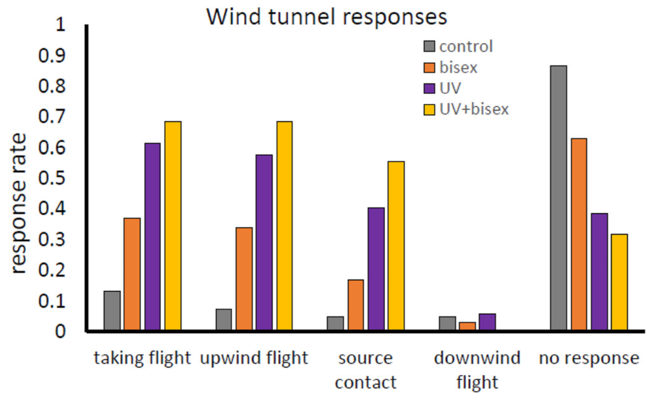

Fig. 4. Responses of *Ostrinia nubilalis* to the UV light stimuli, bisexual lure and their combination

Table 1. Statistical comparisons of effects of light, bisexual lure and their combination on taking flight frequencies

	Chi square value	*P*-value	
All 4 treatments	37.52	<0.0001	
Fisher *post hoc* tests, *P*-values with Benjamini–Hochberg correction	bisexual lure	UV light	bisexual lure + UV light
UV light	0.02	–	–
bisexual lure + UV light	0.05	0.68	–
control	0.004	0.000008	0.00002

differences were statistically significant (Fig. 5; Two-way ANOVA, $F_{3/123} = 17.02$, $P < 0.0001$). Males and females had similar latencies for all treatments (Two-way ANOVA, $F_{1/123} = 0.06$, $P > 0.05$).

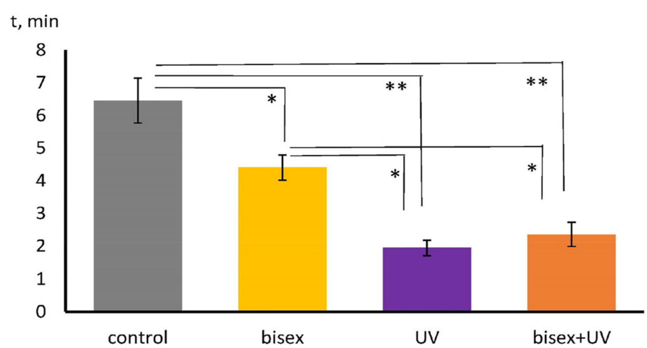

Fig. 5. Latencies of taking flight reaction of *Ostrinia nubilalis* in the wind-tunnel in response to UV light, bisexual lure and combined stimuli. * – $P < 0.05$; ** – $P < 0.01$ (Tukey HSD *post hoc* test)

Field trapping

First, the results of field trapping clearly indicate the complete domination of the ECB adults in catches during the season (Photo 1). Secondly, field trapping clearly shows the prevalence of females caught by any bait other than control. In total 344 moths (from which 83.1 % were females) were captured by light traps; 240 (77.5 % females) by light + bisexual lure; 88 (75.0 % females) by bisexual lure, and 3 males (no females) were found in control traps.

Two-way ANOVA (bait versus sex), performed for all four treatments, revealed strong bias towards females ($F_{1/59}$ = 28.17, $P < 0.0001$; Fig. 6), the factor of baits was significant as well ($F_{3/59}$ = 17.12, $P < 0.0001$). *Post hoc* Tukey's HSD test revealed that differences between sexes were significant for all traps with UV LEDS (with or without bisexual lure), but not the controls. Bisexual lure baited traps showed only slight difference ($P = 0.05$) from control.

Combined sex groups differed from the control for all treatments (Fig. 6; $P < 0.05$, *post hoc* Tukey's HSD test).

The differences between UV light, bisexual lure and combination of them were not statistically significant.

Photo 1. The capture of *Ostrinia nubilalis* adults (females in majority) by the bucket funnel trap equipped by a combination of bisexual lure and LEDs per one night

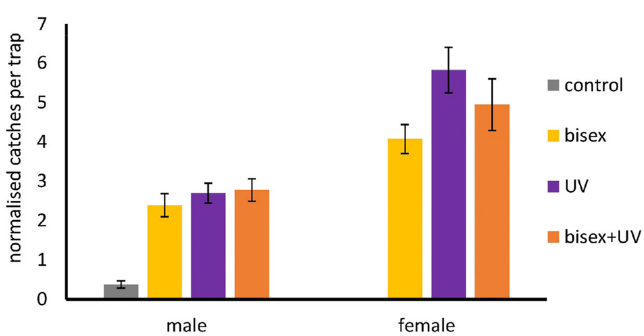

Fig. 6. Field trapping of *Ostrinia nubilalis* by funnel traps equipped with bisexual lure, UV light or both

DISCUSSION

Laboratory testing of *O. nubilalis* adults revealed the most attractive light stimulus, UV light, characterized by the highest attractivity and shortest latency of the response. *O. nubilalis* took flight in response to light stimuli a little more often than shown before for *O. scapulalis* (Zhukovskaya et al., 2023a), but the differences between species were non-significant. Although UV is often the most attractive light stimulus for insects (Cowan and Gries, 2009), there are some exceptions. The light traps equipped with green LEDs, 520 nm, attract oriental armyworm, *Mythimna separata* Walker (Kim et al., 2018). Fall armyworm, *Spodoptera frugiperda* (Smith), had shown the best rate of phototactic responses to blue (420 nm) light. Green and yellow lights attract aquatic insects, probably because these wavelengths are better transmitted through the water (Chou et al., 2020; Kühne et al., 2021). The rice green leafhopper, *Nephotettix cincticeps* Uhler, is attracted to long wavelength light from 480 to 740 nm and further molecular and physiological experiments proved that the action spectrum of behavioral responses corresponds to the sensitivity of broadband L (long wave-sensitive) photoreceptor (Wakakuwa et al., 2014). Similarly, the American cockroach, *Periplaneta americana* (L.), responds faster and is attracted stronger to green light than to UV (Zhukovskaya et al., 2023b) and their green-sensitive photoreceptors of compound eyes had a broadband sensitivity, including UV region (Zhukovskaya et al., 2017). Attraction to green color is sometime interpreted as a search for the foliage for ovipositing and feeding (Hardie, 1989; Isaacs et al., 1999), so the attraction to green could not be excluded *a priori* for the corn borers, ovipositing to leaves and stalks of plants. Installation of the light traps inside dense, tall vegetation (maize plants) prevent propagation of light and attracted insects only from a short distance, i. e. the inhabitants of the corn field. This approach allows to minimize the non-target insects (Frolov et al., 2020). In present data the responses to green light in the wind tunnel were higher than control, but since there was no difference between male (38%) and female (40%) responses to this stimulus, green light cannot be considered as oviposition attractant for ECB. The best attraction of *O. nubilalis* to UV, however, was awaited since the sibling species, *O. scapulalis* was found to fly better to UV than other wavelengths (Zhukovskaya et al., 2023a).

Green light was somehow effective in eliciting moth's responses. Since the blue light was not different from the control, we can conclude, that UV and green-sensitive photoreceptors are both responsible for flight to light response, not some broadband ones. Moreover, low response to blue light is possibly due to green - blue and UV - blue inhibitory mechanisms, which separate between two wavelength-specific behaviors, as was found for the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Stukenberg and Poehling, 2019).

Since one of the components of the bisexual lure, phenylacetaldehyde, attracts many insect species (Cantelo and Jacobson, 1979; Tóth et al., 2009; Landolt et al., 2013), we sometimes found in traps sporadic speciments of Lepidoptera (especially representatives of noctuids, such as *Autographa, Abrostola, Helicoverpa),* Coleoptera, Neuroptera and Orthoptera, but ECB moths absolutely dominated.

We did not find any synergetic effect of UV light and bisexual lure neither in the laboratory tests nor in the field. This result was somehow surprising, since in some cases light and odor attractant together produce stronger effect than each of stimulus alone (Frolov, 2022). For example, western flower thrips, *Frankliniella occidentalis* (Pergande), was attracted to the combination of a commercial semiochemical lure for thrips and blue LED (445 nm) better than to light and odor alone (Otieno et al., 2018). In cases when sex pheromone was used as semiochemical lure, the odor-light traps were better, than light traps alone, but the effect was due to male, but not female increased catches (Miyatake et al., 2016). The Western tarnished plant bugs, *Lygus hesperus* (Knight), tested in a laboratory Y-tube assay, preferred the green (530 nm) light, the addition of hostplant (alfalfa) kairomone significantly enhanced the reaction (Blackmer and Caftas, 2005). Mosquito attraction to human-produced CO_2 substantially decreased after filtering of the long wavelengths (590–660 nm) reflected from the human skin (San Alberto et al., 2022). Sheep-derived odorants improve performance of the light traps against mosquitoes (Tchouassi et al., 2012). Sand flies, *Lutzomyia shannoni* Dyar, are better attracted to the preferred red (660 nm) light traps if CO_2 and the mixture of host-derived attractants 1-octen-3-ol and 1-hexen-3-ol are present (Mann et al., 2009).

Reports on the absence of any improvements by the combination of alluring light and odor stimuli are rather rare, likely because of the bias to publish statistically significant positive effects. The lack of any synergistic or additive effects both in the lab and field condition discovered in the present study may raise a few questions, that should be tested further: Is the odor concentration or the light intensity critically important to the attractivity of combined stimulus? Is there any time shift between natural light and odor guided behaviors? We don't know yet the sequence of reactions in response to combined stimuli, but according to our data the light cause responses with shorter latencies, than the odor, so in the environment the moth should take off when seeing the light and after that smell the odor, which probably can improve the source localization when carefully metered out. We cannot also exclude, that the flight to light and attraction to the host plant volatiles are mutually exclusive behaviors, and our experimental situations favor to choose the response to UV light over odor attractants (Barron et al., 2015; Zhukovskaya et al., 2021).

CONCLUSIONS

1. UV light is the most attractive light stimulus for *O. nubilalis* adults tested in the wind tunnel.
2. Kairomonal attractant, bisexual lure, performs better than control both in the wind tunnel and field trapping experiments.
3. Combination of the two above mentioned stimuli was as good as UV light alone, the synergistic or additive effect was absent both in the field trapping and laboratory tests.

ACKNOWLEDGEMENTS

This research was supported by Russian Science Foundation (Project No. 22-26-00199). The authors are sincerely grateful to Dr S. Maini for his valuable comments that contributed to the improvement of the manuscript.

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