



Violet light is the most effective wavelength for recruiting the predatory bug *Nesidiocoris tenuis*

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Abstract *Nesidiocoris tenuis* (Heteroptera: Miridae) is a zoophytophagous mirid bug that is used as a biological control agent for agricultural pests including whiteflies. *N. tenuis* is mass-reared commercially and can be easily bred on banker plants. However, there are still difficulties in establishing populations on crops. Light illumination is a promising candidate for recruiting *N. tenuis* to crop plants. In this study, we investigated whether *N. tenuis* has a particular spectral preference using a six-arm arena equipped with six monochromatic LED lights: UV (365 nm), violet (405 nm), blue (450 nm), green (525 nm), orange (590 nm), and red (660 nm). Adult bugs were introduced into the arena, and phototactic behavior was

observed. A greater number of male bugs chose violet light, whereas female bugs chose violet and UV light equally. We next examined the effects of violet illumination in a tomato greenhouse. In conventional plots, bugs remained near banker plants, whereas in illuminated plots, bugs immediately dispersed from the plants and became uniformly distributed in the greenhouse. Based on these results, we conclude that violet is a promising wavelength for the recruitment and establishment of *N. tenuis* on crop plants.

Keywords Phototaxis · Behavioral manipulation · IPM · Optical manipulation · Wavelength preference

Takuya Uehara and Takumi Ogino contributed equally to this work.

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Introduction

Phototaxis is exploited to manipulate behavior in various insects to facilitate eco-friendly pest

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management (reviewed by Ben-Yakir et al. 2013; Johansen et al. 2011; Shimoda and Honda 2013). One of the most important aspects of phototaxis is the wavelength preference. Preferred wavelengths are inherent and vary among insect species. This trait has been used to develop light traps with light bulbs and colored adhesive tape. However, studies on wavelength preference are biased toward pest species. Natural enemies that might provide a means of controlling the pest density have been the subject of relatively few studies. Research on the phototactic behavior of natural enemies will support more effective pest management strategies.

Nesidiocoris tenuis Reuter (Heteroptera: Miridae) is a zoophytophagous mirid bug with a global distribution. *N. tenuis* preys on various herbivorous pests, such as whiteflies, thrips, spider mites, and others (Sánchez and Lacasa 2008; Calvo et al. 2009; Itou et al. 2013; Urbaneja et al. 2005; Wei et al. 1998). In crop production sites, multiple pest species can sometimes emerge at the same time. Therefore, omnivorous species such as *N. tenuis* hold great potential as natural control agents. On the other hand, excessive densities of *N. tenuis* on crops can cause crop damage, which is characterized by the appearance of necrotic rings on leaves (Arnó et al. 2010; Calvo et al. 2009; Sánchez and Lacasa 2008; Vänninen et al. 2011). Therefore, it is important to maintain the density of *N. tenuis* at a suitable level for controlling pest infestation.

Nesidiocoris tenuis has been introduced into tomato, eggplant, and green pepper greenhouses, and was observed to reduce whitefly populations (Komi 2016; Nakano et al. 2016). However, a time lag of a few months occurs between introducing *N. tenuis* and achieving the pest control effects (Calvo et al. 2009). Several methods, such as pre-plant release of bugs (Calvo et al. 2012) and augmentation by banker plants (Biondi et al. 2016; Hinomoto et al. 2015; Nakano et al. 2016), have been proposed to address this limitation. Recently, it has been shown that volatiles from infested plants attract *N. tenuis* (Rim et al. 2015, 2017). Thus, olfactory cues are favorable candidates for augmentation. Phototaxis may also be useful for attracting and retaining *N. tenuis*, as reported in the predatory bug *Orius sauteri* Poppius (Ogino et al. 2016). However, the phototactic behavior of *N. tenuis* requires further characterization. In the present study, we observed phototactic responses and

determined wavelength preference in *N. tenuis*. We also investigated the efficacy of violet light in attracting *N. tenuis* to crop plants in a greenhouse setting.

Materials and methods

Wavelength preference test and tracking analysis

Insects

Late instar *N. tenuis* individuals were collected from sesame plants in the experimental field of the Tokushima Agriculture Forestry and Fisheries Technology Support Center (N34°4', E134°30'). According to rearing methods for minute pirate bugs (Maeda et al. 2016; Vacante et al. 1997), *N. tenuis* individuals were reared in a plastic cage (24 × 17 × 5 cm) using *Ephestia kuehniella* Zeller eggs (Ga-Ran®; Agrisect, Inc., Ibaraki, Japan) as a food source and *Plectranthus amboinicus* (Lamiaceae) as an oviposition substrate and moisture source (Tezuka et al., unpublished data). The temperature was maintained at 25 ± 1 °C, with 60–70% RH under a 16:8 L:D photoperiod. To obtain unmated individuals, emerged male and female bugs were separated based on reproductive organ shape and reared separately. Unmated bugs from three days to one week post-emergence were subjected to the wavelength preference test. To obtain mated individuals, emerged male and female bugs were reared in the same cage for more than three days. Mated bugs from three days to two weeks post-emergence were subjected to the test. All bugs used were fed enough to avoid a state of hunger.

Experimental setup

The wavelength preference of adult bugs was tested in a six-arm arena, similar to that previously used for *Orius* bugs and a parasitoid fly (Ogino et al. 2015; Tokushima et al. 2016). This setup enables the bugs to be presented with six lights simultaneously. Two pieces of dodecagonal Plexiglas (150 mm, 5 mm) were separated by six semicircular black spacers (48 mm in diameter, 10 mm) to create an experimental arena. A filter paper (150 mm in diameter) was placed on the arena floor. A hole was made in the

center of the paper, through which the bugs could climb into the experimental arena via a 15-ml conical centrifuge tube (BD Falcon tube, BD Biosciences, MA, USA). Six monochromatic LEDs were used as light sources (LDF 26 series, CCS, Inc, Kyoto, Japan). The emission spectra and photon flux density were measured using a spectrometer (HSU-100S, Asahi Spectra, Tokyo, Japan). The LED was mounted on an optical bench 40 cm from the tip of the spectrometer. The emission spectra of the LEDs peaked at 365 nm (ultra violet; UV), 405 nm (violet; VL), 450 nm (blue; BL), 525 nm (green; GR), 590 nm (orange; OR) and 660 nm (red; RD). The photon flux density was set at 6×10^{16} photons $m^{-2} s^{-1}$ at a distance of 35 cm from the LED surface.

Behavioral experiment

The wavelength preference test was conducted in a dark room at a temperature of 25 °C. The behavior of the bugs was recorded using an infrared camera (Himawari GE60, Library Co., LTD., Tokyo, Japan). The video sequences were analyzed using two-dimensional tracking software (Move-tr/2D, Library Co., Ltd) following a previous study (Uehara et al. 2014). The experimental arena was illuminated by an infrared light to record behavior under dark conditions. The flying behavior of *N. tenuis* can be most conspicuously observed during evening, which is consistent with the locomotor activity rhythm of the bug using an infrared monitoring system (Nakano et al. unpublished data). Therefore, tests were conducted during the 4–5 h before dark. We used new bugs for every experiment. For each trial, eight bugs were placed together in a tube. Recording was started when the first bug entered the arena and continued for 5 min. Data was recorded for all bugs that entered the arena. The experiment was repeated with 10–14 replicates. Light preference among the bugs was assessed based on the bugs' position at the end of this 5-min period. If the bugs crossed an imaginary border of 33 mm from the LED panel, we deemed them to have been attracted to the light source. The ratio of attracted bugs was arc-sine square-root transformed prior to one-way ANOVA followed by a Tukey–Kramer HSD test. Statistical analyses were conducted using R software (R Core Team 2015).

Attraction of *N. tenuis* in the greenhouse

Greenhouse layout

The wavelength that most attracted the bugs in the laboratory choice experiment (i.e., violet) was used in the greenhouse experiment. To evaluate the attractiveness of the violet light, we compared *N. tenuis* recruitment in conventional and light-illuminated tomato greenhouses. All experiments were conducted in two experimental greenhouses (10 m long, 5.4 m wide, 3.15 m tall) (Fig. 1) at the Institute of Agrobiological Sciences, NARO, Tsukuba, Ibaraki, Japan. In Japan, the forced cultivation of tomato plants commonly begins from autumn to winter. Therefore, the experiments were performed from October 17 to December 21, 2016, according to the tomato producer's methods and timing. Black plastic mulch was laid just before transplanting to conserve moisture and to moderate soil temperature. There were four rows in each greenhouse, and ten tomatoes (*Solanum lycopersicum* cv. Housakukigan) were planted in each row. Tomato pruning and watering followed common practices in Ibaraki. According to the biological control guidelines for *N. tenuis* (Abe 2016; NARO manual 2015), we used cleome (*Cleome spinosa*), sesame (*Sesamum indicum*), verbena (*Verbena* cv. Tapien), and scaevola (*Scaevola aemula*) as banker plants. The composition and planting periods of these species were the same in both control and illuminated greenhouses. Banker plants were planted together in a

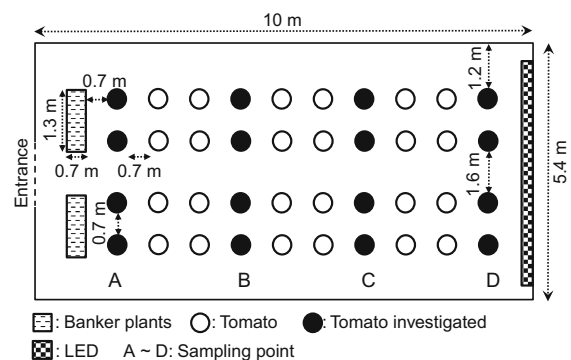


Fig. 1 Greenhouse layout. The greenhouses were 10 m long, 5.4 m wide, and 3.15 m tall. There were four rows, and ten tomatoes were planted in each row. Banker plants were planted at the greenhouse entrance. The LED was placed at the innermost area of the greenhouse, 1.6 m above the ground. The emitting surface was mounted facing the banker plants

small area at the entrances of greenhouses. Tomatoes and banker plants were automatically watered twice a day through an irrigation tube laid under the mulch. When the temperature was below 15 °C at night, the greenhouse was heated with an oil heater located at the center.

Light control

We used custom-ordered rope LED (5 m, SHIGRAY, Inc., Tokyo, Japan), which contained LED chips placed 1.7 cm apart with peaks at 405 nm as a source of violet light to illuminate the tomato plants. The rope LED was placed in the innermost area of the greenhouse (Figs. 1, 2), fixed horizontally to supporting poles 1.6 m tall. The light was switched on from 5:00 pm to 8:00 pm, the period during which *N. tenuis* is most active (Nakano et al. unpublished data). The light-emitting surface was oriented toward the banker plants. The photon flux density of single LED chips was 48×10^{16} photons $\text{m}^{-2} \text{s}^{-1}$ at 30 cm away from the LED surface. To determine the amount of photons reaching the banker plants from the rope LED (10 m away), the photon flux density of a LED with 405 nm was measured in a large dark room at 5, 10, 15, and 20 m from the light source using a spectrometer (HSU-100S).

Sampling procedure

Mass-reared *N. tenuis* were provided by Agri-Soken, Inc. (Inashiki, Ibaraki, Japan). Two-hundred adult bugs were released on the banker plants. The plants were then enclosed in plastic mesh (mesh size 1.0 mm) for two days to prevent bugs from escaping. At the same time as the mesh was removed, violet illumination was started. Bugs that moved to the tomato shoot apex (0–50 cm from the top) were counted during the daytime (from 9:00 am to 12:00 pm, when the bugs remain still) for five days from the onset of the lighting experiment. We sampled at points A, B, C and D, which were 0.7, 2.8, 4.9, and 7.0 m from the banker plants, respectively. The cumulative number of bugs at each sampling point over the five days was treated as a value for one trial. We conducted eight trials, including a trial shortened to four days due to a heavy snow. The means of the cumulative numbers for the eight trials in conventional and illuminated greenhouses were compared using Welch *t* test.



Fig. 2 Photos of the greenhouse. Daytime and nighttime views of tomato greenhouse with LED illumination. The light was switched on from 5:00 pm to 8:00 pm. The light-emitting surface was oriented toward the banker plants

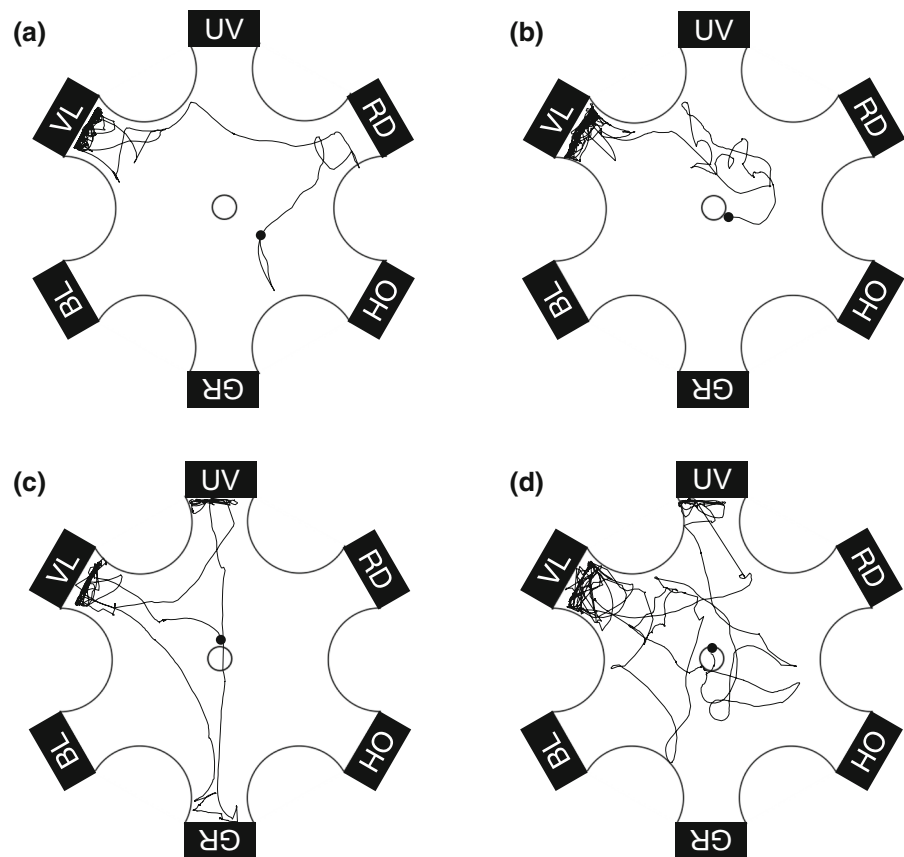
Results

Wavelength preference test

On average, 61.4% of bugs climbed up into the arena (virgin males: 68/96, virgin females: 52/80, mated males: 54/88, mated females: 54/112). Typical bug trajectories are shown in Fig. 3. Some bugs wandered around in the arena and subsequently approached a light (Fig. 3a, b). Others visited several lights before ultimately staying near one (Fig. 3c, d). Bugs that approached lights tended to remain in their vicinity. These results suggest that the positions of bugs after a period of 5 min reflected the effects of particular wavelengths on phototaxis.

The attractiveness of different wavelengths to bugs varied significantly, irrespective of sex or mating experience: virgin males (ANOVA, $F_{5,66} = 10.81$, $P < 0.001$), virgin females (ANOVA, $F_{5,54} = 11.11$, $P < 0.001$), mated males (ANOVA, $F_{5,66} = 11.78$,

Fig. 3 Typical trajectories of bugs attracted to violet light. Views of experimental field and trajectories from above. The bugs wandered around in the arena and eventually reached the light source. Some bugs went directly to the light (**a, b**). Other bugs visited several lights before finally settling near a light source (**c, d**). Black enclosures represent the locations of each LED. Open circles show holes through which bugs entered. Filled circles show the starting points of trajectories. UV: ultra violet, VL: violet, BL: blue, GR: green, OH: orange, RD: red



$P < 0.001$), mated females (ANOVA, $F_{5,88} = 14.73$, $P < 0.001$). VL light strongly attracted bugs (Fig. 4). Among mated males, the largest number were attracted to VL ($P < 0.05$), and the second-largest number to UV light (Fig. 4c). Most mated females were also attracted to VL, but were equally attracted to UV (Fig. 4d). VL appeared to be the most strongly preferred wavelength for virgin bugs, although UV and BL were also attractive (Fig. 4a, b). Bugs were not attracted to lights of longer wavelength, such as OR and RD.

Greenhouse experiment

The preference of *N. tenuis* for VL was also observed in the greenhouse experiment. During the experimental period, the number of bugs on tomato plants was always higher in the LED-illuminated greenhouse than in the conventional greenhouse. The mean cumulative counts per five days were 14.63 ± 1.49 (mean \pm SE) in the illuminated plots and 4.25 ± 0.66

in the control plots. Additionally, the number of bugs varied among sampling points (Fig. 5). The number of bugs observed on tomato plants near banker plants (A) did not differ between the conventional and illuminated plots. In the illuminated plots, the number of bugs on tomato plants was significantly different at the site most distant from the banker plants.

The relationship between photon flux density and the distance from the LED is presented in Fig. 6. The photon flux density reaching the banker plants located 10 m from the LED was 7.95×10^{13} photons $m^{-2} s^{-1}$. With point sources of light, the illuminated light intensity decreases according to the inverse-square law. However, in the case of LED lighting, the actual photon flux density was higher than the theoretical amount of photons, due to the high directivity toward the front of the LED. Therefore, the photon flux density reaching points 20 m from the LED was 2.91×10^{13} photons $m^{-2} s^{-1}$, which was still enough to attract the bugs.

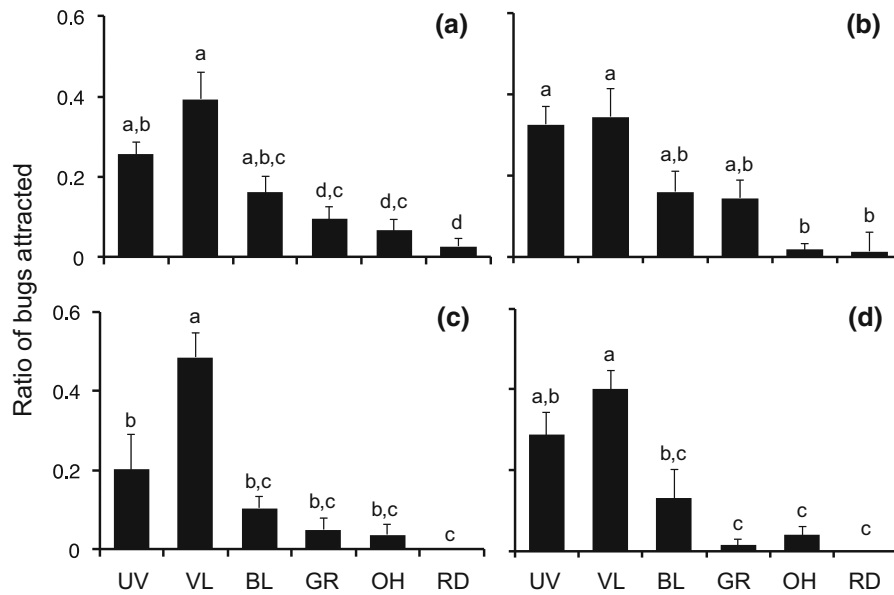


Fig. 4 Spectral preference of *Nesidiocoris tenuis*. Ratio of insects attracted to each wavelength in virgin males (a), virgin females (b), mated males (c), and mated females (d). Each bar represents the mean ± SE. Bars with the same letters are not significantly different at $P > 0.05$, determined using the Tukey–

Kramer HSD test after ANOVA. The proportion of attracted bugs was normalized by arc-sine square-root transformation prior to the test. UV: ultra violet, VL: violet, BL: blue, GR: green, OH: orange, RD: red

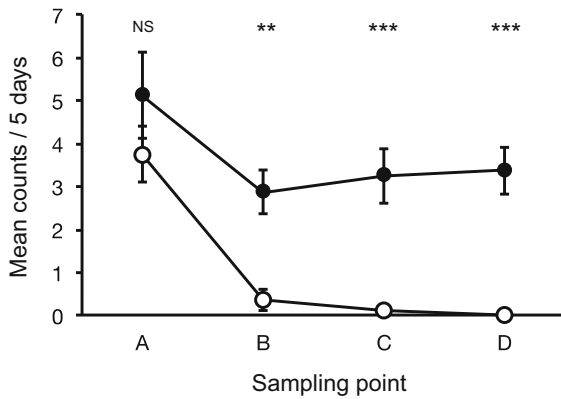


Fig. 5 Comparison of the number of *Nesidiocoris tenuis* in conventional and illuminated tomato greenhouses. Mean number of *N. tenuis* observed at each sampling point and SE. Open and filled circles show data for control and illuminated plots, respectively. Data were compared using Student’s t -test ($N = 8$, **a** $t = -1.0824$, $df = 12.14$, $P = 0.3001$, **b** $t = -4.107$, $df = 10.054$, $P < 0.01$, **c** $t = -5.3391$, $df = 7.6152$, $P < 0.001$, **d** $t = -5.6652$, $df = 7$, $P < 0.001$). NS: not significantly different, $**P < 0.01$, $***P < 0.001$

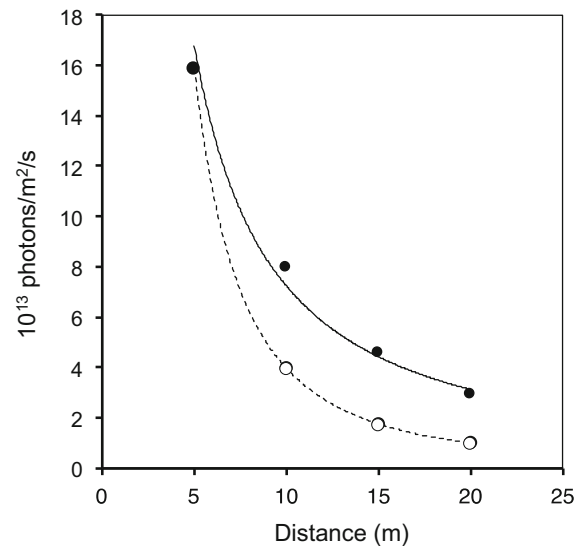


Fig. 6 Relationship between illuminated light intensity and distance from the LED light source. Filled circles show the measured photon flux density of the LED at various distances from the light. Open circles show the theoretical photon flux density. Solid and dashed lines show power approximations (measured: $y = 1 \times 10^{15} \times x^{-1.2}$, theoretical: $y = 4 \times 10^{15} \times x^{-2}$)

Discussion

In general, the visible spectrum for insects ranges from UV to red, and insects are particularly sensitive to UV light (Warrant and Nilsson 2006). The light sources used in the present study covered the entire range of the visible spectrum for insects. Based on our results, we concluded that VL is the most strongly preferred visible wavelength for *N. tenuis*. UV or green light have previously been reported as the preferred wavelengths for many other insects (Briscoe and Chittka 2001; Johansen et al. 2011; Shimoda and Honda 2013). We previously revealed a preference for violet light in two predacious species: the predatory stinkbug *O. sauteri* and the parasitoid fly *Exorista japonica* Townsend (Ogino et al. 2015; Tokushima et al. 2016). Although further research is required, we speculate that the preference for violet light is related to the visual behaviors involved in predation, such as the detection and/or tracking of prey.

A preference for violet light is rare in insects. Therefore, violet illumination can selectively attract specific species to crops. We previously reported that violet illumination was able to attract and maintain populations of an indigenous natural enemy, *O. sauteri*, on eggplants in the field (Ogino et al. 2016). In the present study, we demonstrated that violet illumination is also effective in recruiting another natural enemy, *N. tenuis*, to tomato plants in a greenhouse setting. In the conventional plot, bugs did not move from the banker plants, even by five days after release. Conversely, in illuminated plots, lights attracted bugs from banker plants to tomato plants. *N. tenuis* introduced on banker plants can effectively control whitefly infestation (NARO manual 2015). However, once the bugs settle on the banker plants, they tend to remain there. To address this problem, farmers in Japan cut off the foliage of the banker plant and hang its leaves among crop plants (Nakano et al. 2016). Violet illumination may become an effective and less labor-intensive means of dispersing *N. tenuis* for pest control.

In addition to helping establish *N. tenuis* on crops, illumination can be used to control and monitor insect density. Because *N. tenuis* is zoophytophagous, excessive density of insects on crops can cause crop damage (Arnó et al. 2010; Calvo et al. 2009; Sánchez and Lacasa 2008; Vänninen et al. 2011). In the case of *Macrolophus pygmaeus* Rambur, which is also a

zoophytophagous natural enemy, blue sticky traps are recommended to monitor density (Böckmann and Meyhöfer 2017). In addition to colored sticky traps, light traps may be useful for maintaining *N. tenuis* density at a suitable level for controlling whitefly infestation.

In greenhouse-based tomato cultivation, blue (~ 450 nm) and red LED (~ 650 nm) supplemental lighting are commonly used to manipulate seedling growth (Nanya et al. 2012; Watanabe et al. 2016). Although *N. tenuis* most strongly preferred violet light, the insects also exhibited a degree of phototaxis toward blue light. Therefore, supplemental lighting with blue light may also be effective in attracting *N. tenuis* if no competing light source is present in a greenhouse. However, violet light is the most effective wavelength for recruiting *N. tenuis* in greenhouse environments.

According to studies of leafhoppers and flower thrips, the minimum light intensity required to evoke positive phototaxis is approximately 10^{12} – 10^{13} photons $\text{m}^{-2} \text{s}^{-1}$ (Wakakuwa et al. 2014; Otani et al. 2014). We have no information about the threshold intensity of phototaxis in *N. tenuis*. However, assuming that *N. tenuis* has a sensitivity similar to those of leafhoppers and thrips, a sufficient amount of photons to attract bugs reaches as far as 20 m from the light source (2.91×10^{13} photons $\text{m}^{-2} \text{s}^{-1}$, Fig. 6). Hence, our rope-type LED illumination can also be applied in larger greenhouses.

On the other hand, in a larger greenhouse, the cultivated plants themselves may obstruct the light pathway and cause blind areas. Thus, further improvements to the LED design and the illumination arrangement are necessary. For example, the location/height of the light device and the number of light devices must be optimized to cover the entire greenhouse. In this situation, a portable-type LED may also be useful. To promote this violet LED technology in pest control, we are currently developing a portable-type LED unit with a small solar battery that can be utilized within a greenhouse without electrical access. This approach will reduce the initial installation and maintenance costs. The price of LED devices is decreasing annually, and our violet LED method will only become more practical for use in vegetable production when taking total costs into consideration.

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