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ORIGINAL ARTICLE

Behavioral responses of *Nesidiocoris tenuis* (Reuter) to different monochromatic lights and light intensities

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Abstract

Nesidiocoris tenuis (Reuter) is an important omnivorous insect in tobacco fields. It is of practical scientific significance to regulate the population of *N. tenuis* by employing insect lure strategies with specific light spectra. In this study, the phototactic behavior of *N. tenuis* adults and nymphs to 15 kinds of monochromatic lights was examined. *N. tenuis* adults have a higher response rate and a longer displacement to different light spectra and different light intensities compared with nymphs. *N. tenuis* adults were more attractived by monochromatic lights at 628, 504, 568 and 589 nm among the 15 monochromatic lights. While *N. tenuis* nymphs were more attractived by monochromatic lights at 628, 549 and 577 nm, and there was no significant difference between the nymphs' response displacements to monochromatic lights except for red light at 628 nm and darkness treatments. The light intensity significantly affected the phototactic behavior of *N. tenuis* adults but not nymphs. These results suggested that insect traps with a red light at 628 nm, blue-green light at 504 nm, yellow-green light at 568 nm and yellow-green light at 589 nm can be used to monitor and regulate *N. tenuis* population.

Keywords *Nesidiocoris tenuis* (Reuter) · Phototaxis · Sensitive spectrum · Light intensity · Visual control

Introduction

Nesidiocoris tenuis (Reuter) is a common omnivore in the agricultural ecosystem of tobacco fields in Yunnan Province, China. This species sucks plant juices, including flue-cured tobacco, paulownia, soybean, tomato, rapeseed, sesame, and cucurbitaceous plants. It spreads viruses, inducing many symptoms, such as leaf spots, flower dropping, fruit dropping, mosaic leaves, and witches' broom. In addition, this insect can also prey on tobacco field pests such as tobacco aphids, *Bemisia tabaci*, thrips, spider mites, and young larvae of some Lepidoptera species. It is a farmland insect that is both harmful and beneficial (Li and Zhang 2005; Varshney and Ballal 2017; Hou et al. 2020; Moerkens et al. 2020; Yano et al. 2020). *N. tenuis* can damage tobacco leaves in the hot and dry valley of Yunnan Province in spring and summer. While it only spreads after harvest and causes little harm to agricultural production in the cold highland areas (Shao et al. 1997). Therefore, adjusting *N. tenuis* population control measures to local conditions is of practical significance for supporting the production of flue-cured tobacco.

The use of phototaxis to regulate insect populations has the advantages of low cost, simple operation, and environmental friendliness. However, the compound eyes of different insect species have different sensitivities to lights with different wavelengths (Lebhardt and Desplan 2017; Park and Lee 2017). Moreover, most current commercial insect trap lights and color plates have a broad-spectrum range with inconsistent light color standards and values. Consequently, the lure control effect is attenuated, and accidental injury to natural insect enemies may occur (Li et al. 2015; Fu et al. 2017). So far there have been no reports on the phototaxis characteristics of *N. tenuis*. Therefore, in this study, we investigated the phototactic behaviors of *N. tenuis* adults and nymphs to the spectrum with different wavelength and light intensity. Our results provide a theoretical foundation for developing specific *N. tenuis* light color traps.

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Materials and methods

Insects

N. tenuis adults and nymphs were collected from the Maqiao Aphidius gifuensis Breeding Center in Hongta District, Yuxi City, Yunnan Province (24°22′51.09″ N, 102°31′32.78″ E), China. The experimental populations of *N. tenuis* adults and nymphs were obtained from a greenhouse (temperature: 20.0 ± 2.0 °C, relative humidity: 60 ± 5%) and were fed on tobacco (the K326 variety) leaves. Healthy and active adults and 5th instar nymphs were selected from the experimental populations for phototactic behavior testing.

Apparatus

The insect phototaxis behavior testing setup by Chen et al. (2020) was used, which mainly comprises a light source section (a halogen lamp cup, a light path, and a monochromatic light filter) and a testing area (a set of testing tubes and a dark room). The testing area is a tube structure composed of two opaque plastic outer tubes and a transparent acrylic inner tube. The black box is composed of an opaque plastic outer tube and a transparent acrylic inner tube. The function of the outer tube is to prevent the external light source from filtering through the testing area. The inner tube acts as the crawling area for the experimental insects. The black box connects the testing casings on both sides to serve as a dark adaptation area for the insects before the experiment and insect releasing area during the experiment.

Phototactic behavior response to wavelength stimulation

The phototactic behavior response of *N. tenuis* adults and nymphs to different monochromatic lights was tested using the phototaxis testing device mentioned above. During the whole experiment, the indoor temperature was controlled at 20.0 ± 2.0 °C, and the relative humidity was maintained at 60.0 ± 5.0%. The experiment encompassed a total of 17 treatments, including complete darkness control treatment (CK), full-spectrum natural light treatment (WL), and 15 monochromatic lights with different wavelengths (340, 380, 414, 450, 492, 504, 510, 538, 549, 568, 577, 589, 601, 628 and 649 nm). In each treatment (or CK), 20 insects (10 adults and 10 nymphs) were tested, with one insect for each test, and the response rate was calculated according to Formula (1). The specific methods for various treatments were as follows:

(1) *Complete darkness control treatment* This treatment did not involve the light source area and only used the testing area. In addition, the two ends of the testing tubes were sealed with opaque black plugs to check the crawling state of *N. tenuis* in the absence of light, serving as a control treatment in the experiment.

(2) *Full-spectrum natural light treatment* This treatment was carried out using the full testing setup described above. To check whether *N. tenuis* displayed phototaxis, the opening at the left end was sealed with an opaque black plug. In contrast, at the right end, full-spectrum natural light generated by the halogen lamp cup directly entered the testing area through the central hole of the silicone rubber plug.

(3) *Treatment with monochromatic lights of different wavelengths* This treatment was carried out using the aforementioned complete testing setup. The opening at the left end was blocked with an opaque black plug to examine the phototactic behavior of *N. tenuis* to monochromatic light of different wavelengths. At the opening of the right end, silicon interference filters of different wavelengths were placed at the rubber interface to obtain monochromatic lights of different spectra. Treatment process: first, the experimental insects were placed in a black box, and then both ends of the black box were quickly sealed off. Subsequently, the black box was wrapped with black light-shielding cloth to prevent light from filtering through the black box. The shade cloth and the plugs on both sides of the black box were removed after the insects were placed in the darkroom and adapted to darkness for one hour. Immediately, the black box was connected to the testing tube, and shade clothes were wrapped at the interface of the outer tubes to prevent light leakage at the connection. The light intensity was constant at 10 lx, and the testing time was 5 min. After 5 min, the inner tube was pulled out quickly, and the displacement of *N. tenuis* was recorded. After each test, the tube wall was wiped first with alcohol and then cleaned with pure water, and the tube was dried with a hair dryer before proceeding to the next test. The tested *N. tenuis* insects were not reused.

For CK, WL, and treatment with 15 monochromatic lights of different wavelengths, 20 insects (10 adults and 10 nymphs) were used, with one insect tested each time. The response rates of adults and nymphs were calculated for each treatment.

$$\text{Response Rate} = \frac{\text{Number of insects with effective phototaxis}}{\text{Total insects}} \times 100\% \tag{1}$$

Measurement of phototactic response to light intensity stimulation

In the experiment above, both *N. tenuis* adults and nymphs displayed a strong phototactic response to red light (628 nm), and the adults, but not the nymphs, showed a strong phototactic response to yellow-green light (568 nm). Therefore, these two lights were used as the light sources to measure the phototactic behaviors of *N. tenuis* adults and nymphs to a series of six light intensities (1, 10, 50, 100, 150 and 200 lx) and reveal the influence of light intensity on the phototactic behavior of *N. tenuis*. Each light-intensity treatment included 20 insects (10 adults and 10 nymphs), and the number of insects was one for each test. The experimental setup, methods, and environmental conditions are shown in the experiment above.

Statistical analyses

SPSS 21.0 was used to conduct a one-way analysis of variance on the data, and the least significant difference (LSD) method was used to test the significance of the difference on different data. Student's *t*-test was performed to determine the significance of the difference between the two data groups.

Results

Phototactic behavior response under wavelength stimulation

N. tenuis adults subjected to the WL treatment, the CK treatment, and 15 monochromatic light treatments showed significantly different phototactic responses (ANOVA, $F_{16,153} = 2.336$, $P = 0.003$; Fig. 1), but no significant difference was found for the nymphs (ANOVA, $F_{16,153} = 0.929$, $P = 0.276$; Fig. 1). There was no significant correlation between the phototactic responses of *N. tenuis* adults and nymphs to the above treatments (Pearson correlation, $r = 0.473$, $P = 0.055$).

After the CK treatment, 90% of the insects did not respond (i.e., not moving). Nevertheless, 20% of the experimental insects moved toward the light source after the WL treatment. The response displacements of the adults and nymphs were 4.4 and 5.3 cm, respectively (Figs. 1 and 2).

Responses of *N. tenuis* adults to 15 different monochromatic light treatments were more pronounced than those of the nymphs (Tukey's test, $F = 22.843$, $P = 0.000$; Figs. 1 and 2). For the monochromatic lights with 15 wavelengths, there was a significant difference in the response displacements of the adults (LSD, $F_{14,135} = 2.252$, $P = 0.009$; Figs. 1 and 2). Among the 15 monochromatic lights tested, red light at 628 nm (response rate = 60%; response displacement = 26.7 cm), blue-green light at 504 nm (response rate = 60%; response displacement = 24.0 cm), yellow-green light at 568 nm (response rate = 60%; response displacement = 23.7 cm), and yellow light at 589 nm (response rate = 70%; response displacement = 22.3 cm) were more attractive to *N. tenuis* adults. Ranked according to the response displacement, for the other lights, the effect of yellow light (577 nm) on *N. tenuis* adults > blue light (45 nm) > ultraviolet light (380 nm) > green light (549 nm) > red light (649 nm) > red light (601 nm) > green light (510 nm) > green light (538 nm) > ultraviolet light (340 nm) > blue light (492 nm) > purple light (414 nm). The response displacement of *N. tenuis* adults to red light at 628 nm was significantly larger than that to nine monochromatic lights, namely ultraviolet light (340 nm and 380 nm), purple light (414 nm), blue light (492 nm), green light (510 nm, 538 nm, and 549 nm), and red light (601 nm and 649 nm) (LSD, $F_{8,81} = 2.254$, $P = 0.032$; Fig. 1). There was no significant difference in the nymphs' response displacements after being treated

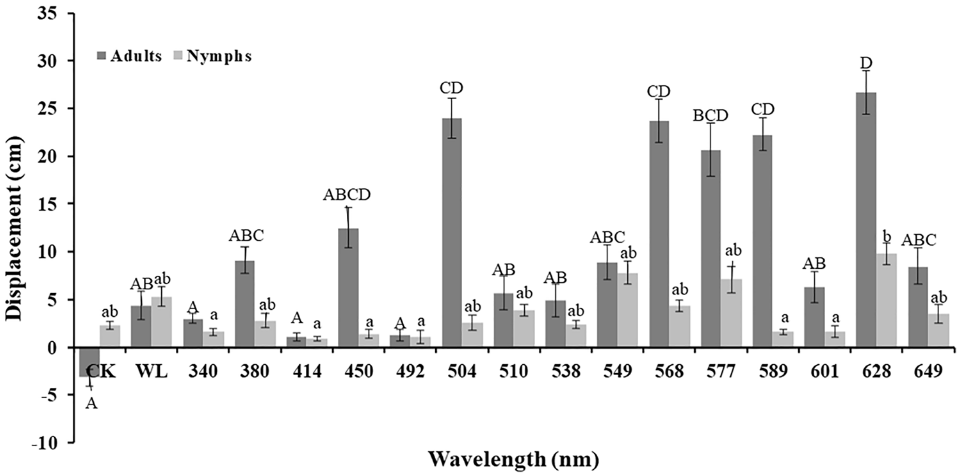

Fig. 1 Comparison of the response displacements of *N. tenuis* adults and nymphs under the stimulation of full-spectrum, dark and different monochromatic lights

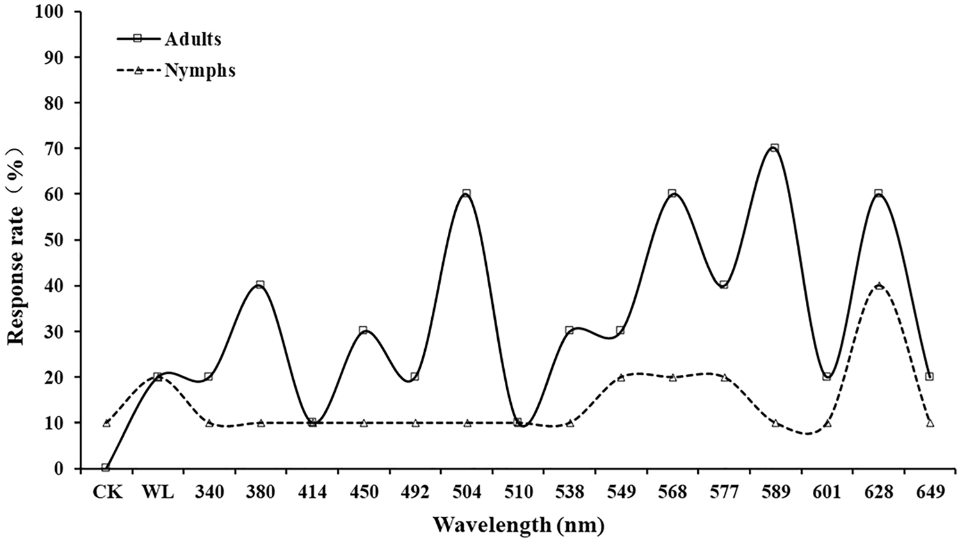

Fig. 2 Comparison of the response rates of *N. tenuis* adults and nymphs under the stimulation of full-spectrum, dark and different monochromatic lights

with 15 monochromatic lights with different wavelengths (LSD, $F_{14,135} = 1.006$, $P = 0.451$; Fig. 1). Among the 15 monochromatic lights tested, red light at 628 nm (response rate = 40%; response displacement = 9.8 cm), green light at 549 nm (response rate = 20%; response displacement = 7.8 cm), and yellow light at 577 nm (response rate = 20%; response displacement = 7.1 cm) were more attractive to *N. tenuis* nymphs. Ranked according to the response displacement, for the other lights, the effect of yellow-green light (569 nm) on *N. tenuis* nymphs > green light (510 nm) > red light (649 nm) > ultraviolet light (380 nm) > blue-green light (504 nm) > green light (538 nm) > ultraviolet light (340 nm) > yellow light (589 nm) > red light (601 nm) > blue light (450 nm) > blue light (492 nm) > purple light (414 nm). There was no significant difference between the response displacements of *N. tenuis* nymphs treated with different monochromatic lights except the red light (628 nm) group and that of the CK group (LSD, $F_{14,135} = 0.663$, $P = 0.807$; Fig. 1).

Phototactic behavior response under light intensity stimulation

Phototactic response to different light intensities of yellow-green light (568 nm)

Significant differences were found in the response displacements of *N. tenuis* adults under the stimulation of different light intensities of yellow-green light (568 nm) (ANOVA, $F_{5,54} = 1.810$, $P = 0.002$; Fig. 3A). However, no significant difference was found for the nymphs (ANOVA, $F_{5,54} = 0.949$, $P = 0.457$; Fig. 3A). There was no significant correlation between the phototactic responses of *N. tenuis* adults and nymphs to different light intensities (Pearson correlation, $r = 0.158$, $P = 0.764$). The phototactic responses of *N. tenuis* adults to different light intensities were significantly greater than those of nymphs (Tukey's test, $F = 9.437$, $P = 0.012$; Fig. 3). The phototactic responses of *N. tenuis* adults were ranked as follows: 10 > 50 > 100 > 150 > 200 >

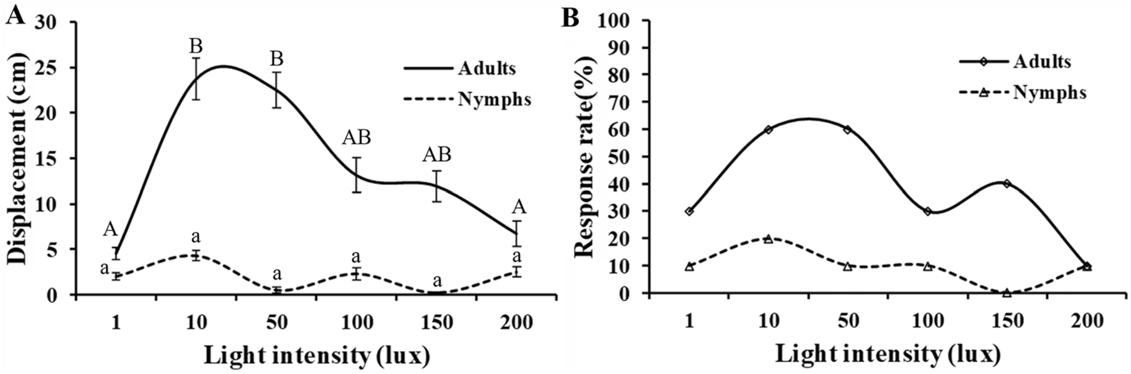

Fig. 3 Comparison of the response displacements (**A**) and response rates (**B**) of *N. tenuis* adults and nymphs treated with different light intensities of yellow-green light (568 nm)

1 lx. The phototactic response of *N. tenuis* adults stimulated by 10 lx light intensity (response rate = 60%; response displacement = 23.7 cm) was significantly greater than those under the stimulation of 1 and 200 lx light intensity (LSD, $F_{2,27} = 4.204$, $P = 0.026$; Fig. 3). The phototactic responses of *N. tenuis* nymphs were ranked as follows: 10 > 200 > 100 > 1 > 50 > 150 lx. The phototactic response of *N. tenuis* nymphs under the stimulation of 10 lx light intensity (response rate = 20%; response displacement = 4.3 cm) was not significantly different from those under the stimulation of other light intensities (LSD, $F_{5,54} = 0.949$, $P = 0.457$; Fig. 3).

Phototactic response to different light intensities of red light (628 nm)

Significant differences were found in the response displacements of *N. tenuis* adults under the stimulation of different light intensities of red light (628 nm) (ANOVA, $F_{5,54} = 4.826$, $P = 0.001$; Fig. 4A), but no significant difference was found for the nymphs (ANOVA, $F_{5,54} = 1.648$, $P = 0.163$; Fig. 4A). There was no significant correlation between the phototactic responses of *N. tenuis* adults and nymphs to different light intensities (Pearson correlation, $r = 0.014$, $P = 0.979$). The phototactic responses of *N. tenuis* adults to different light intensities were significantly greater than those of the nymphs (Tukey's test, $F = 7.860$, $P = 0.019$; Fig. 4). The phototaxis responses of *N. tenuis* adults were ranked as follows: 50 > 100 > 10 > 150 > 200 > 1 lx. The phototactic response of *N. tenuis* adults stimulated by 50 lx light intensity (response rate = 90%, response displacement = 36.3 cm) was significantly greater than that under the 1 lx light stimulation (Tukey's test, $F = 15.965$, $P = 0.001$; Fig. 4). The phototactic responses of *N. tenuis* nymphs were ranked as follows: 10 > 200 > 1 > 50 > 100 > 150 lx. The phototactic response of *N. tenuis* nymphs under the stimulation of 10 lx light intensity (response rate = 40%, response displacement = 9.8 cm) was significantly greater than those under the stimulation of 50, 100, and 150 lx light intensities (LSD, $F_{2,27} = 5.619$, $P = 0.009$; Fig. 4).

Discussion

In this study, we found that *N. tenuis* adults and nymphs display positive phototaxis to full-spectrum natural light and 15 monochromatic lights of different wavelengths. The phototactic responses of *N. tenuis* adults to different light spectra and intensities were significantly stronger than those of *N. tenuis* nymphs. Moreover, there were significant differences in the phototactic responses when comparing the full-spectral treatment group and the 15 monochromatic light treatment group with the control (complete darkness treatment group). Nonetheless, no significant difference was found in the nymphs. The light intensity significantly affected the phototactic behavior of *N. tenuis* adults, while not in the nymphs. *N. tenuis* adults displayed stronger phototaxis than nymphs, a typical phenomenon observed in other insects. For example, *Harmonia axyridis* adults responds more significantly to natural light and 15 monochromatic lights than the nymphs (Chen et al. 2020). The winged aphid *Myzus persicae* has a stronger phototaxis response to natural light and 14 monochromatic lights, while neither adults nor nymphs of the wingless *M. persicae* have an obvious phototaxis response (Fu et al. 2009). The high photosensitivity of the adults is related to their biological activities, such as flight orientation, dispersal and migration, host searching, oviposition, and mating.

Up to now, the photosensitivity of compound eyes in most insects studied is a typical "ultraviolet-blue-green" three-primary-color vision system (Briscoe and Chittka 2001; Stavenga 2002), while there are also a few insects that have a strong phototaxis response to red light. For example, *Frankliniella occidentalis* has a strong phototaxis response to red

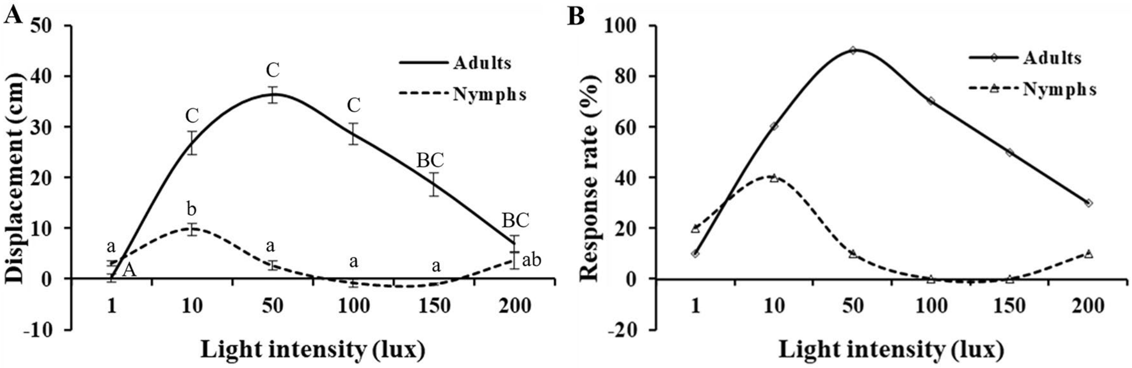

Fig. 4 Comparison of the response displacements (**A**) and response rates (**B**) of *N. tenuis* adults and nymphs treated with different light intensities of red light (628 nm)

light at 652 nm (Fan et al. 2012), *Coccinella septempunctata* to red light at 649 nm (Zhou et al. 2013), and *Aphidius gifuensis* to red light at 628 nm (Chen et al. 2012). Our study showed that both *N. tenuis* adults and nymphs have the strongest phototactic response to red light at 628 nm. Leonard and Cordoba-Aguilar held that the color vision-associated response of many animals is mainly affected by factors such as habitat, food, natural enemies, competitors, and mates (Leonard and Cordoba-Aguilar 2010). Interestingly, the field population of the pea aphid *Acyrthosiphon pisum* usually changes its color gradually from red to green during the growth process (Tsuchida 2010). Previous studies have shown that the color change of pea aphids from red to green is beneficial for avoiding predatory insects, because their natural enemies, such as ladybugs, prefer to prey on red-colored aphids (Losey et al. 1997; Libbrecht et al. 2007). In nature, there are also two ecotypes of plant aphids, i.e., the red ecotype and the green ecotype (Wu et al. 2005). So, whether the phototaxis response of *N. tenuis* to red light at 628 nm is related to the foraging for the red ecotype aphids needs to be validated through further experiments.

In sum, here we found that *N. tenuis* adults are sensitive to red light (628 nm), blue-green light (504 nm), yellow-green light (568 nm) and yellow light (589 nm). Based on these findings, *N. tenuis*-specific photochromic lure devices can be developed to regulate the aggregation and oviposition of *N. tenuis*, and to achieve the targeted regulation of its population for pest control (mainly aphids) in tobacco fields. This is likely a direction for future research on pest control strategies for tobacco.

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Declarations

Competing interests The authors declare no competing or financial interests.

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