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Xinwei Zhang
Shandong academy of forestry
Mengxiao Wang
Shandong academy of forestry
Yongyun Qu
Shandong academy of forestry
Haiwei Wu (haiweiw@126.com)
Shandong academy of forestry

Article

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STUDY ON THE HOST LOCALIZATION CHARACTERISTICS OF *CORYTHUCHA CILIATE* SAY， IN CHINA

Xinwei Zhang[1*], Mengxiao Wang[1], Yongyun Qu[1], Haiwei Wu[1**]

([1] Shandong Academy of Forestry, Jinan, 250014, P. R. China)

(* First author，E-mail：zhangxinwei892@163.com ** Corresponding author，E-mail：haiweiw@126.com)

ABSTRACT: In 2006, *Corythucha ciliate* was first discovered in China. It was an important invasive pest and had spread from the Yangtze River basin to most of northern China for more than ten years.In this paper, the artificial release method was used to determine the role of visual and olfactory signals in locating the host by the *C. ciliate*. The results showed that nearly 80% of the adults of *C. ciliate* tended to coexist visual and olfactory signals compared with single olfactory signals. After the removal of the main olfactory organs of *C. ciliate*, more than 77% of individuals still tended to visual signals, proving that visual signals play a major role in the process of locating host in the *C. ciliate*. This result could provide a new theoretical basis for the prevention and control of the *C. ciliate*.

KEY WORDS: *Corythucha ciliate* Say, host localization, visual signal, olfactory signal

Introduction

Among insects，the important relationship between insects and plants is herbivorous insects and their feeding hosts. One of the important aspects is how these insects identify their host plants for spawning or feeding in a multi-plant environment, and then to meet the needs of reproduction and survival (Wang Fugui et al., 2000). And some insects can also transfer from one host to another along with seasonal changes, such as *Aphis gossypii* (Zhang Kebin et al., 1987), *Diuraphis noxia* (Zhang Guangxue and Zhang Runzhi, 1994), *Apolygus lucorum* (Ma Xingli et al., 2016), *Sagra femorata* (Zhang Zongfu, 1985), *Grapholita molester* (Liu Jinli et al., 2017), etc. Some insects can also distinguish between oviposition hosts and supplementary nutrient hosts, such as *Anoplophora chinensis* (Peng Han et al., 2020), *Apriona germari* (Tang Yanping, 2013), etc.

In the process of host localization, herbivorous pests make tropism toward the preferred stimulus mainly by recognizing external visual signals or olfactory signals. Visual information mainly includes the color, shape, size, etc. Olfactory information mainly includes the composition and proportion of volatiles released by the host plant. Most insects discover hosts by identifying their olfactory signals. The physiological state of host plant are determined by their volatile constituents and release characteristics among some insects, and the insects can locate their hosts form distant areas by volatiles of host plant, to fulfill its nutritional requirements and to find suitable oviposition sites (Harrewijn *et al.,* 1994; Lou Yonggen & Cheng Jia'an, 2000; Bruce *et al.,* 2005; Baldwin, 2010). Such as *Hippophaecolus hippophaecolus* (Liu Shujing, 2010), *Anoplophora glabripennis* (Cao Bing *et al.*, 2004), *Tetranychus urticae* (Dicke *et al.,* 1990), *Leptinotarsa decemlineata* (Kalberer *et al.* , 2001), *Empoasca vitis* (Cai *et al.,* 2014), etc. Some insects can locate hosts by identifying the visual signals of host plants, such as the *Dendroctonus ponderosae* finds hosts in which the color of the trunk plays a major role (Campbell & Borden, 2006). The host plans are localized by leaf color in *Trialeurodes vaporariorum*, with yellow leaves being preferred and transitioning to green leaves after a period of exposure (Vaishampayan *et al.,* 1975). The shape and size of the host fruit are the main visual positioning information for the mating and egg-laying of *Rhagoletis pomonella* (Boller & Prokopy, 1976). Of course, the recognition of these visual and olfactory signals in insects is usually affected by many abiotic factors, such as the distance between the host plant and the insect, and climatic factors (wind, precipitation), etc., which all play an important role in the process of host positioning.

The sycamore lace bug, *Corythucha ciliata* (Say, 1832) (Tingidae, Hemiptera) was originally distributed in central and eastern North-America (Froeschner, 1988). It first invaded Padva (Padova in Italian) in the 1860s, and gradually invaded and spread to more than 10 countries in central and southern Europe, and then invaded South Korea in1996 and spread to Japan in 2001.In 2006, it was first discovered because of its serious harm in Wuhan, Hubei ,China . The adults and nymphs of *C. ciliata* with piercing-sucking mouthparts feed on plant sap by piercing and extracting plan the host leaf fluids. Initially it can cause yellow-white spots and leaf chlorosis, and in severe cases the leaves begin to wither from the veins to the whole leaf and fall off the plant. As a result, trees fall leaves in advance, tree growth interruption, tree weakness, and even death (Maceljski *et al*. 1972; Battisti *et al*. 1985; Soria *et al*. 1991）. In addition, *C. ciliata* is also the transmission vector of *Gnomonia platani* and *Ceratocystis fimbriata*, and it makes *Platanus* *acerifolia* easy to be infected by the *Gnomonia veneta, Ceratocystis fimbriata*(Ellis. et Halsted) and *Ceratocystis fimbriata* f.sp. Platani （Halperin,1989; Nikusch,1992）thereby leads to the aggravation of aging and death of *P. acerifolia.* In 2007, the State Forestry Administration added it to the "List of Dangerous Pests in Forestry" by the Office of Prevention of Invasion of Exotic Forest Pests.

Artificial carrying is the main ways of transmission and diffusionthe (Halbert & Meeker, 1998). Regardless of the way of spreading and dispersing into new habitats, *C. ciliata* all needs to adapt to the new habitat, and on the other hand, it needs to complete the process of positioning and adaptation of host plants as soon as possible. How did the worm find and successfully reach the host in the new habitat? Is it the role of visual signals or olfactory signals? Clarifying this feature not only helps to understand the ecological mechanism of the outbreak of herbivorous pests, but also provides a theoretical basis for how to use plant volatile substances or physical barriers to control pests.

1 Materials and methods

1.1 Insects

Tested *C. ciliata* adults and nymphs were collected from London plane trees (*Platanus acerifolia* (Ait.) Willd.) at a nursery of Zhongjihuajing Garden Co., Ltd, in an experimental field of the Shandong Academy of Forestry and in the Licheng District of Ji'nan City (36.846°N, 117.32°E). All the *P. acerifolia* trees and insects were kept pesticide-free in the field. The tree was planted artificially and 8 years old, about 6.5 m in height, 13-18 cm in diameter at breast height, and 2 m×3 m between rows. In 2014, the experimental forest was infested by *C. ciliata*, and no control measures were taken. Before the beginning of the experiment, the damaged branches were cut off with high branch scissors, and the leaves with the adults of *C. ciliata* on the branches were cut off with the branch shears, and then the adults were gently swept to a petri dish (φ=10cm) with a fine brush, 300-400 heads per petri dish, which were used in the following experiments after the adults were starved for 12 h.

1.2 Methods

1.2.1 Setting of Physical Barriers with Different Transparency

Pre-experiment: 4 pieces of transparent glass are vertically enclosed into a cuboid with a length of 1.5 m×1.5 m×2 m. The top of the cuboid is covered with transparent glass. The petri dish of 50 24-hour-starved adults of *C. ciliata* was directly above, and the petri dish was in the center of the bottom of the cuboid. After 24 hours, whether the adults sticked to the inside and top of the transparent glass were examined, and no adults with a height of more than 2 m were found.

On the horizontal ground, four pieces of transparent glass were vertically enclosed into a bottomless and cover less cuboid with a length of 1.5m×1.5m×0.8m, and the four sides are fixed with wooden wedges inserted into the ground. The inside surfaces of the cuboid were coated evenly with sticky shellac and the outside of the two opposite sides of the four outside surfaces were sticked with opaque black film (Figure 1). At a distance of 50 cm from the cuboid, a glass plate with sticky shellac were erected in each of the four directions. The length of the glass plate was the same as the length of the cuboid, and the height was not less than 2 m. The glass plate was used to capture the adults of *C.ciliate* which do not land on the host plant.

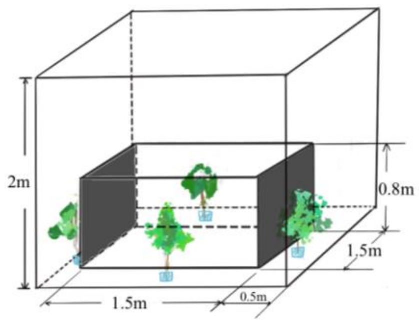

Fig.1 The model of the test installation

1.2.2 Test method

In trial 1, four one-year-old branches of *P. orientalis* with a length of 0.5 m were placed vertically in the middle between the outside of the cuboid and the inside of the vertical glass. And the branches were not harmed by *C.ciliate* , and inserted into a glass bottle filled with pure water. The adults that had been starved for 12 h in the petri dish were placed on the ground in the center of the cuboid with the petri dish, and then the numbers of female and male adults which landed on the glass in each direction were counted for 24 h. The aim was to determine the tendency behavior of adult in both cases. One case was to retain both visual and olfactory signals (the direction of transparent glass), the other was to retain only olfactory signal (the direction of opaque glass).

In trial 2, The antennae of adults of *C.ciliate* that had been starved for 12 h in the petri dish were excised, and then repeated the trial 1. The aim was to determine the tendency behavior of adult that had no olfactory organs in both cases. One case was to retain both visual and olfactory signals (the direction of transparent glass), the other was to retain only olfactory signal (the direction of opaque glass).

In trial 3， Remove the host plants around the cuboid, and then repeated the trial 1. That was to what was the tendency behavior of adult in the absence of olfactory and visual signals.

Each tests were repeated tree times. The branches and the glass used for the test were replaced after each test

1.3 Data Analysis Methods

All data was processed using the SPSS software for statistical analysis. The quantity difference of *C.ciliate* landing in different positions and different directions, and the difference in the number of landings of male and female adult were treated by univariate ANOVA.

1.4 Author declarations

we confrm that all experiments were performed in accordance with guidelines and regulations of Shandong Academy of Forestry, Shandong, China. We confrm that all experimental protocols were approved by Shandong Academy of Forestry, Shandong, China. We confrm that all methods were carried out in accordance with relevant guidelines and regulations of Shandong Academy of Forestry, Shandong, China.

2 Results and analysis

2.1 Trend characteristics of adult insects under different signal sources

In three trials, 74.76%, 90.23% and 73.05% of the adult insects with intact antennae flew to the light-transmitting direction with both visual and olfactory signals, and the average proportion was 79.35%. 25.24%, 9.77% and 26.96% of the adult insects with intact antennae flew to the proportion in the opaque direction of the olfactory signal, and the average proportion was only 20.65% **(Figure 2 A)**. The results of variance analysis showed that the number of adult insects flying in the translucent direction was significantly higher than that flying in the opaque direction ($F_{1, 2}$=57.678, P=0.002).

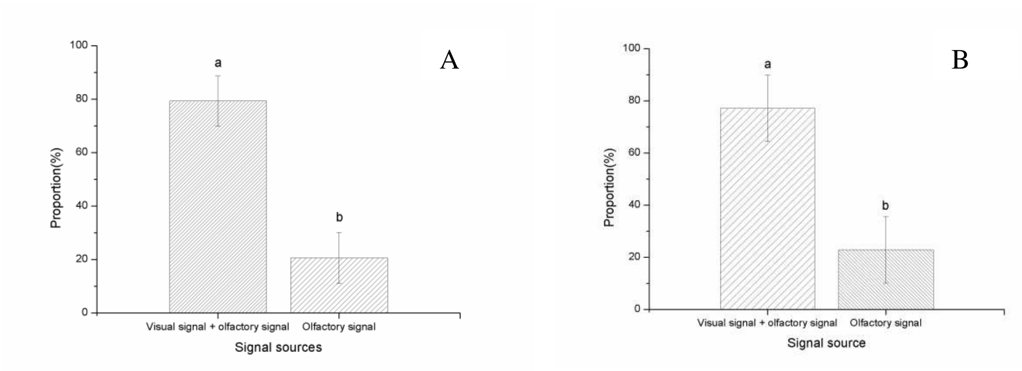

Fig. 2 The proportion of adult insects tending to different signal sources

(A is the intact antennae adult insects, B is the antenna excised adult insects)

In three trials, 63.79%, 89.13% and 78.57% of antenna excised adult insects flew in the direction of transparent glass, with an average proportion of 77.16%, while the proportion of adult insects flying in the direction of opaque glass was 36.21%, 10.87% and 21.43%, with an average proportion of only 22.84% **(Figure 2 B)**. ANOVA results showed that the number of adult insects flying in the light-transmitting direction was significantly higher than that in the opaque direction ($F_{1,2}$=27.335, P=0.006).

2.2 Landing characteristics of adult insects under different signal source conditions

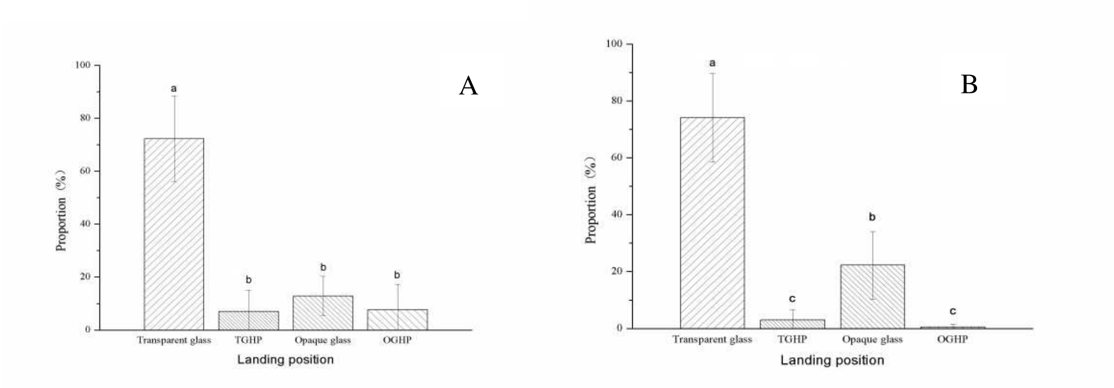

Fig. 3 Landing characteristics of adult insects under different signal sources

(A is the intact adult insects. B is the excised antennae adult insects; TGHP represents the host plant with both visual signal and olfactory signal direction, OGHP represents the host plant with only the direction of olfactory signal)

The proportion of adult insects who has intact antennae with both visual and olfactory signals landed on the glass in three trials was 70.87%, 89.06% and 56.89%, with an average of 72.27%.

The proportion of landing on host plants was 3.88%, 1.17%, and 16.17%, and the average was 7.07% **(Figure 3A)**. The number of adult insects landings on glass was significantly higher than that on host plants ($F_{1,2}$=39.34, P=0.003). The proportion of *C.ciliata* with only olfactory signal landed on the glass in three trials was 21.36%, 8.98% and 8.38% , accounting for 12.91% on average. The proportion of landing on host plants was 3.88%, 0.78%, and 18.6% , with an average proportion of 7.74% **(Figure 3A)**. The number of adult insects landings on glass was significantly higher than that on host plants ($F_{1,2}$=0.557, P=0.497). There was no significant difference between that *C.ciliata* settled on the host plants in the direction of the transparent glass with on host plants in the opaque glass orientation in different signal directions ($F_{1,2}$=0.009, P=0.930).

The proportion of released antenna excised adult insects with both visual and olfactory signals landed on the glass in three trials was 56.90%, 86.96% and 78.57%, with an average of 74.14%. The proportion of landings on host plants was 6.90%, 2.17% and 0.00%, with an average of 3.02% **(Figure 3B)**. The number of adult insects landings on glass was significantly higher than that on host plants ($F_{1, 2}$=59.962, P=0.001). The proportion of *C.ciliata* with only olfactory signal landed on the glass in three trials was 34.48%, 10.87% and 21.43%, accounting for 22.26% on average. The proportion of landing on host plants was 1.72%, 0.00% and 0.00% , with an average proportion of 0.57% **(Figure 3B).** The number of adult insects landings on glass was significantly higher than that on host plants ($F_{1,2}$=10.012, P=0.034). The number of adult insects landings on glass was significantly higher than that on host plants ($F_{1,2}$ =1.340, P=0.311).

2.3 Trend characteristics of adult insects under the condition of no signal source

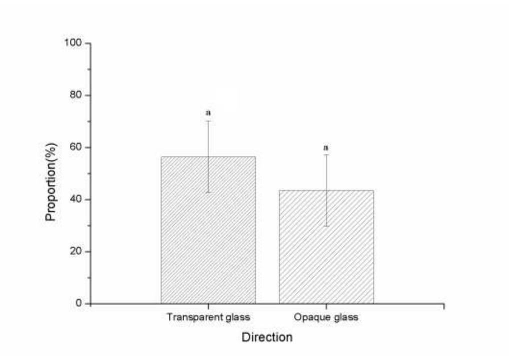

Fig. 4 Tendentious characteristics of adult insects without visual and olfactory signal sources

When there were neither visual nor olfactory signals, the proportion of adult *C.ciliata* tending to the transparent glass direction was 41.94%, 69.23% and 58.16% in the three trials, and the average proportion was 56.44%. The proportion of adults tended to the direction of transparent glass was 58.06%, 30.77% and 41.84%, accounting for 43.56% on average(**Figure 4**). There was no significant difference between two directions ($F_{1,2}$=1.322, P=0.314).

2.4 Sex differences in the tendency of adult *C.ciliata*

In the presence of host plants, the take off proportion of adult male insects with intact antennae were 70.87%, 64.45% and 65.87%, and the average was 67.07%, while the take off proportion of adult female insects were 29.17%, 35.55% and 34.13%, and the average was 32.93% (Fig. 5A). The take off proportion of female was significantly higher than male ($F_{1, 2}$=153.54, P=0.000). In the absence of host plants, the take off proportion of adult male insects were 59.14%, 60.26% and 58.16%, and the average proportion was 59.19%, while the take off proportion of adult female insects were 40.86%, 39.74% and 41.84%, with an average of 40.81% **(Figure 5 B)**. The take off proportion of female was significantly higher than male ($F_{1, 2}$=153.54, P=0.000). For adult insects with excised antennae, in three trials, the take off proportion of adult male insects were 74.14%, 56.52% and 77.63%, with an average proportion of 69.43%, while the take off proportion of adult female insects were 25.86%, 43.48% and 45.79%, with an average of 38.38%

(Figure 5C).The take off proportion of female was significantly higher than male ($F_{1,2}$=11.722, P=0.027).

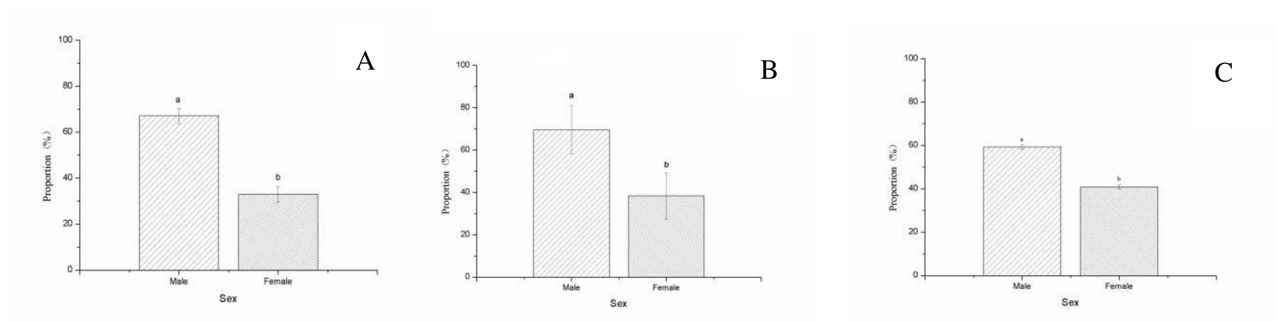

Fig. 5 The take-off proportion of adult insects of *C.ciliata* with different sexes under different experimental conditions

3 Conclusion and discussion

It has been documented that the insects have poor vision and can only perceive light (Schoonhoven et al., 2005), and multiple images of the surrounding environment (Borst, 2009; Land & Nilsson, 2012), and cannot identify host plants at all (Land, 1997).In recent years, studies of insect visual organs, responses to light, and molecular biology have shown that insects have excellent visual abilities and are able to distinguish the color, size, and contour of plant hosts (Wen Chao et al., 2020). For example, *Cydia strobilella* can distinguish cones in different physiological states by the color of the cones (Jakobsson et al., 2017). Reeves et al. (2009) believe that insect vision can sometimes directly locate hosts or with chemical odors to locate host plants. which is confirmed by Qin Junde (1987), Li Dejia et al. (1999) and Liu Bo et al. (2012), arguing that vision plays a key role in the localization and identification of hosts in Longicorn. Our study found that the proportion of *C.ciliata* tending to a single signal (olfactory signal) was significantly lower than the proportion tending to a composite signal (both olfactory signal and visual signal), indicating that although the olfactory signal has a certain role, the visual signal is the driving force. After the main olfactory organ was cut off, the *C.ciliata* mainly relied on visual signals to find the host, and the proportion of the single signal (olfactory signal) at this time was more significantly lower than the proportion of the tendency to the composite signal (both olfactory signal and visual signal), which indicates that the vision of *C.ciliata* could directly help to find the host, and its vision played a major role in the locating of the host.

Insects use vision to orient and locate host plants, mainly through the ability to distinguish colors and shapes and sizes of objects. Such as, *Liriomyza huidobrensis* (Blan-chard) has a yellowing response (Meng Shigui et al., 2003), and *Pieris rapae* is selective to the size of cabbage leaves (Renwick & Radke, 1988). Proving the behavior of herbivorous insects to find host plants can provide new control strategies and methods for integrated pest management. The use of visual stimulation in production to control and monitor pests has been very extensive. The most common is the use of plant colors, such as the use of blue to induce the western flower thrips *Frankliniella occidentalis* Pergande (Li Jiangtao et al., 2008), the green to induce the blunt-nosed leafhopper *Limotettix vaccinii* (Van Duzee) (Rodriguez-Saona et al. 2012b), the yellow to induce *Enbosca vitis* Gothe et al. (Zhao Dongxiang et al., 2001). Although we have preliminarily confirmed that the adults of *C.ciliata* tended to locate host plan mainly through visual signals, it is still unclear what kind of plant information it obtains visual signals, and further research is needed. It is certain that setting isolation barriers on the periphery of the host could significantly reduce the selection effect of *C.ciliata*. Under the conditions of this experiment, only 7.07% of the adults landed on the host plants. In production, how should the barriers be? The setup still needs further exploration.

It is generally believed that oligotropic and mono-eating insects usually orient their hosts through their olfactory. For example, the adult of *Anthonomus pomorum* Linnaeus after overwintering only harms one apple variety in apple orchards, and its host search process is due to the effect of chemical factors (Blanka et al. 2010). *Delia brassicae* Bouche located the cruciferae plant with the information compound allylisothiocyanate (Hawkes & COAKER, 2011).The *C.ciliata.* only harms the *Platanus* of Platanaceae (Li Chuanren et al., 2007) and is also an oligophagous pest, but does not rely on olfactory sensation to locate host plant. This needs to be further clarified for its deep-seated reasons.

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