A THESIS

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Eco-friendly pest management strategy for *Frankliniella occidentalis* and *Bemisia tabaci* in tomato smart greenhouse of Korea

토마토 스마트 온실에서 꽃노랑총채벌레와 담배가루이의 친환경 관리 전략

By

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ENTOMOLOGY PROGRAM

DEPARTMENT OF AGRICULTURAL BIOTECHNOLOGY

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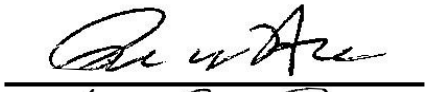
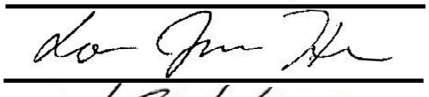
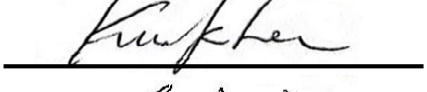
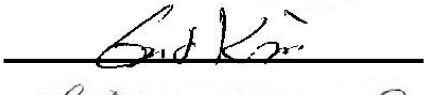
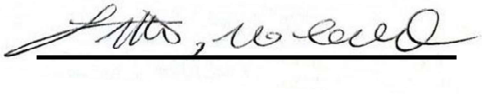
Chairman	**Yeon Ho Je**	
Vice Chairman	**Joon-Ho Lee**	
Member	**Kwang Pum Lee**	
Member	**Gil-Hah Kim**	
Member	**Un Taek Lim**	

ABSTRACT

Eco-friendly pest management strategy for *Frankliniella occidentalis* and *Bemisia tabaci* in tomato smart greenhouse of Korea

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The smart greenhouse refers to a greenhouse in which the crop growth environment can be managed remotely by incorporating ICT, and is a system that enables labor reduction and high efficiency production through automatic environmental control and environmental optimization by computers. In Korea, tomato is a major plant in smart greenhouses, and *Frankliniella occidentalis* and *Bemisia tabaci* are major insect pests in tomato greenhouses. Chemical control has been the most frequently used method for insect pest control in greenhouses. However, in addition to environmental and health problems due to excessive use of chemicals, its control efficacy has been also hampered by insecticide resistance

development in insect pests including *F. occidentalis* and *B. tabaci*. Thus, strategies enhancing eco-friendly pest management such as cultural and biological control methods have been increasingly considered.

To explore the eco-friendly management strategy for *F. occidentalis* and *B. tabaci* in tomato smart greenhouses, following studies were conducted. I examined relationship between occurrence of thrips and whitefly and environmental conditions in tomato smart greenhouses to determine which factors should be considered to manage populations of these two pests. *F. occidentalis* was the dominant thrips species, and *B. tabaci* was the dominant whitefly species in investigated greenhouses. For thrips, its population density in the greenhouse was highly related with its outside population, indicating prohibition of inflow of thrips from outside of the greenhouse is important. Also, its population was correlated with variation of temperature and humidity in greenhouses. On the contrary, whitefly density in the greenhouse was not significantly correlated with greenhouse environmental conditions, but was also related with its outside population.

The life history characteristics of *F. occidentalis* were investigated at control temperature and humidity (27.3 ± 0.54 °C, 79.9 ± 2.79% RH) (mean ± SD), a 10 °C-range fluctuation in temperature (27.1 ± 5.28 °C, 81.5 ± 4.03%

RH), a 20 °C-range fluctuation in temperature (26.5 ± 10.09 °C, 80.4 ± 5.76% RH), a 20%-range fluctuation in humidity (26.8 ± 0.37 °C, 80.7 ± 9.55% RH) and a 30%-range fluctuation in humidity (27.3 ± 0.41 °C, 76.3 ± 15.28% RH). Overall, the life history traits of *F. occidentalis* were more negatively affected by fluctuating environmental conditions. The impact of temperature fluctuation was more severe than that of humidity fluctuation. Additionally, the degree of impact increased as the fluctuation range of the temperature increased, while the reverse trend was observed with humidity fluctuations. With the 20 °C-range fluctuation in temperature, *F. occidentalis* died at the 1st instar larval stage. The offspring's sex ratio was significantly higher at the 20%- and 30%-range fluctuations in humidity (0.47 and 0.49, respectively). From the fertility life table analysis, the intrinsic rate of increase (*r*) was higher at the 30%-range fluctuation in humidity and control conditions as 0.218 and 0.205, respectively. At the 10 °C-range fluctuation in temperature conditions, *r* was significantly lower as 0.169 than other conditions. High fluctuations in temperature and low fluctuations in humidity appear to be the best conditions for controlling *F. occidentalis* populations in greenhouses.

Nesidiocoris tenuis is a biological control agent for controlling *B. tabaci*. Successful establishment of a biological control agent and its spatial coherence with pest in the target area is essential for effective biological

control. To explore effective wavelength which can be used for enhancing spatial coherence of *B. tabaci* and *N. tenuis*, Y-tube test was conducted for various wavelengths. The 385 nm wavelength was found to be best. The incubator test was conducted to verify effect of 385 nm wavelength on *N. tenuis*, and enhanced establishment rate of *N. tenuis* was observed at 385 nm wavelength treatment. The 385 nm wavelength LED light significantly affected population dynamics of *N. tenuis* and *B. tabaci* in greenhouses. In the plots of 385 nm wavelength LED with release of *N. tenuis* and *B. tabaci*, the 385 nm wavelength appeared to enhance establishment of *N. tenuis* and control of *B. tabaci*.

In conclusion, control of *F. occidentalis* might be enhanced by humidity control in smart greenhouses. Enhanced establishment rate of *N. tenuis* by 385 nm wavelength would help to control the *B. tabaci* population in smart greenhouses.

Key words: *Bemisia tabaci*, Biological control, Cultural control, Environmental control, *Frankliniella occidentalis*, Life table, *Nesidiocoris tenuis*, Phototaxis, Relative humidity, Ultraviolet

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General introduction

The smart farm was introduced in Korea from 2014 for the following purposes: 1) stable supply of safe agri-food, 2) strengthening the competitiveness of the agri-food industry, 3) farm household income and management stabilization, 4) promotion of farmers' welfare and development of rural areas, and 5) improving the way the agricultural/rural sector works (MAFRA, 2016). Development of smart farm was planned in three phases: the first-generation smart farms for improving convenience and reducing labor force by 2016, second-generation smart farms to improve productivity by computers and farmer by 2018, and third-generation smart farms for mass production through computer-based decisions by 2020 (MAFRA et al., 2016).

The smart farm refers to a farm in which the growth environment for crops and livestock can be managed automatically and remotely by incorporating ICT, and is a system that enables labor reduction and high production efficiency through automatic environmental control and environmental optimization. The acceleration of research on precision agriculture and automated agricultural production in developed countries from more than a decade ago also stimulated the activation of 'Smart Farm' diffusion in Korea (Adrian et al., 2005; McBratney et al., 2005; Choe and Jang, 2019). Precision farming and automated production are emerging as alternatives to solve the growing problem of agricultural land and labor aging

(MAFRA, 2016; Choe and Jang, 2019). The Netherlands and Israel, which are advanced countries in greenhouse technologies, have long been engaged in cutting-edge farming using ICT, such as automation of cultivation and measuring plant growth information using sensors, and Korea has also been developing, applying, and conducting various researches by various related institutions (Chaudhary et al., 2011; MAFRA, 2016).

Major crops in smart greenhouses of Korea are tomato, sweet pepper and strawberry (Park et al., 2020). The components of a smart greenhouse include sensors to record the environmental conditions in greenhouse and an automatic environment control system (Park et al., 2013; MAFRA, 2016). The automatic environment control system controls all factors such as ventilation, temperature/humidity, and irrigation according to the environmental conditions set by the user, and ensures that the optimal environment for crop cultivation is maintained. (Park et al., 2013; MAFRA, 2016). The biggest difference between smart greenhouses and conventional greenhouses is the difference in the precision of environmental management inside greenhouses, such as temperature and humidity. Smart greenhouses can maintain a relatively constant temperature/humidity through an automated environmental control system, which can also affect the occurrence of pests. In fact, it is known that major greenhouse pests

such as thrips and whiteflies can multiply at a faster rate at a constant temperature (Xu, 1999; Ullah and Lim, 2015). In Korea, tomato is a major plant in smart greenhouses, and *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) are major insect pests in tomato greenhouses (MAFRA, 2016; MAFRA et al., 2016; Park et al., 2020).

In Korea, the most frequent pest control method in most of greenhouses, including smart greenhouses, is chemical control (Cha et al., 2014; Park et al., 2020). Chemical control is very useful, convenient and effective but its excessive use causes environmental and health problems (Jeyaratnam, 1990; Bertolote et al., 2006; Konstantinou et al. 2006; Eddleston et al., 2008; Zhang et al., 2011), and effectiveness has been hampered by resistance development in most of insect pests including thrips and whiteflies (Prabhaker et al., 1985; Zhao et al., 1995; Denholm et al., 1998; Jensen, 2000; Bielza, 2008).

Eco-friendly pest management methods include the cultural control and the biological control methods (Van Lenteren and Woets, 1988; Quisenberry and Schotzko, 1994; All, 1999; Naranjo, 2001; Nomikou et al., 2001; Bale et al., 2008; Pimentel and Perkins, 2019). Cultural control aims to reduce insect pest populations by creating environment unfavorable to pest. However, this should be done not to harm the crop growing conditions.

This method is thought to be more appropriate in smart greenhouses where precise environmental control is possible. Biological control aims to manage pest density by using natural enemies such as predators and parasitoids. For example, for control of *B. tabaci* and other pests such as thrips, aphids, mites, and moths in greenhouses, a generalist predator, *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae), is often used for controlling whiteflies. For improving biological control in greenhouses, successful establishment of natural enemies in the target area must occur. The establishment rate of natural enemies could be reduced by the chemical pesticides, low pest density, and environmental conditions (Holland et al., 2008; Straub et al., 2008; Thomson et al., 2010; Messelink et al., 2014).

By introduction of smart greenhouse, automated and accurate environmental management in greenhouse has become possible. And also, application of advanced facility for plant growth and pest control in greenhouse would be possible. Thus, it needs to explore appropriate ways to manage pests in smart greenhouse by pest species. The relationship between environmental variables and occurrence of thrips and whitefly in tomato smart greenhouses were identified. According to the results, study about cultural control method for controlling *F. occidentalis* was conducted. And, for effective biological control of *B. tabaci*, enhancing establishment of *N. tenuis* in greenhouse was studied.

Chapter I.

Correlation analysis between environmental factors and insect pest density in the smart greenhouse

Abstract

In smart greenhouses, the optimal environment for crop growth can be maintained and managed stably by the automated environmental management system. To explore the eco-friendly management strategy for *F. occidentalis* and *B. tabaci* in tomato smart greenhouses, I examined relationship between occurrence of thrips and whitefly and environmental conditions in tomato smart greenhouses to determine what factors should be considered to manage populations of these two species. The environmental data and occurrence of whiteflies and thrips in the greenhouse were investigated using temperature/relative humidity data logger and yellow sticky traps in four smart greenhouses in Wanju-gun, Korea. *F. occidentalis* was the dominant thrips species, and *B. tabaci* was the dominant whitefly species in investigated greenhouses. For thrips, its population density in the greenhouse was highly related with its outside population, indicating prohibition of inflow of thrips from outside of the greenhouse is important. Also, its population was correlated with variation of temperature and humidity in greenhouses. On the contrary, whitefly density in the greenhouse was not significantly correlated with greenhouse environmental conditions, but was also related with its outside population.

1.1. Introduction

Insect populations are affected by environmental conditions such as temperature and humidity (Karuppaiah and Sujayanad, 2012; Estay et al., 2014; Khaliq et al., 2014). If the temperature and humidity variation is low by the automatic environmental control system in the smart greenhouses, a stable environment would be provided for both plants and pests. It was reported that the population growth rate was higher in the conditions of low temperature variation for thrips (Ullah and Lim, 2015) and whiteflies (Lee et al., 2020). Park et al. (2020) conducted a survey for major insect pest species and environmental conditions in the tomato smart greenhouses, and found that there was no significant change in the major pest species and their problem. This might be because most current smart greenhouses are basically conventional greenhouses equipped with an automatic environmental control system, and thus greenhouse environmental control might not be properly operated (Park et al., 2020).

Thrips can damage to plant leaves, stems, flowers, etc. (Lewis, 1973; Morse and Hoddle, 2006; Kirk, 2002; Reitz, 2009). Some species are virus vectors in tomatoes. In particular, tomato spotted wilt virus (TSWV) is mediated by *Frankliniella* occidentalis (Pergande) (Thysanoptera: Thripidae) (Stobbs et al., 1992; Ullman et al., 1992; Boonham et al., 2002). *F.*

occidentalis is one of the major species of thrips and is distributed worldwide (Han et al., 1998; Lee et al., 2001; Kirk, 2002; Kirk and Terry, 2003). Whiteflies are the most difficult species to control in tomato smart greenhouses in Korea (Park et al., 2020). Major whiteflies species that occurred in greenhouses are *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) (Costa, 1976; Van Lenteren and Noldus, 1990; Byrne and Bellows Jr, 1991; Naranjo et al., 2009). Whiteflies causes plant weakness by sucking and sooty mold by discharging their honey dew (Van Lenteren and Noldus, 1990; Byrne and Bellows Jr, 1991; Rodriguez-Saona et al., 2003; McCollum et al., 2004). In particular, *B. tabaci* is a major species that causes serious economic damage to tomatoes by mediating tomato yellow leaf curl virus (TYLCV) (Costa, 1976, Mehta et al., 1994; Sanchez-Campos et al., 1999; Ghanim and Czosnek, 2000; Antignus, 2009; Li et al., 2010).

In this study, I examined the relationship between environmental variables and occurrence of insect pests such as thrips and whiteflies. One of the main purpose of smart greenhouse is plant cultivation in stable environment. The precision of environmental control in smart greenhouse has not yet reached a remarkable level. However, through continuous research and development, environmental variation would decrease, and precision would increase. It needs to study the management strategies for

major pests in line with the smart greenhouse development and introduction plan. Thus, significant factors that related to thrips and whiteflies occurrence in smart greenhouse were identified, and the pest control strategies has been established.

1.2. Materials and Methods

1.2.1. Data collection

The environmental conditions and occurrence of whiteflies and thrips were monitored in four tomato smart greenhouses in Wanju-gun, Korea. Table 1 shows information on basic specifications of smart greenhouse. The greenhouse A did not have side windows, and roof windows were used for ventilation while side windows and roof windows were used in other greenhouses. Greenhouse C-1 and C-2 were operated by the same farmer and were adjacent to each other.

Table 1. Location and basic specifications of the investigation greenhouses.

Greenhouse	Location	Area (m^2)	Eaves height (m)	Ventilation
A	Bondong-eup, Wanju-gun	3,305	6	Roof
B	Bibong-myeon, Wanju-gun	2,000	3.8	side, roof
C-1	Samnye-eup, Wanju-gun	1,820	3	side, roof
C-2		1,120		

Whitefly and thrips density were monitored by using yellow sticky traps (10 x 15 cm). The locations of temperature/humidity data loggers (HOBO, Onset Computer, Pocasset, MA, USA) and traps in each greenhouse are shown in Figs. 1, 2, and 3. Traps inside the greenhouse were installed at about 1.8 m height, and traps outside the greenhouse were installed at about 1 m height. Due to the small area of greenhouse C-2, only six traps were installed inside, and 12 traps were installed inside of greenhouse A, B and C-1. Outside of the greenhouse A, only two traps were installed due to lack of available space. For other greenhouses, six traps were installed outside of each greenhouse. Traps were replaced about one-week interval. Thrips and whiteflies caught on the traps were counted and identified under the microscope ($\times$ 45) in the laboratory.

The temperature and relative humidity data inside the greenhouse were collected at one hour intervals using data loggers installed inside the greenhouse. Monitoring of environmental condition was conducted during the period of insect monitoring. Mean and variation of temperature/humidity were used as environmental variables to evaluate if variation of environment variables were related with densities of thrips and whitefly.

Traps and data logger were installed in each greenhouse on January 24. Monitoring ended on December 26. From July to mid-September, muskmelons were cultivated in the greenhouse A, and thus, monitoring was

not conducted in the greenhouse A during that period. Then, tomato growing was resumed on late September in the greenhouse A. In the greenhouse B, the first season of tomato cultivation ended on July 4, and on the next day, tomato seedlings were planted for the summer cultivation, which ended on October 10. The next cultivation began in late October. Greenhouse C-1 and C-2 had fallow season in August to September.

All greenhouses that I conducted investigation were first generation smart greenhouses. Around the greenhouse A, there were paddy fields and tomato greenhouses. And, there were bean field, grass land, and small stream around the greenhouse B. And, there were paddy field and bean field around the greenhouse C.

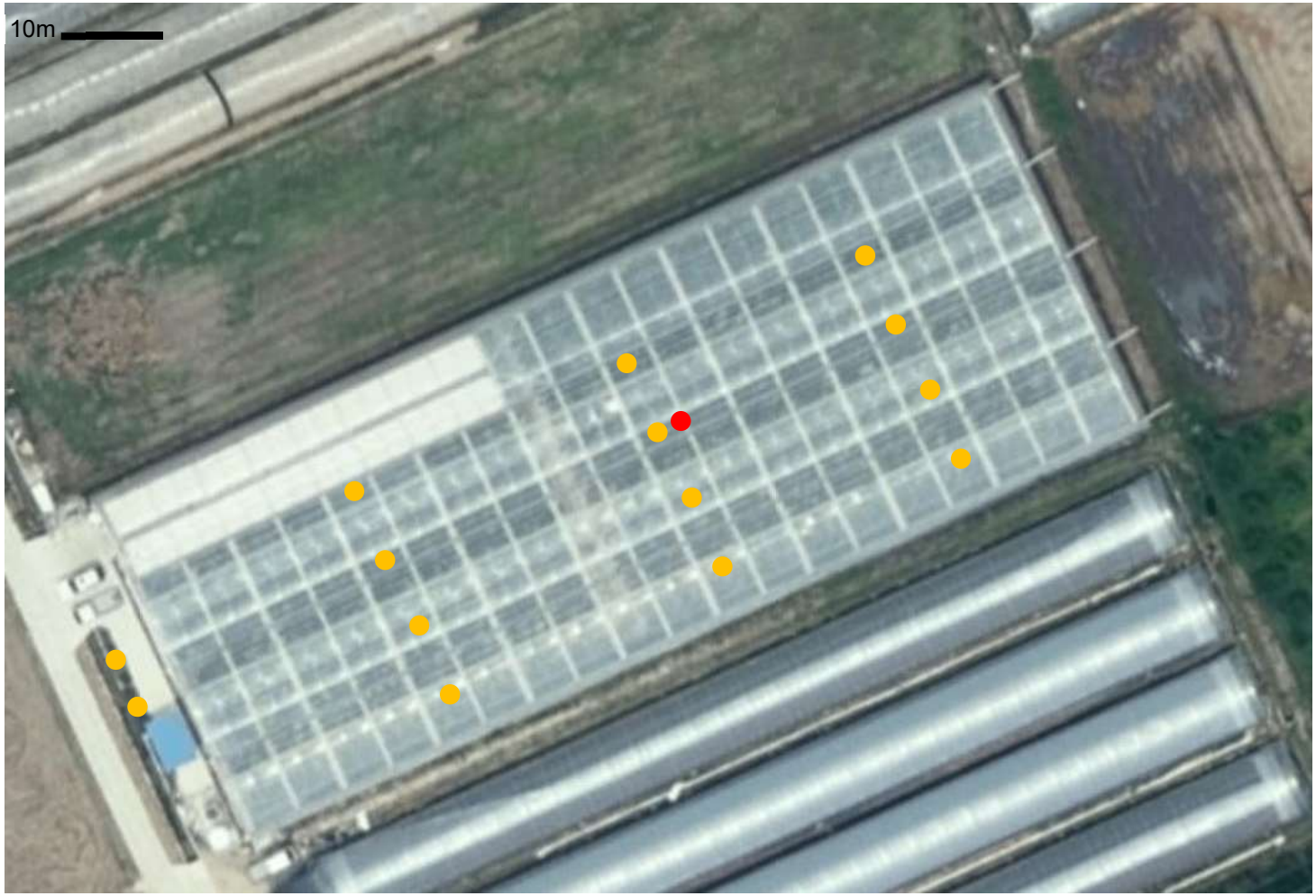


Figure 1. Location of trap (●) and data logger (●) in the greenhouse A.

Figure 2. Location of trap (●) and data logger (●) in the greenhouse B.

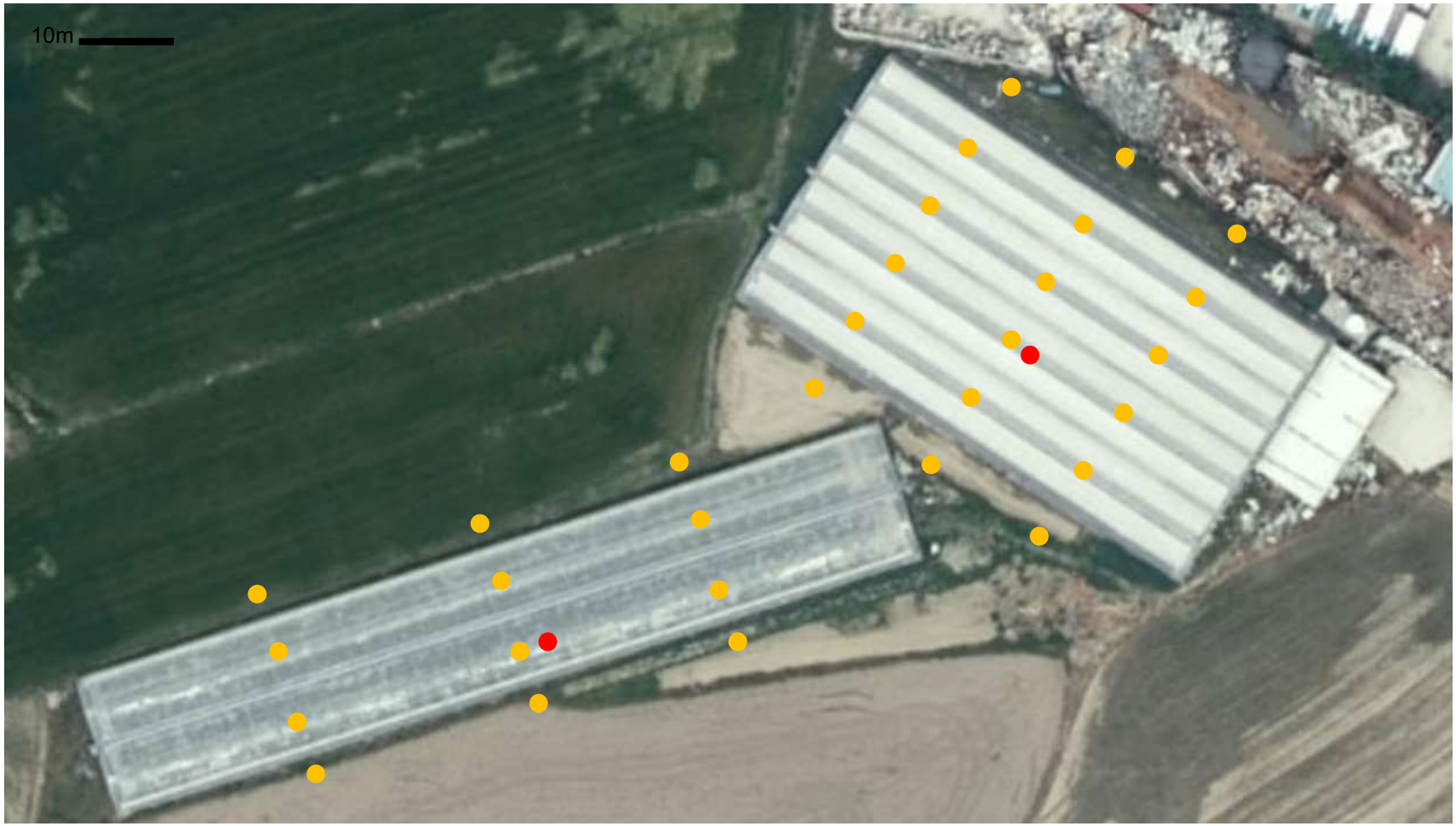


Figure 3. Location of trap (●) and data logger (●) in the greenhouse C-1 and C-2.

1.2.2. Data analysis

Correlation analysis between density of each pest in the greenhouse and environmental conditions in the greenhouse such as temperature, relative humidity and their variation (i.e., standard deviation), and outside pest densities by using SPSS 23 (IBM Corporation, 2016). Data analysis was conducted separately for each greenhouse and conducted using combined data of all greenhouses. By using PROC NPAR1WAY in SAS (SAS Institute, 2013), Wilcoxon rank-sum test was conducted for analyzing difference of pest density between inside and outside in greenhouse A. And that of greenhouse B, C-1 and C-2 was analyzed by using PROC TTEST in SAS (SAS Institute, 2013).

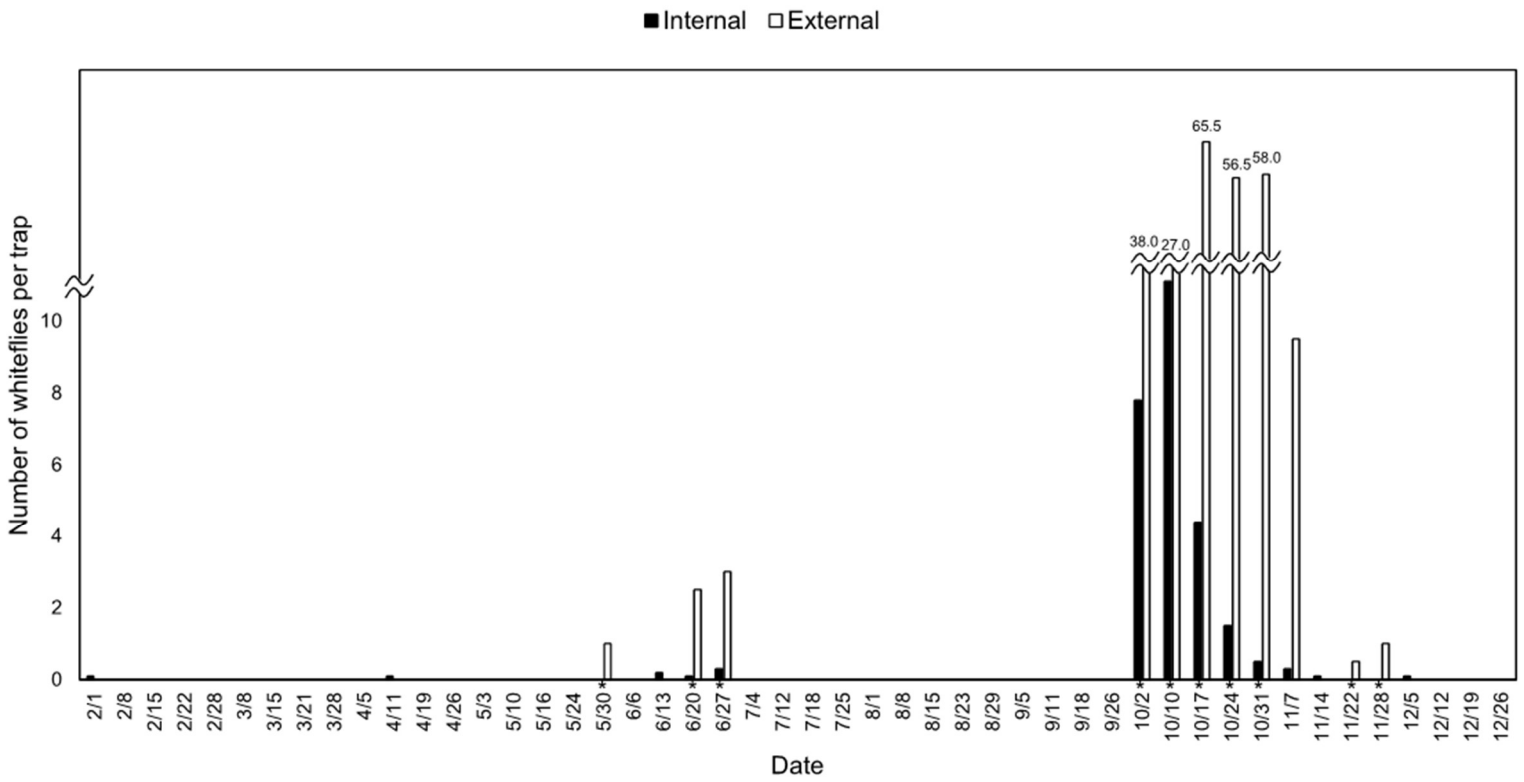
1.3. Results

1.3.1. Whitefly and thrips density in greenhouses

Greenhouse A

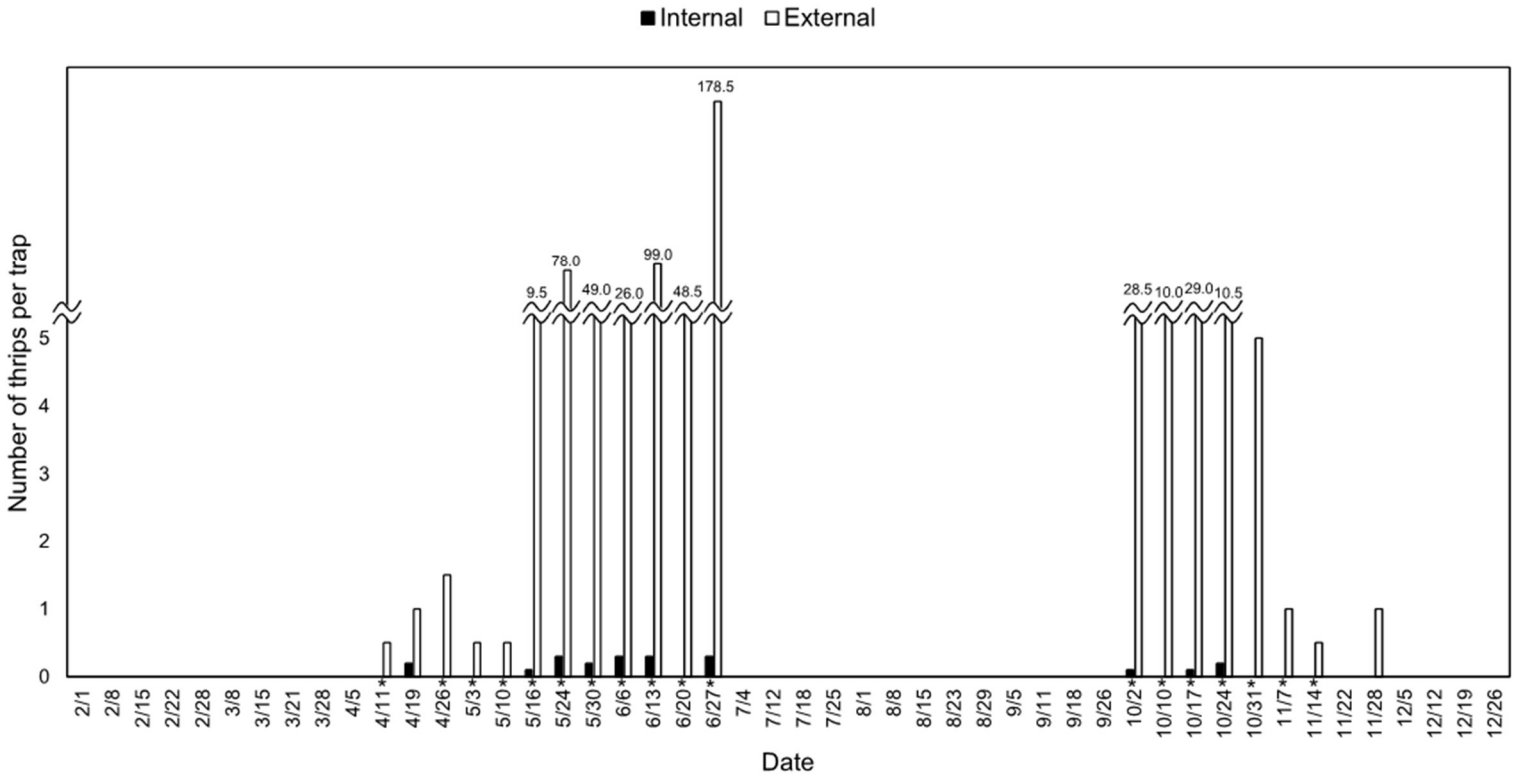
Whitefly and thrips density, temperature and relative humidity in the greenhouse A are presented in Figs. 4 to 7. Whiteflies (*B. tabaci* – over 95%) were rare until the next cropping season, and occurred at low density on early October. Thrips (*F. occidentalis* – 57.9%, *F. intonsa* – 19.2%, others – 22.9%) were rare during all investigation period. Density of whiteflies outside of the greenhouse was highest in October and that of thrips was highest in May to June, it was significantly higher than inside (Whitefly - 2/1, *P*=0.8383; 2/8, *P*=1.0000; 2/15, *P*=1.0000; 2/22, *P*=1.0000; 2/28, *P*=1.0000; 3/8, *P*= 1.0000; 3/15, *P*=1.0000; 3/21, *P*=1.0000; 3/28, *P*=1.0000; 4/5, *P*=1.0000; 4/11, *P*=0.8312; 4/26, *P*=1.0000; 5/3, *P*=1.0000; 5/10, *P*=1.0000; 5/16, *P*=1.0000; 5/24, *P*=1.0000; 5/30, *P*=0.0247; 6/6, *P*=1.0000; 6/13, *P*=0.8383; 6/20, *P*=0.0074; 6/27, *P*=0.0420; 10/2, *P*=0.0348; 10/10, *P*=0.0352; 10/17, *P*=0.0346, 10/24, *P*=0.0322; 10/31, *P*=0.0194; 11/7, *P*=0.3570; 11/14, *P*=0.8312; 11/22, *P*=0.0247; 11/28, *P*=0.0005; 12/5, *P*=0.8383; 12/12, *P*=1.0000; 12/19, *P*=1.0000; 12/26, *P*=1.0000; Thrips -

2/1, *P*=1.0000; 2/8, *P*=1.0000; 2/15, *P*=1.0000; 2/22, *P*=1.0000; 2/28, *P*=1.0000; 3/8, *P*= 1.0000; 3/15, *P*=1.0000; 3/21, *P*=1.0000; 3/28, *P*=1.0000; 4/5, *P*=1.0000; 4/11, *P*=0.0330; 4/26, *P*=0.0006; 5/3, *P*=0.0247; 5/10, *P*=0.0247; 5/16, *P*=0.0035; 5/24, *P*=0.0085; 5/30, *P*=0.0084; 6/6, *P*=0.0085; 6/13, *P*=0.0138; 6/20, *P*=0.0010; 6/27, *P*=0.0138; 10/2, *P*=0.0035; 10/10, *P*=0.0006; 10/17, *P*=0.0035, 10/24, *P*=0.0084; 10/31, *P*=0.0006; 11/7, *P*=0.0247; 11/14, *P*=0.0330; 11/22, *P*=1.0000; 11/28, *P*=1.0000; 12/5, *P*=1.0000; 12/12, *P*=1.0000; 12/19, *P*=1.0000; 12/26, *P*=1.0000). The pest density of the greenhouse A was lowest among the four greenhouses, and it might be because the side windows were absent.



* Statistically significant at $P<0.05$, Wilcoxon rank-sum test.

Figure 4. Internal and external density of whiteflies in the greenhouse A.



* Statistically significant at $P<0.05$, Wilcoxon rank-sum test.

Figure 5. Internal and external density of thrips in the greenhouse A.

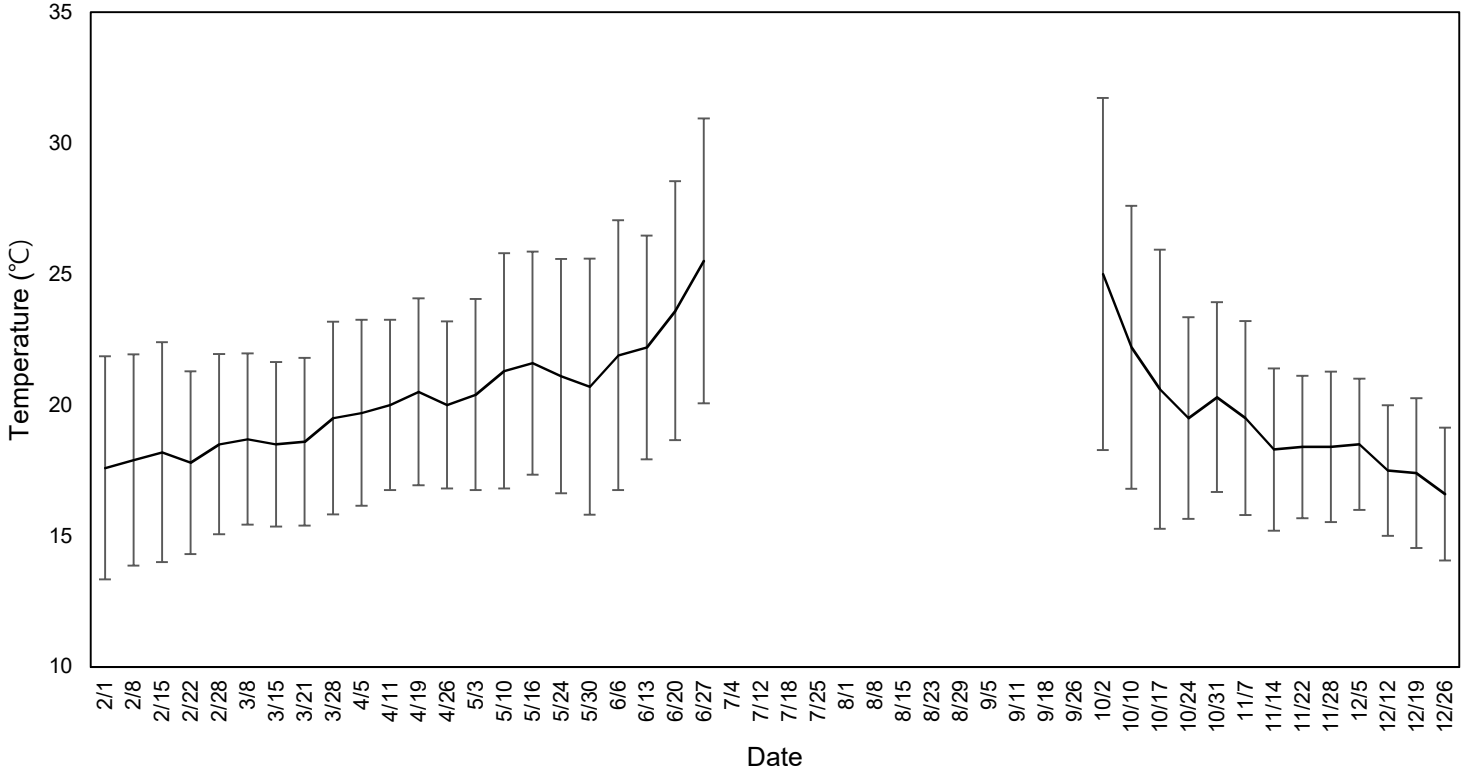


Figure 6. Internal temperature and standard deviation of the greenhouse A.

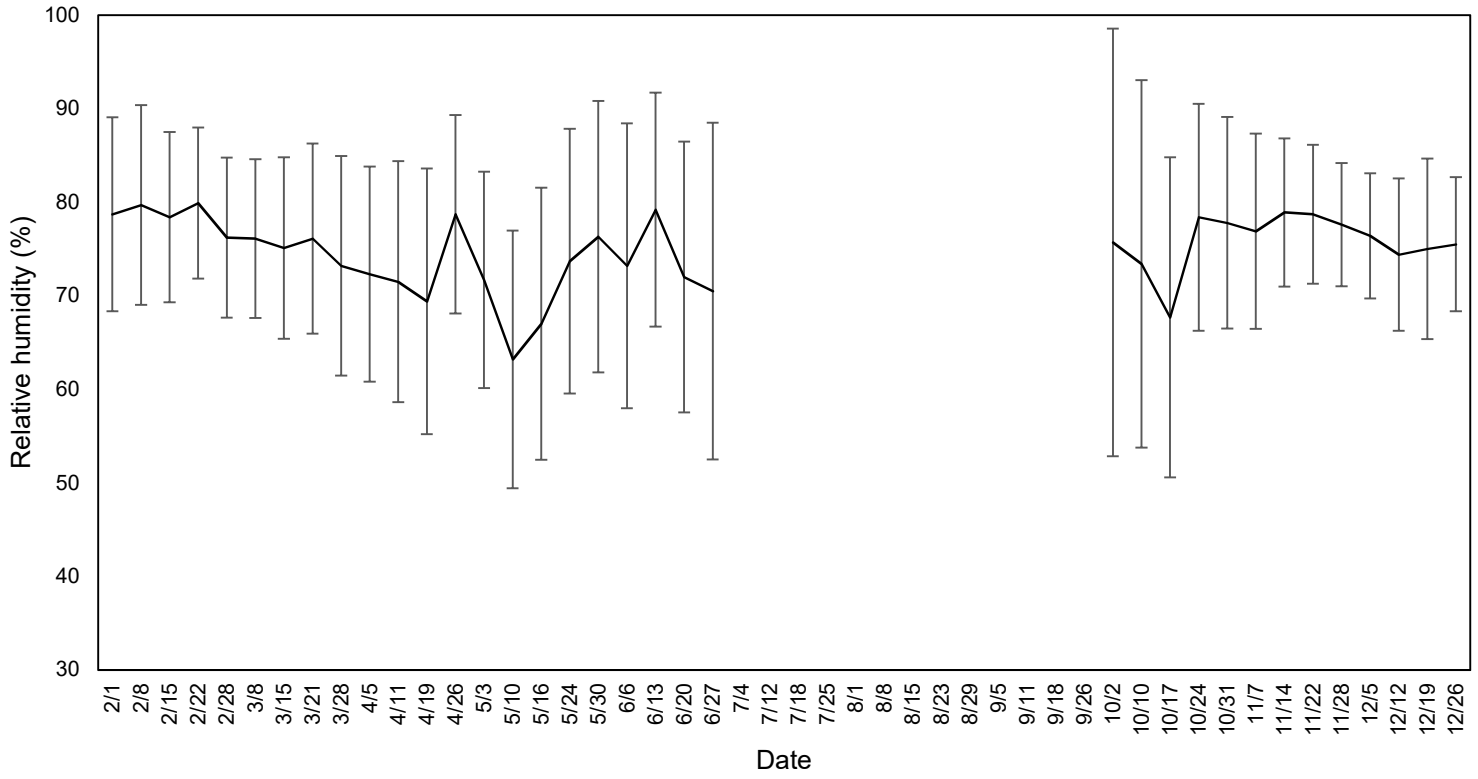


Figure 7. Internal relative humidity and standard deviation of the greenhouse A.

Greenhouse B

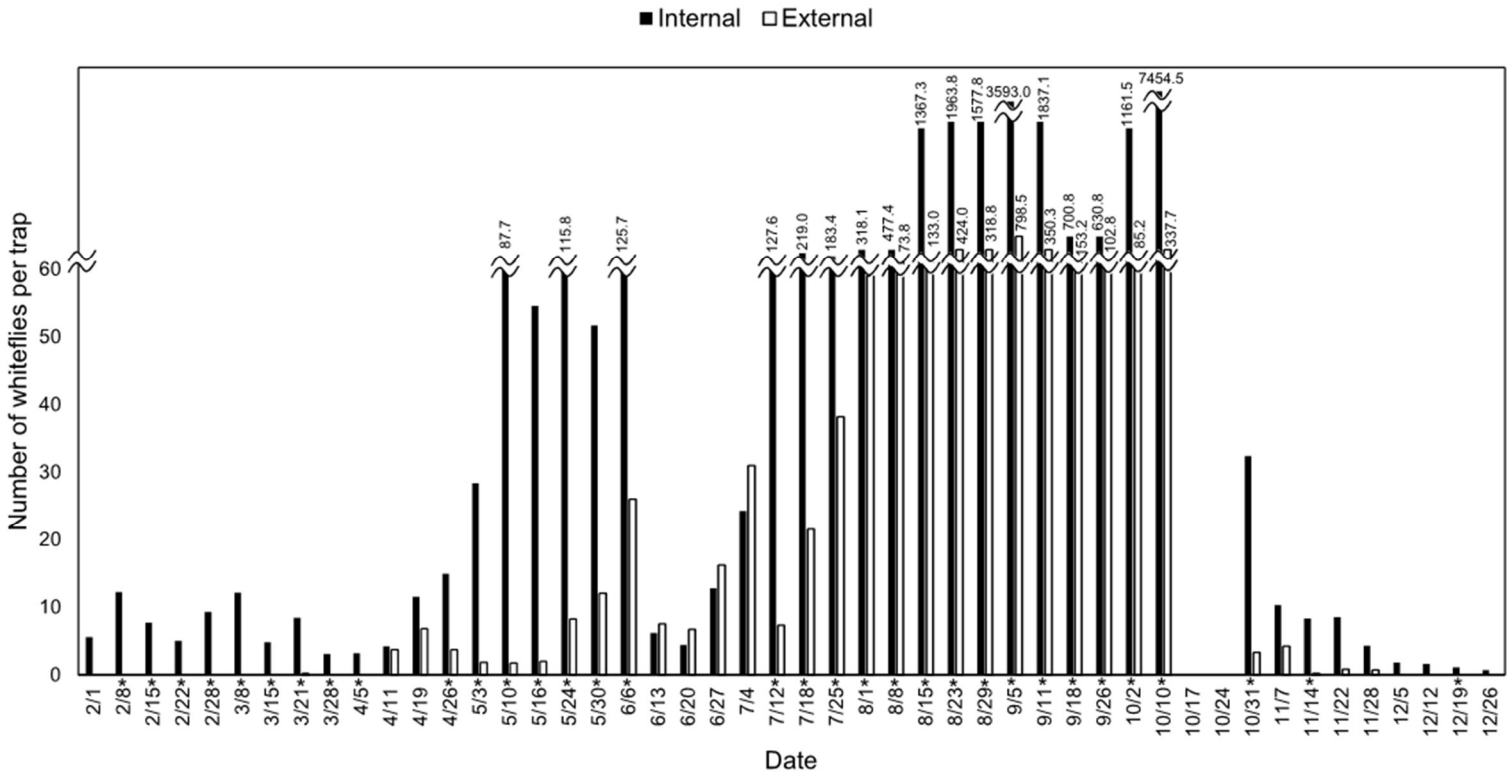
Whitefly and thrips density, temperature and relative humidity in greenhouse B are presented in Figs. 8 to 11. In the greenhouse B, the whiteflies (*B. tabaci* – over 95%) inside the greenhouse occurred from February 1, and external populations were observed from April 11. The internal density of whiteflies peaked in July to October, and the peak of external densities was in August to September in the greenhouse B. After the fallow season, the whitefly density was lower than the previous season, and gradually decreased. The external density of whitefly was also gradually decreased, and was zero on December.

The thrips (*F. occidentalis* – 53.5%, *F. intonsa* – 27.0%, others – 10.4%) population outside of the greenhouse began to develop from late March, and peaked in July and August. Its internal population increased from late April, and was highest in June to July.

The internal density of whitefly was significantly higher than external, and thrips showed opposite trend (whitefly - 2/1, $T_{10.00}$=1.73, P=0.1136; 2/8, $T_{10.00}$=2.29, P=0.0452; 2/15, $T_{11.00}$=6.25, P<0.0001; 2/22, $T_{11.00}$=2.64, P=0.0231; 2/28, $T_{11.00}$=3.91, P=0.0024; 3/8, $T_{10.00}$=4.46, P=0.0012; 3/15, $T_{11.00}$=2.90, P=0.0143; 3/21, $T_{11.08}$=2.95, P=0.0131; 3/28, $T_{11.00}$=3.53, P=0.0047; 4/5, $T_{11.00}$=2.44, P=0.0326; 4/11, $T_{14.22}$=0.29, P=0.7782; 4/19,

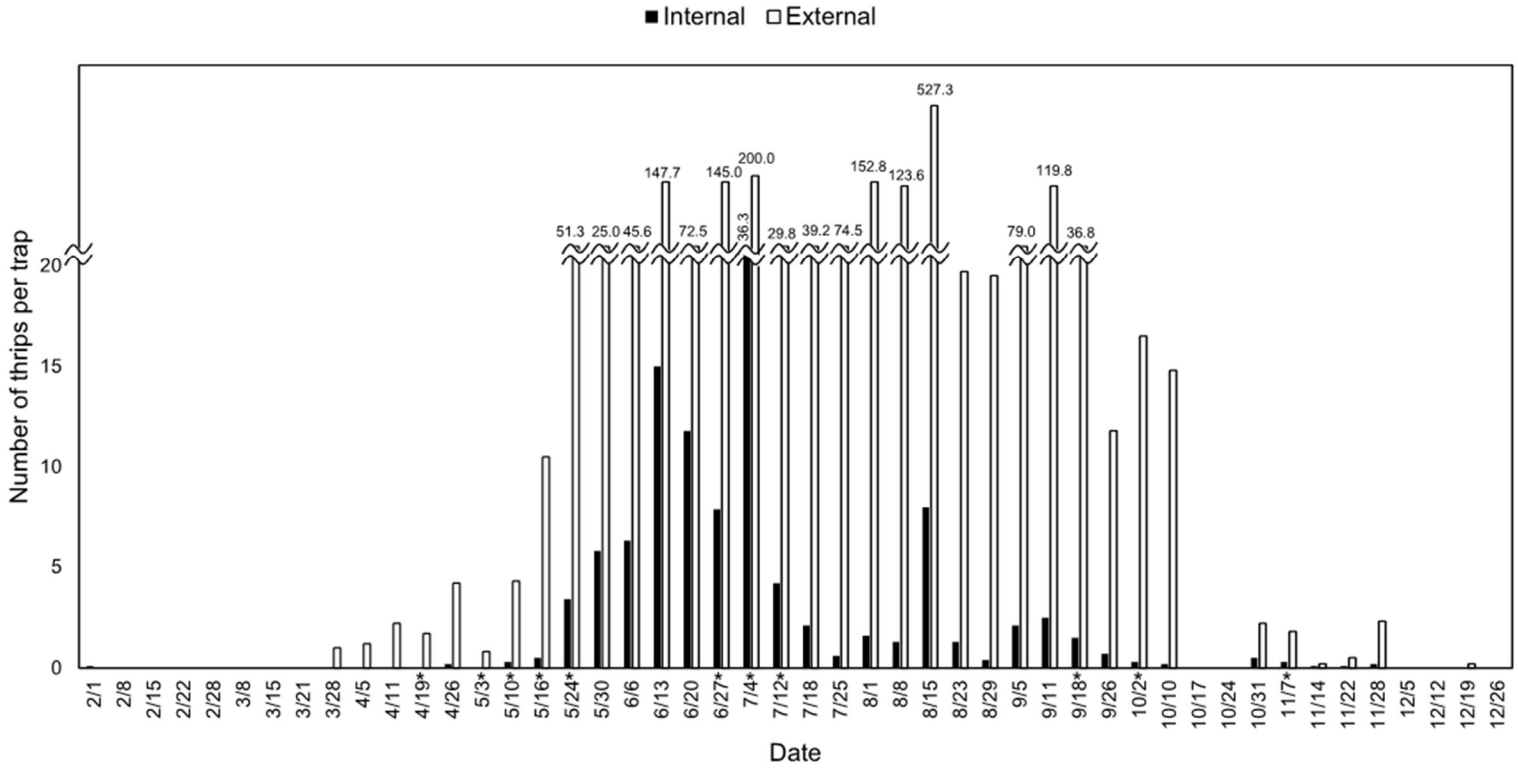
$T_{14.89}$=1.36, P=0.1942; 4/26, $T_{11.54}$=2.45, P=0.0312; 5/3, $T_{10.80}$=3.95, P=0.0023; 5/10, $T_{10.01}$=4.36, P=0.0014; 5/16, $T_{10.03}$=3.81, P=0.0034; 5/24, $T_{10.11}$=5.55, P=0.0002; 5/30, $T_{14.48}$=3.27, P=0.0054; 6/6, $T_{13.80}$=3.62, P=0.0028; 6/13, T_{14}=-0.58, P=0.5735; 6/20, T_{16}=-1.00, P=0.3309; 6/27, T_{16}=-0.87, P=0.3958; 7/4, $T_{5.46}$=-0.68, P=0.5260; 7/12, $T_{11.97}$=10.29, P<0.0001; 7/18, $T_{10.97}$=9.38, P<0.0001; 7/25, $T_{14.16}$=4.10, P=0.0011; 8/1, $T_{13.56}$=4.04, P=0.0013; 8/8, $T_{10.34}$=3.09, P=0.0110; 8/15, $T_{12.54}$=6.05, P<0.0001; 8/23, $T_{14.82}$=6.10, P<0.0001; 8/29, $T_{13.12}$=4.30, P=0.0008; 9/5, $T_{14.87}$=6.29, P<0.0001; 9/11, $T_{15.25}$=8.99, P<0.0001; 9/18, $T_{12.55}$=5.14, P=0.0002; 9/26, $T_{11.67}$=6.23, P<0.0001; 10/2, $T_{11.18}$=5.67, P=0.0001; 10/10, $T_{10.10}$=12.45, P<0.0001; 10/31, $T_{10.20}$=4.75, P=0.0007; 11/7, $T_{13.72}$=2.01, P=0.0645; 11/14, $T_{11.23}$=4.91, P=0.0004; 11/22, $T_{11.15}$=2.05, P=0.0642; 11/28, $T_{11.40}$=1.47, P=0.1682; 12/5, $T_{11.00}$=1.33, P=0.2120; 12/12, $T_{11.00}$=1.57, P=0.1456; 12/19, $T_{11.00}$=2.40, P=0.0353; 12/26, $T_{11.00}$=1.68, P=0.1201; thrips - 2/1, $T_{10.00}$=1.00, P=0.3409; 2/8, T_{15}=0, P=0; 2/15, T_{16}=0, P=0; 2/22, T_{16}=0, P=0; 2/28, T_{16}=0, P=0; 3/8, T_{16}=0, P=0; 3/15, T_{16}=0, P=0; 3/21, T_{16}=0, P=0; 3/28, $T_{2.00}$=-1.73, P=0.2254; 4/5, $T_{5.00}$=-2.44, P=0.0583; 4/11, $T_{5.00}$=-2.38, P=0.0631; 4/19, $T_{5.00}$=-3.95, P=0.0108; 4/26, $T_{5.02}$=-1.74, P=0.1427; 5/3, $T_{5.00}$=-5.00, P=0.0041; 5/10, $T_{5.16}$=-2.61, P=0.0461; 5/16, $T_{5.07}$=-4.03, P=0.0097; 5/24, $T_{5.08}$=-3.73, P=0.0131; 5/30, $T_{4.06}$=-1.76, P=0.1521; 6/6, $T_{4.04}$=-2.38, P=0.0750; 6/13, $T_{5.02}$=-1.65, P=0.1588; 6/20,

$T_{5.04}$=-1.93, P=0.1115; 6/27, $T_{5.01}$=-2.91, P=0.0334; 7/4, $T_{5.03}$=-2.77, P=0.0389; 7/12, $T_{5.15}$=-3.17, P=0.0238; 7/18, $T_{5.10}$=-2.39, P=0.0614; 7/25, $T_{5.00}$=-2.31, P=0.0690; 8/1, $T_{5.00}$=-2.07, P=0.0934; 8/8, $T_{4.00}$=-2.37, P=0.0769; 8/15, $T_{5.00}$=-1.92, P=0.1128; 8/23, $T_{5.04}$=-2.24, P=0.0744; 8/29, $T_{5.01}$=-2.20, P=0.0794; 9/5, $T_{5.01}$=-1.68, P=0.1543; 9/11, $T_{5.01}$=-2.18, P=0.0811; 9/18, $T_{5.08}$=-3.13, P=0.0254; 9/26, $T_{5.05}$=-1.82, P=0.1277; 10/2, $T_{5.01}$=-2.65, P=0.0453; 10/10, $T_{5.00}$=-2.10, P=0.0896; 10/31, $T_{5.23}$=-1.62, P=0.1640; 11/7, $T_{6.29}$=-3.52, P=0.0116; 11/14, $T_{7.60}$=-0.45, P=0.6672; 11/22, $T_{6.43}$=-1.75, P=0.1281; 11/28, $T_{5.08}$=-1.76, P=0.1386; 12/5, T_{16}=0, P=0; 12/12, T_{16}=0, P=0; 12/19, T_{16}=0, P=0; 12/26, T_{16}=0, P=0).



* Statistically significant at $P<0.05$, *T*-test.

Figure 8. Internal and external density of whiteflies in the greenhouse B.



* Statistically significant at $P<0.05$, T-test.

Figure 9. Internal and external density of thrips in the greenhouse B.

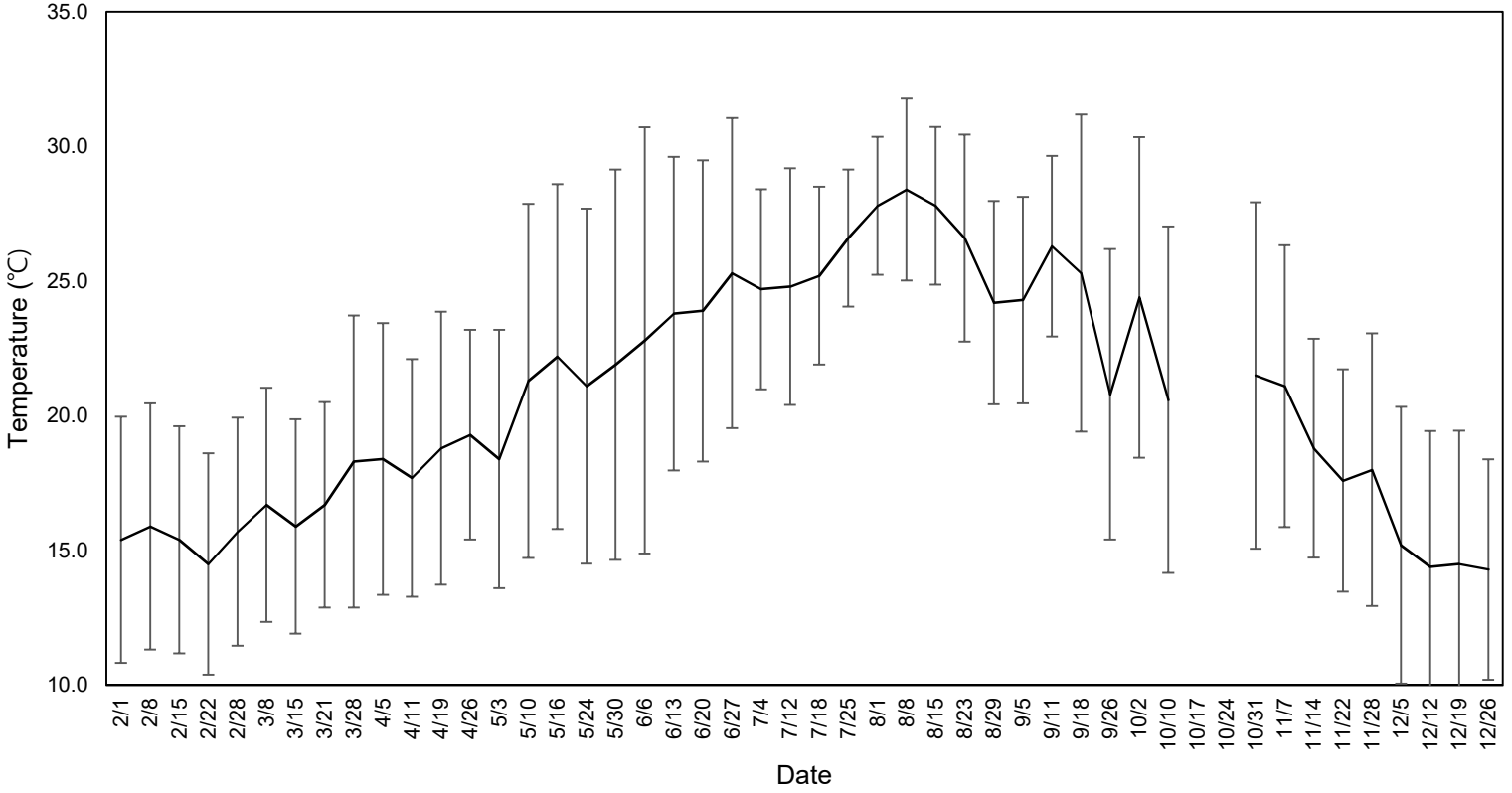


Figure 10. Internal temperature and standard deviation of the greenhouse B.

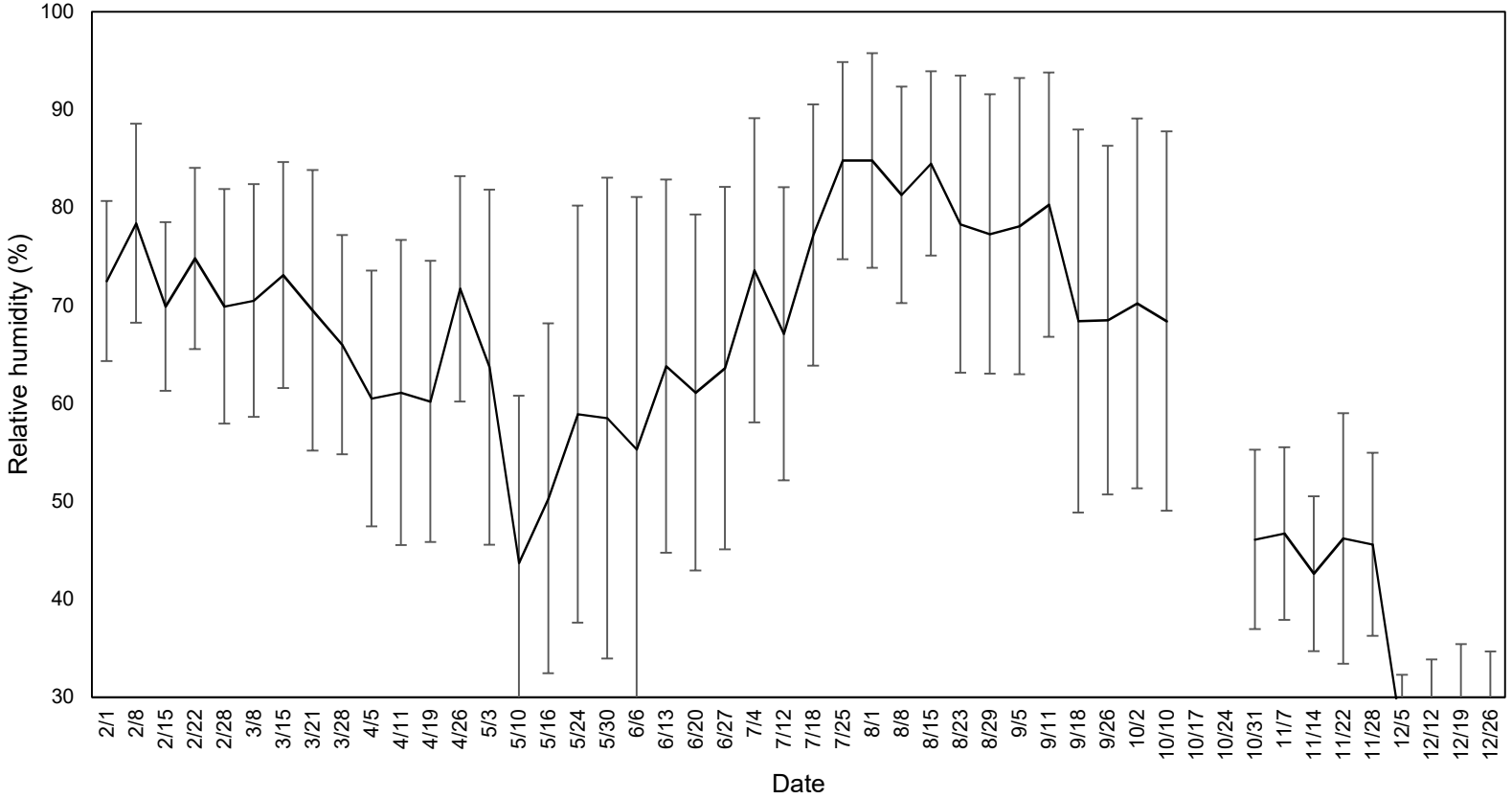


Figure 11. Internal relative humidity and standard deviation of the greenhouse B.

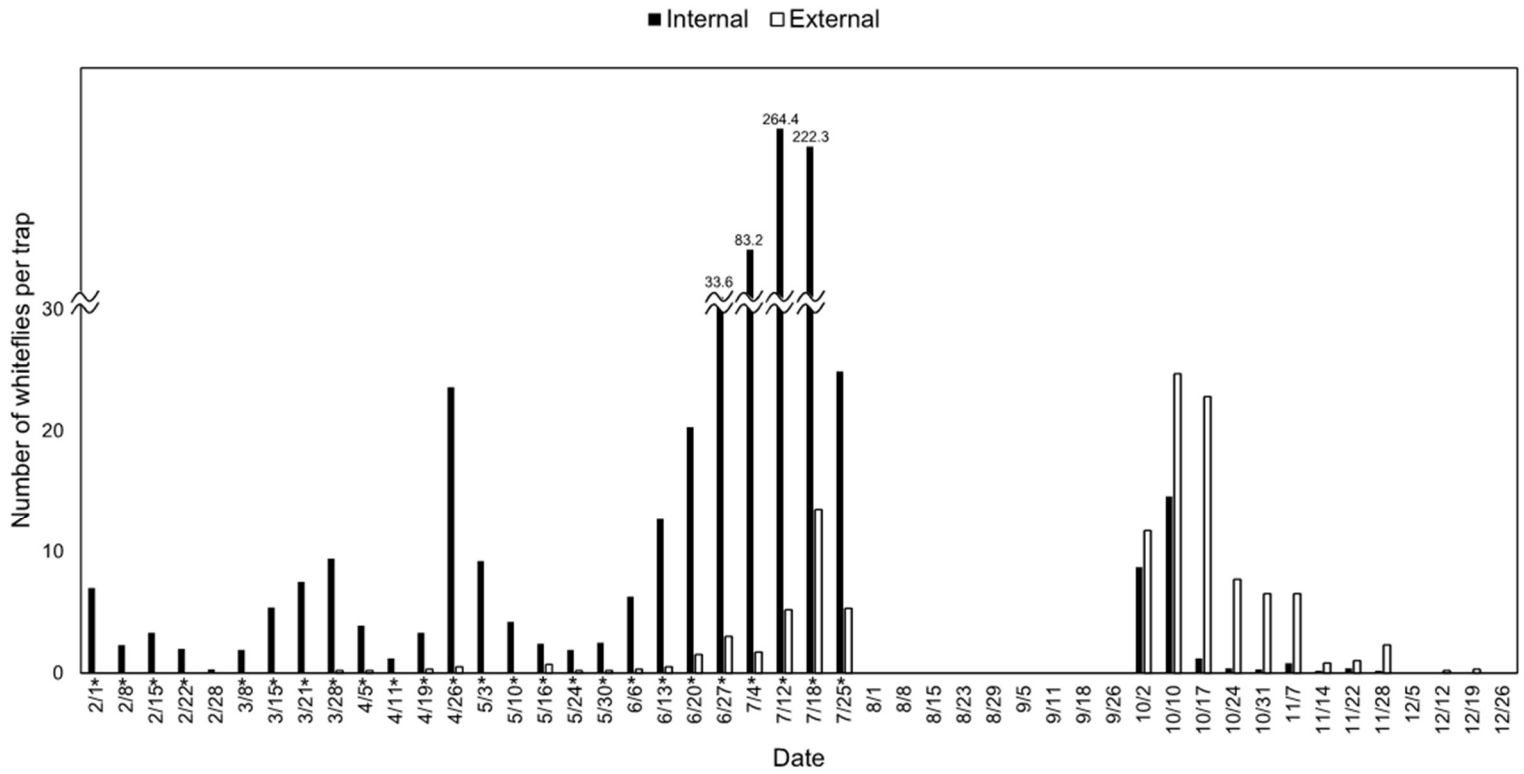
Greenhouse C-1 and C-2

Whitefly and thrips density, temperature and relative humidity in greenhouse C-1 are presented in Figs. 12 to 15. Whitefly and thrips density, temperature and relative humidity in greenhouse C-2 were presented in Figs. 16 to 19. Although greenhouse C-1 and C-2 were cultivated by the same farmer, internal whiteflies (*B. tabaci* - over 95% in both greenhouses) density was generally higher in the greenhouse C-1 during the whole season. The occurrence pattern of thrips (greenhouse C-1: *F. occidentalis* – 57.0%, *F. intonsa* – 28.4%, others – 5.5%; greenhouse C-2: *F. occidentalis* – 52.0%, *F. intonsa* – 30.9%, others – 7.7%) in both greenhouses was similar. The whiteflies and thrips began to disappear almost in November. Like in all other greenhouses, thrips density was higher outside the greenhouse than inside. And, whitefly density was lower outside the greenhouse than inside (whitefly of greenhouse C-1 - 2/1, $T_{11.00}$=3.55, P=0.0045; 2/8, $T_{11.00}$=3.65, P=0.0038; 2/15, $T_{11.00}$=2.69, P=0.0210; 2/22, $T_{11.00}$=2.83, P=0.0164; 2/28, $T_{11.00}$=1.91, P=0.0819; 3/8, $T_{10.00}$=2.75, P=0.0204; 3/15, $T_{11.00}$=2.93, P=0.0137; 3/21, $T_{11.00}$=3.35, P=0.0065; 3/28, $T_{11.19}$=5.17, P=0.0003; 4/5, $T_{11.47}$=3.28, P=0.0070; 4/11, $T_{11.00}$=3.19, P=0.0086; 4/19, $T_{14.51}$=3.65, P=0.0025; 4/26, $T_{11.04}$=4.39, P=0.0011; 5/3, $T_{11.00}$=4.06, P=0.0019; 5/10, $T_{11.00}$=4.75, P=0.0006; 5/16, $T_{13.55}$=2.82, P=0.0139; 5/24, $T_{13.82}$=3.76,

P=0.0022; 5/30, $T_{12.27}$=3.33, *P*=0.0058; 6/6, $T_{11.69}$=3.13, *P*=0.0089; 6/13, $T_{11.18}$=4.91, *P*=0.0004; 6/20, $T_{11.53}$=4.67, *P*=0.0006; 6/27, $T_{10.09}$=4.04, *P*=0.0023; 7/4, $T_{5.28}$=5.28, *P*=0.0003; 7/12, $T_{11.02}$=11.02, *P*=0.0002; 7/18, $T_{11.06}$=4.61, *P*=0.0007; 7/25, $T_{10.67}$=2.89, *P*=0.0151; 10/2, $T_{6.43}$=-0.72, *P*=0.4981; 10/10, $T_{6.98}$=-1.89, *P*=0.1005; 10/17, $T_{5.00}$=-1.11, *P*=0.3160; 10/24, $T_{5.03}$=-1.76, *P*=0.1383; 10/31, $T_{5.03}$=-1.78, *P*=0.1342; 11/7, $T_{5.06}$=-1.37, *P*=0.2269; 11/14, $T_{5.30}$=-1.00, *P*=0.3588; 11/22, $T_{5.57}$=-0.70, *P*=0.5148; 11/28, $T_{5.04}$=-1.23, *P*=0.2744; 12/5, T_{16}=0, *P*=0; 12/12, $T_{5.00}$=-1.00, *P*=0.3632; 12/19, $T_{5.00}$=-1.58, *P*=0.1747; 12/26, T_{16}=0, *P*=0; thrips of greenhouse C-1 - 2/1, T_{16}=0, *P*=0; 2/8, T_{16}=0, *P*=0; 2/15, T_{16}=0, *P*=0; 2/22, T_{16}=0, *P*=0; 2/28, T_{16}=0, *P*=0; 3/8, T_{16}=0, *P*=0; 3/15, T_{16}=0, *P*=0; 3/21, T_{16}=0, *P*=0; 3/28, T_{16}=0, *P*=0; 4/5, $T_{5.00}$=-1.00, *P*=0.3632; 4/11, $T_{11.37}$=-0.87, *P*=0.4013; 4/19, $T_{5.00}$=-2.31, *P*=0.0686; 4/26, $T_{5.24}$=-8.65, *P*=0.0003; 5/3, $T_{3.06}$=-1.63, *P*=0.2003; 5/10, $T_{5.32}$=-2.36, *P*=0.0617; 5/16, $T_{5.62}$=-6.22, *P*=0.0010; 5/24, $T_{5.07}$=-2.93, *P*=0.0321; 5/30, $T_{5.49}$=-4.78, *P*=0.0039; 6/6, $T_{5.01}$=-1.77, *P*=0.1368; 6/13, $T_{5.01}$=-2.65, *P*=0.0456; 6/20, $T_{5.08}$=-5.43, *P*=0.0027; 6/27, $T_{5.04}$=-3.42, *P*=0.0187; 7/4, $T_{5.06}$=-5.33, *P*=0.0030; 7/12, $T_{5.05}$=-2.54, *P*=0.0512; 7/18, $T_{5.38}$=-2.78, *P*=0.0361; 7/25, $T_{5.48}$=-3.92, *P*=0.0094; 10/2, $T_{5.24}$=-3.13, *P*=0.0242; 10/10, $T_{6.00}$=-4.20, *P*=0.0057; 10/17, $T_{5.00}$=-2.52, *P*=0.0531; 10/24, $T_{5.14}$=-3.59, *P*=0.0150; 10/31, $T_{5.13}$=-2.61, *P*=0.0466; 11/7, $T_{5.75}$=-6.54, *P*=0.0007; 11/14, T_{16}=0, *P*=0; 11/22, T_{16}=0,

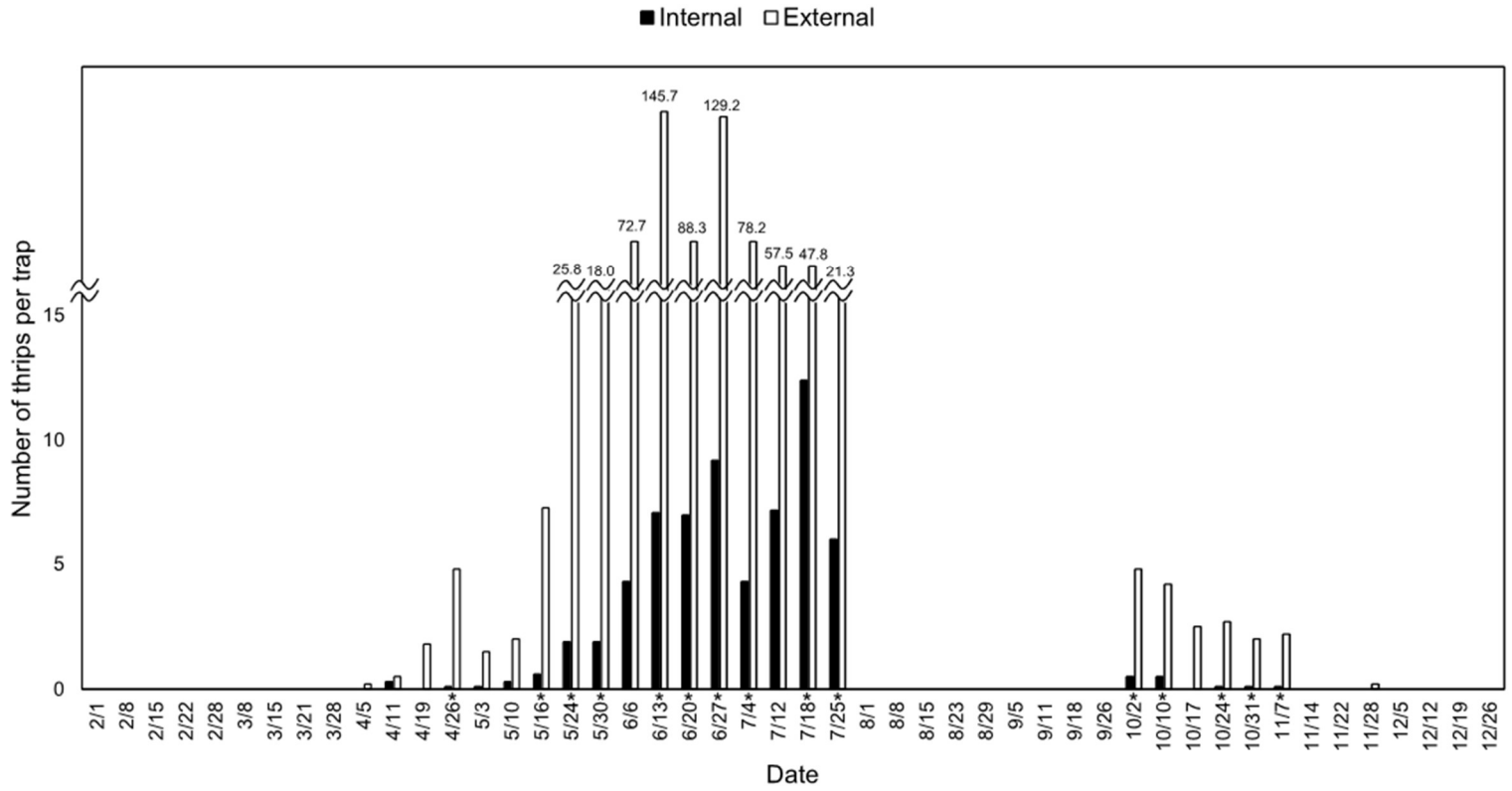
P=0; 11/28, $T_{5.00}$=-1.00, P=0.3632; 12/5, T_{16}=0, P=0; 12/12, T_{16}=0, P=0; 12/19, T_{15}=0, P=0; 12/26, T_{16}=0, P=0; whitefly of greenhouse C-2 - 2/1, $T_{5.00}$=1.00, P=0.3632; 2/8, T_{0}=0, P=0; 2/15, $T_{5.00}$=1.46, P=0.2031; 2/22, T_{10}=0, P=0; 2/28, T_{10}=0, P=0; 3/8, T_{9}=0, P=0; 3/15, T_{10}=0, P=0; 3/21, $T_{5.00}$=1.00, P=0.3632; 3/28, $T_{5.00}$=-1.00, P=0.3632; 4/5, T_{10}=0, P=0; 4/11, $T_{5.00}$=-1.00, P=0.3632; 4/19, $T_{5.00}$=1.00, P=0.3632; 4/26, T_{10}=0, P=0; 5/3, T_{10}=0, P=0; 5/10, T_{10}=0, P=0; 5/16, $T_{5.15}$=-1.38, P=0.2239; 5/24, T_{10}=0.00, P=1.0000; 5/30, $T_{5.00}$=-2.24, P=0.0756; 6/6, T_{10}=-0.54, P=0.5995; 6/13, T_{10}=1.86, P=0.0924; 6/20, T_{10}=-1.54, P=0.1556; 6/27, T_{10}=-3.08, P=0.0116; 7/4, T_{10}=0.78, P=0.4530; 7/12, T_{10}=0.89, P=0.3951; 7/18, T_{10}=2.82, P=0.0182; 7/25, T_{10}=1.21, P=0.2537; 10/2, $T_{6.23}$=3.82, P=0.0081; 10/10, T_{10}=2.40, P=0.0371; 10/17, $T_{5.60}$=-0.32, P=0.7604; 10/24, $T_{5.28}$=-1.27, P=0.2562; 10/31, $T_{5.36}$=-0.89, P=0.4131; 11/7, T_{10}=0.06, P=0.9557; 11/14, $T_{4.00}$=1.63, P=0.1778; 11/22, T_{10}=0.31, P=0.7650; 11/28, $T_{5.12}$=-0.96, P=0.3800; 12/5, T_{10}=0, P=0; 12/12, T_{10}=0, P=0; 12/19, $T_{5.00}$=2.24, P=0.0756; 12/26, $T_{4.00}$=1.50, P=0.2080; thrips of greenhouse C-2 - 2/1, T_{10}=0, P=0; 2/8, T_{10}=0, P=0; 2/15, T_{10}=0, P=0; 2/22, T_{10}=0, P=0; 2/28, T_{10}=0, P=0; 3/8, T_{10}=0, P=0; 3/15, T_{10}=0, P=0; 3/21, T_{10}=0, P=0; 3/28, $T_{6.03}$=-1.54, P=0.1753; 4/5, $T_{5.34}$=-1.80, P=0.1278; 4/11, $T_{5.11}$=-1.81, P=0.1291; 4/19, $T_{5.18}$=-2.40, P=0.0602; 4/26, $T_{5.56}$=-2.77, P=0.0352; 5/3, T_{10}=-1.94, P=0.0809; 5/10, T_{10}=-4.34, P=0.0015; 5/16, $T_{5.44}$=-5.74, P=0.0017; 5/24, $T_{5.01}$=-4.53,

P=0.0062; 5/30, $T_{5.13}$=-7.80, P=0.0005; 6/6, $T_{5.01}$=-3.12, P=0.0261; 6/13, $T_{5.01}$=-3.41, P=0.0190; 6/20, $T_{5.08}$=-2.17, P=0.0817; 6/27, $T_{5.01}$=-2.30, P=0.0697; 7/4, $T_{5.00}$=-1.84, P=0.1248; 7/12, $T_{5.02}$=-2.48, P=0.0557; 7/18, $T_{8.29}$=-0.39, P=0.7059; 7/25, T_{10}=-1.12, P=0.2873; 10/2, $T_{6.06}$=0.27, P=0.7967; 10/10, $T_{5.82}$=-2.15, P=0.0766; 10/17, $T_{5.00}$=-2.82, P=0.0372; 10/24, $T_{5.00}$=-6.64, P=0.0012; 10/31, T_{10}=-0.21, P=0.8347; 11/7, T_{10}=0.22, P=0.8284; 11/14, T_{9}=0, P=0; 11/22, T_{10}=0, P=0; 11/28, $T_{6.12}$=-0.96, P=0.3742; 12/5, T_{10}=0, P=0; 12/12, T_{10}=0, P=0; 12/19, T_{9}=0, P=0; 12/26, T_{9}=0, P=0).



* Statistically significant at $P<0.05$, *T*-test.

Figure 12. Internal and external density of whiteflies in the greenhouse C-1.



* Statistically significant at $P<0.05$, *T*-test.

Figure 13. Internal and external density of thrips in the greenhouse C-1.

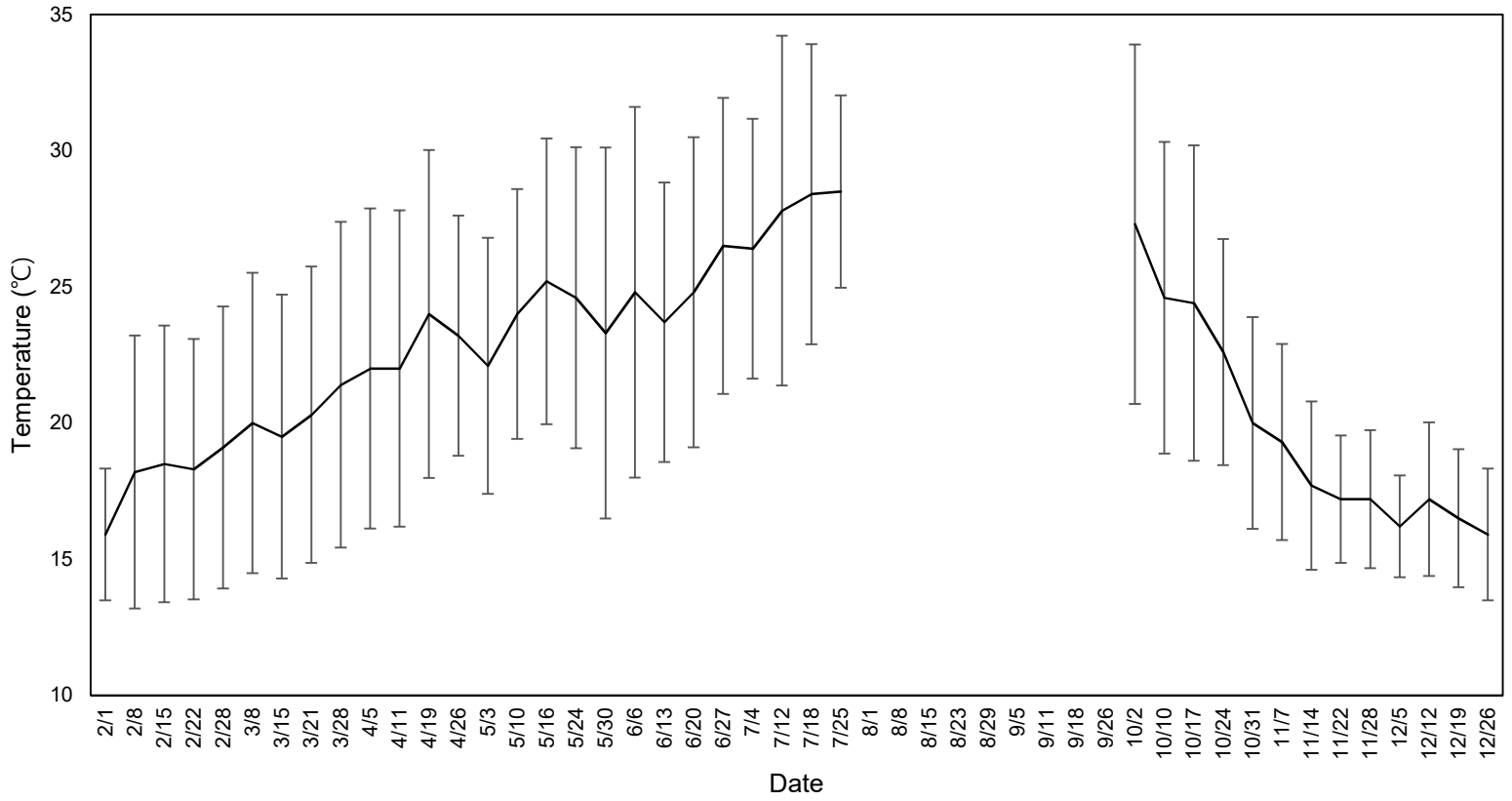


Figure 14. Internal temperature and standard deviation of the greenhouse C-1.

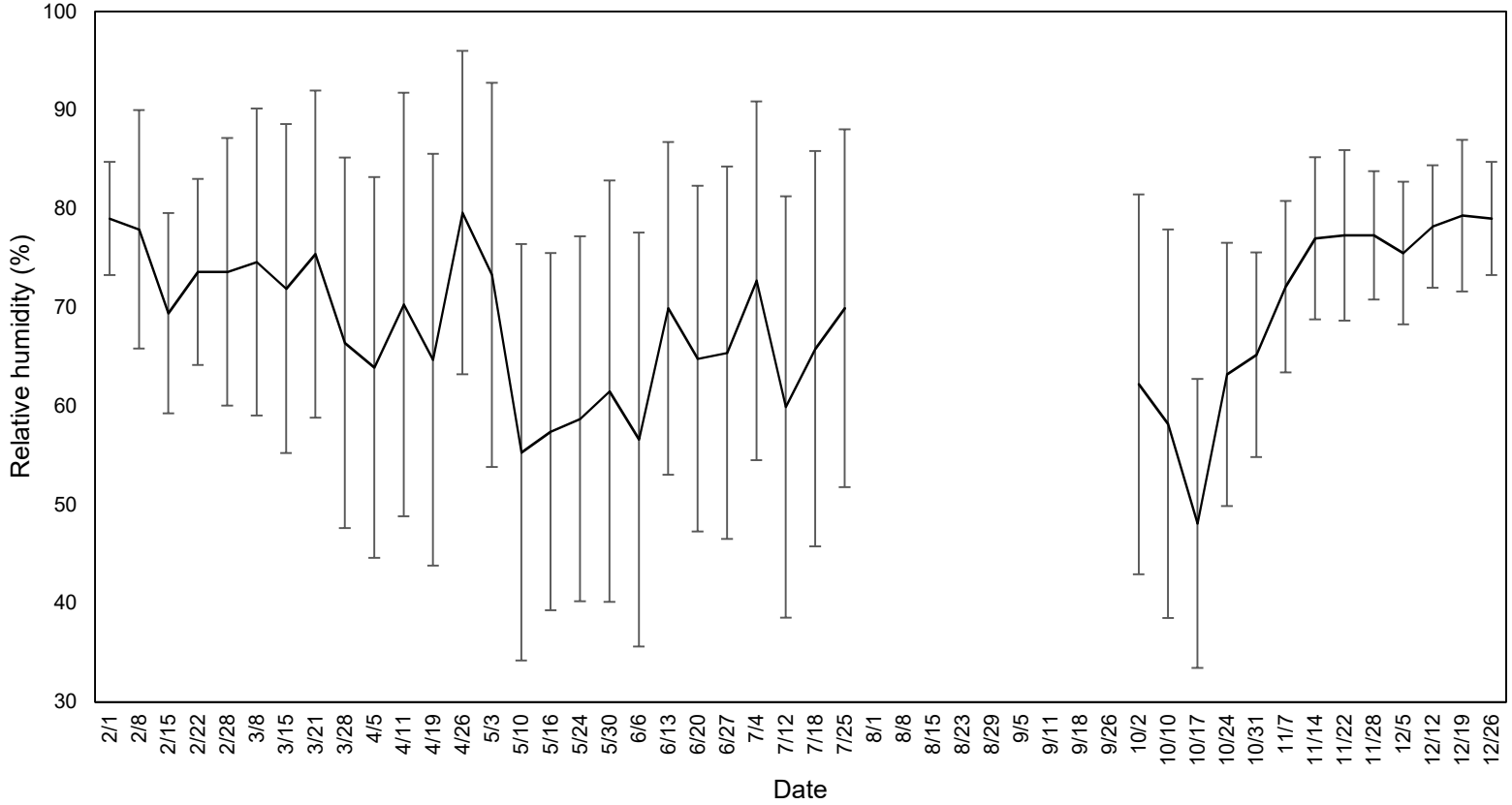
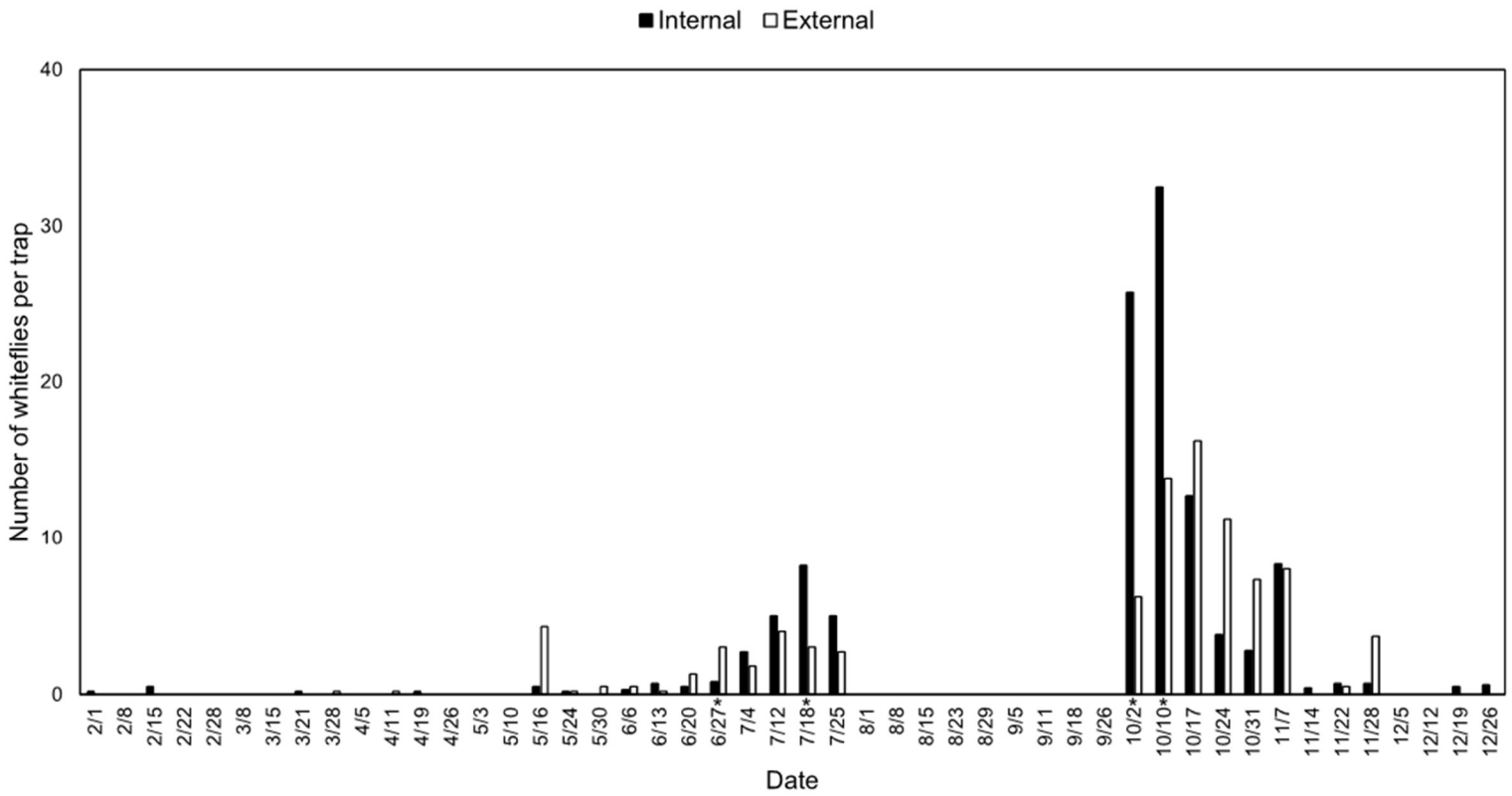
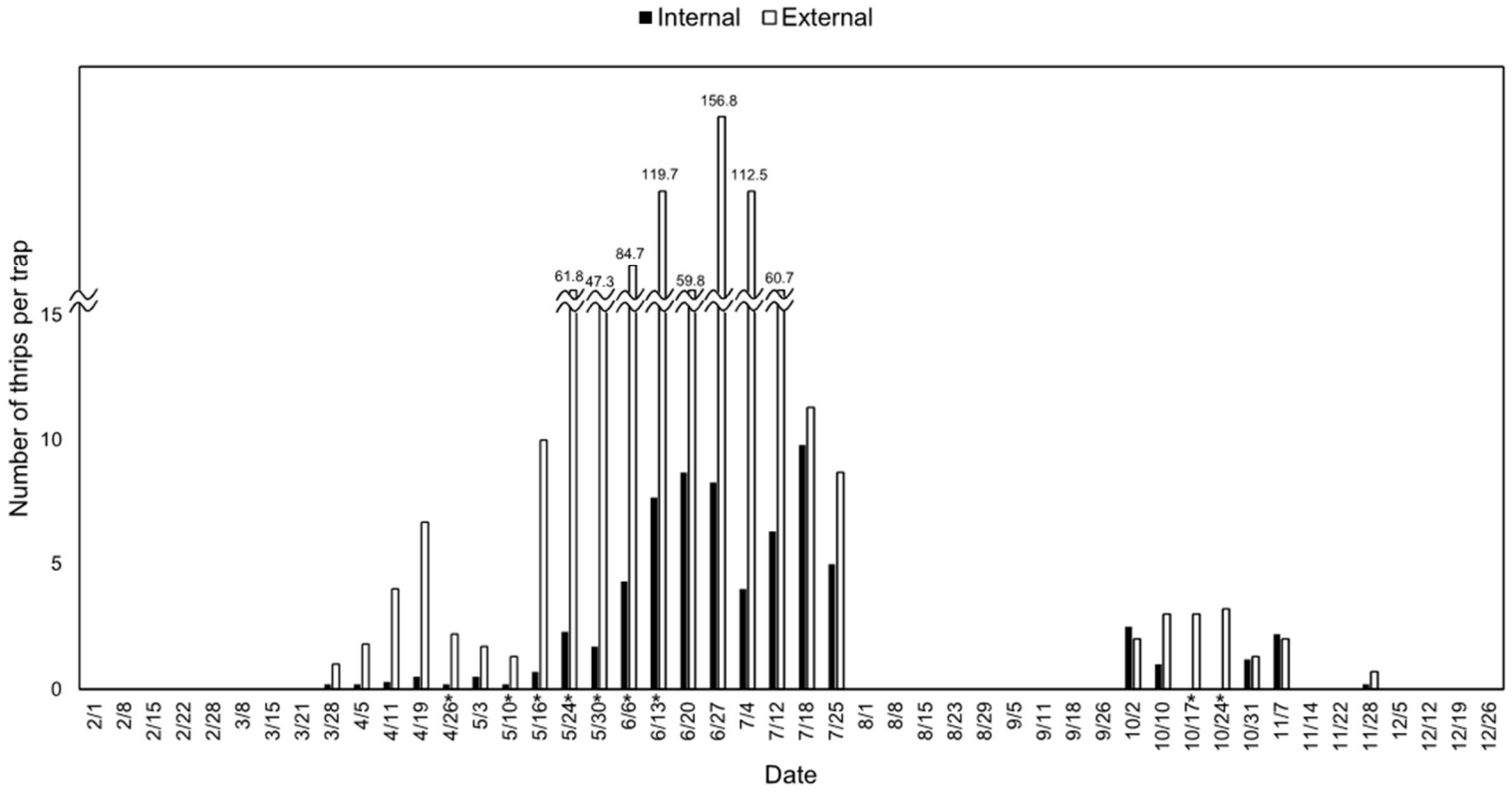


Figure 15. Internal relative humidity and standard deviation of the greenhouse C-1.



* Statistically significant at $P<0.05$, *T*-test.

Figure 16. Internal and external density of whiteflies in the greenhouse C-2.



* Statistically significant at $P<0.05$, *T*-test.

Figure 17. Internal and external density of thrips in the greenhouse C-2.

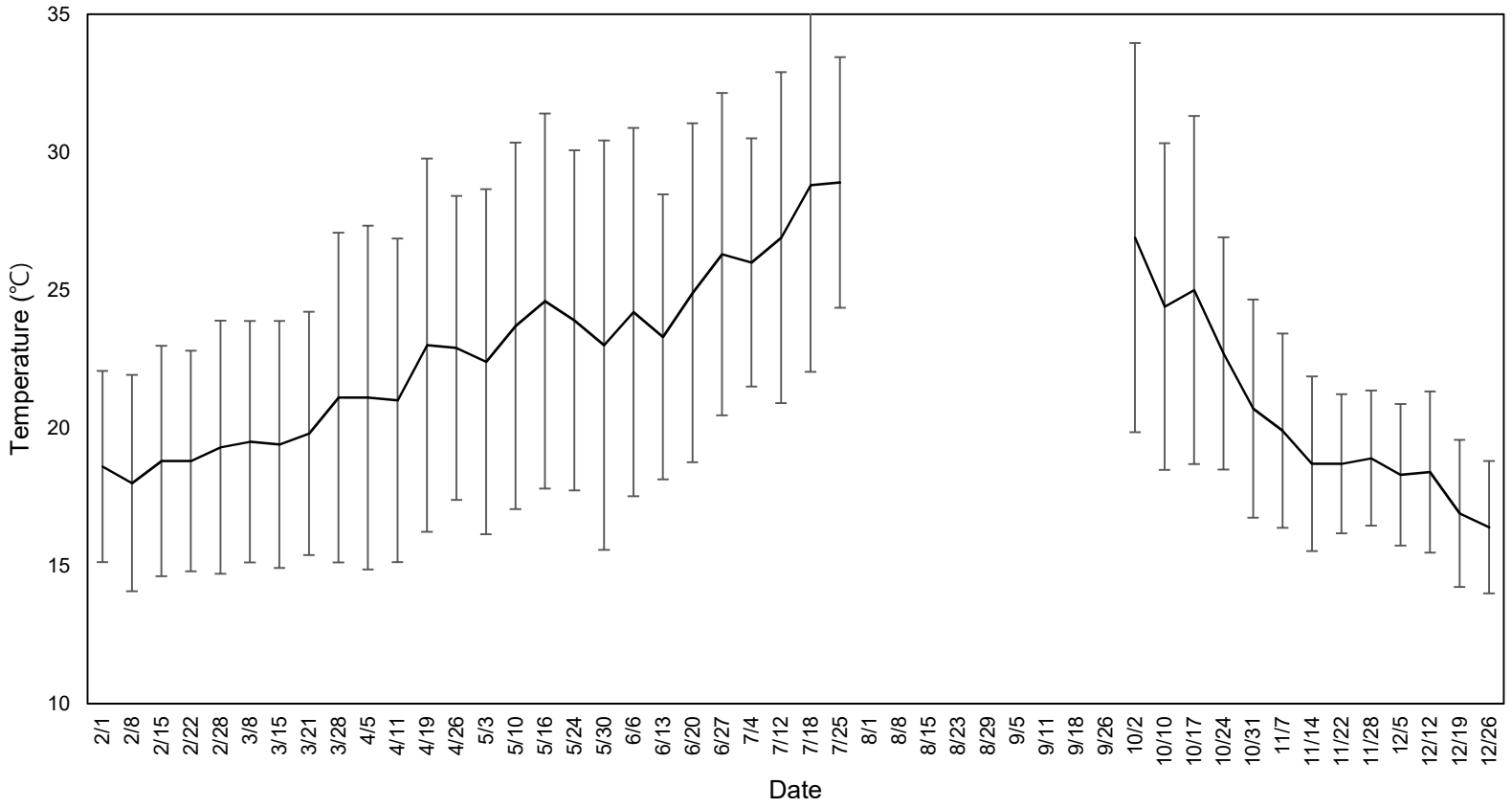


Figure 18. Internal temperature and standard deviation of the greenhouse C-2.

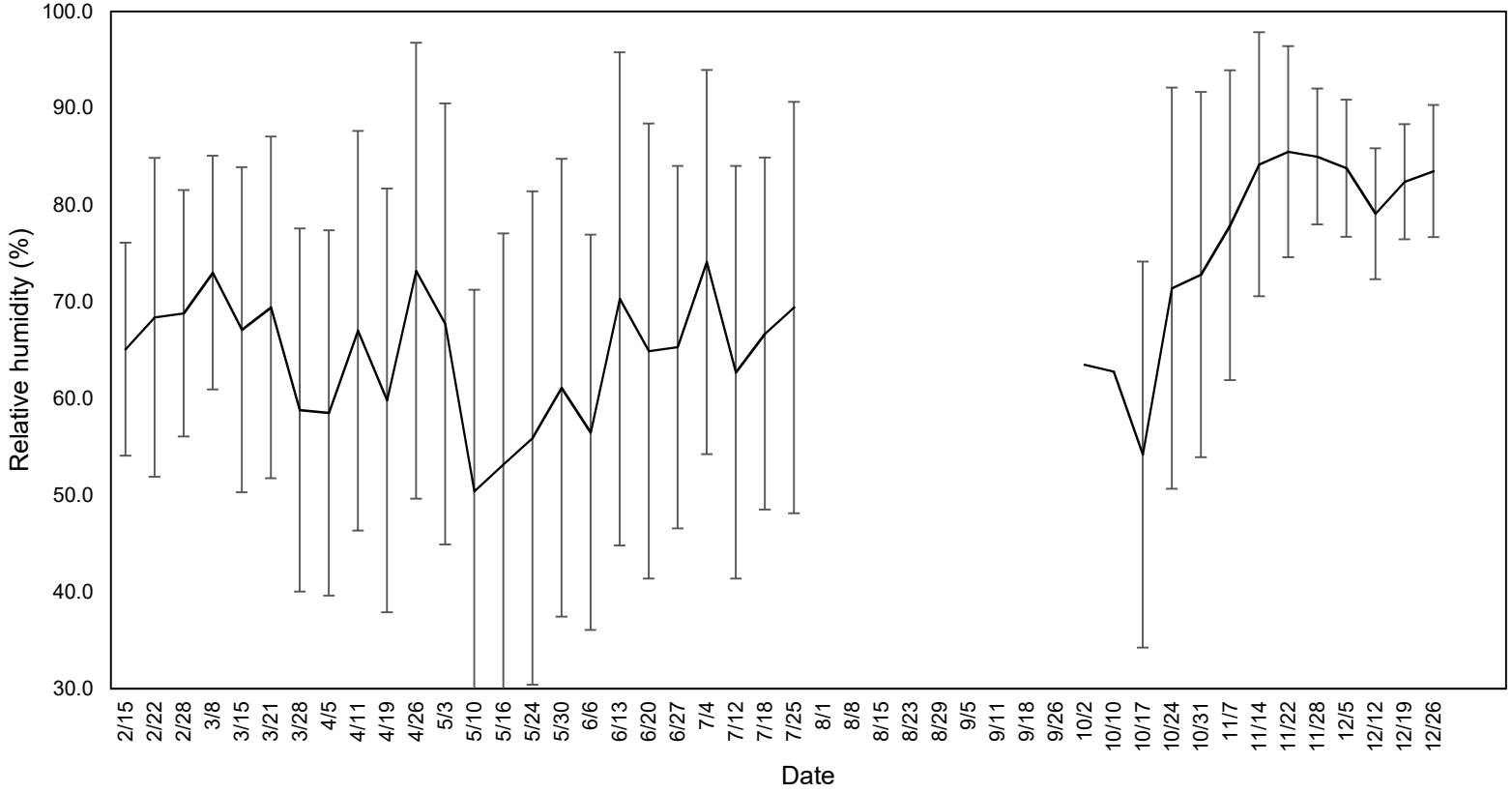


Figure 19. Internal relative humidity and standard deviation of the greenhouse C-2.

1.3.2. Correlation analysis

The results of correlation analysis are presented in Table 2. Internal whitefly density was highly correlated with external density in all greenhouses except for in the greenhouse C-1. Also, it was correlated with internal temperature except for in the greenhouse B. Internal thrips showed correlation coefficient of over 0.5 with external density. It was also correlated with internal temperature and variation of relative humidity in all investigated greenhouses.

Table 2. Correlation coefficient between pests and related variables.

Pest	Greenhouse	External density	Internal temperature	S.D. of internal temperature	Internal relative humidity	S.D. of internal R.H.
Whitefly	A	0.557**	0.421*	0.588***	-0.104	0.662***
	B	0.747***	0.269	0.025	0.270	0.223
	C-1	0.240	0.503**	0.246	-0.172	0.330*
	C-2	0.727***	0.412**	0.257	-0.162	0.138
	All	0.767***	0.155	0.044	0.095	0.079
Thrips	A	0.745***	0.568***	0.497**	-0.160	0.503**
	B	0.519***	0.372*	0.018	0.156	0.301*
	C-1	0.819***	0.659**	0.292	-0.247	0.421**
	C-2	0.720***	0.705***	0.378*	-0.175	0.357*
	All	0.567***	0.465***	0.199*	-0.044	0.306***

* significant at $P < 0.05$

** significant at $P < 0.01$

*** significant at $P < 0.001$

1.4. Discussion

For greenhouse pest management, it is very important to keep initial density of pest low or delay its occurrence as long as possible. Although general conditions inside the greenhouse are expected to be probably favorable to insect pests, it is important to find what environmental variables are closely related with the occurrence of specific insect pest species, and to find significant factors among them. Since insects are poikilotherms, temperature has a great influence on their population growth (Osborne, 1982; van Rijn et al.,1995; Wagner, 1995; Wang and Tsai, 1996; McDonald et al., 1998; Park et al., 2010; Park et al., 2011; Yadav and Chang, 2012). Therefore, temperature is commonly used to predict the population density of pests (Osborne, 1982; van Rijn et al.,1995; Wagner, 1995; Wang and Tsai, 1996; McDonald et al., 1998; Park et al., 2010; Park et al., 2011; Yadav and Chang, 2012). However, in greenhouse condition, temperature could not fully explain the population dynamics of pest species. In greenhouse conditions, not only temperature but also relative humidity or external inflow affected the population dynamics of pest species.

In addition to birth and death, insect population size is greatly affected by immigration and emigration. In most smart greenhouses in

Korea, including greenhouses investigated in this study, chemical control is frequently implemented to control pests (Park et al., 2020). In a situation that frequent control action is operated for pests, immigration could be more important than birth (Higgins, 1992; Pearsall and Myers, 2001; Gabarra et al., 2004; Riis and Nachman, 2006, Naranjo et al., 2009). It has been emphasized that prohibition of pest infestation is the first priority for greenhouse pest management (Antignus et al., 2001; Bailey et al., 2003; Teitel, 2007; Ben-Yakir et al., 2008; Martínez et al., 2014; Tosh and Brogan, 2015). In this study, I found a high correlation between the internal and external density of the two pests. Thus, suppression of external inflow of thrips and whitefly would be important for effective pest management in greenhouse.

In this study, densities of whitefly and thrips were significantly lower in the greenhouse A, in which ventilation was operated through the roof windows than other greenhouses, in which ventilation was operated through the roof windows and side windows. It appears that absence of side windows in the greenhouse A delayed the infestation of these insect pests, resulting in their lower occurrence during the season. In this study, the thrips population occurred first in the outside of greenhouses and was higher in the outside of the greenhouse than in the inside of the greenhouse. Thus,

main source of thrips seems to be outside of the greenhouse. The whitefly had the opposite trend. This seems to be due to the fact that thrips can overwinter in the fields (Cho et al., 1995). It was known that whitefly is possible to overwinter in weeds around the greenhouse, but they have very low survival rate (Lin et al., 2007). Therefore, the whitefly populations identified in the external traps in early spring were more likely to be those that escaped the greenhouse. Also, it appears that after the external whitefly population built up, they can be an additional source for the whitefly population in the greenhouse. In this study, whitefly density in the greenhouse was not, in general, related with greenhouse environmental conditions, but was related with its population in the outside of the greenhouse. Thus, it might be difficult to control whitefly population by environmental control through an automated system.

In correlation analysis, the internal density of thrips had a relationship to external density, internal temperature and humidity variation. Many studies have been conducted on temperature in thrips, but no studies have been conducted on variation of relative humidity (van Rijn et al., 1995; Park et al., 2010; Yadav and Chang, 2012; Ullah and Lim, 2015). Of course, frequent ventilation increases the variation in humidity, and it also would increase the inflow of external populations. Since the environment favorable

for plant growth is probably also favorable for the occurrence of pests, environmental control in greenhouses should be careful studied for pest control. For example, in smart greenhouses with precise environmental control system, control of humidity variation would be possible. If the effect of humidity variation on thrips population is identified by related study, the possibility of thrips management through humidity control could be explored.

In conclusion, *B. tabaci* was the dominant whitefly species, and *F. occidentalis* was the dominant thrips species. Occurrence of major pest in tomato greenhouse such as *F. occidentalis* and *B. tabaci* had a high correlation with their external density, and thus, it needs to reduce external inflow for pest management in greenhouse. For effective eco-friendly pest management in smart greenhouses, environmental control may not be working for whitefly while thrips may be the proper subject. For control of *B. tabaci,* the method to enhance biological control efficacy may be needed, instead.

Chapter II.

Cultural control method (Environmental control) – Thrips

Life history characteristics of the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), under fluctuating conditions of temperature or relative humidity

Abstract

Frankliniella occidentalis (Pergande) is a major insect pest of greenhouse crops such as leaf vegetables, flowers and vegetable fruits worldwide. The life history characteristics of *F. occidentalis* were investigated at control temperature and humidity (27.3 ± 0.54 °C, 79.9 ± 2.79% RH) (mean ± SD), a 10 °C-range fluctuation in temperature (27.1 ± 5.28 °C, 81.5 ± 4.03% RH), a 20 °C-range fluctuation in temperature (26.5 ± 10.09 °C, 80.4 ± 5.76% RH), a 20%-range fluctuation in humidity (26.8 ± 0.37 °C, 80.7 ± 9.55% RH) and a 30%-range fluctuation in humidity (27.3 ± 0.41 °C, 76.3 ± 15.28% RH). Overall, the life history traits of *F. occidentalis* were more negatively affected by fluctuating environmental conditions. The impact of temperature fluctuation was more severe than that of humidity fluctuation. Additionally, the degree of impact increased as the fluctuation range of the temperature increased, while the reverse trend was observed with humidity fluctuations. With the 20 °C-range fluctuation in temperature, *F. occidentalis* died at the 1st instar larval stage. The offspring's sex ratio was significantly higher at the 20%- and 30%-range fluctuations in humidity (0.47 and 0.49, respectively) compared to the control (0.35) and at the 10

°C-range fluctuation in temperature (0.33). From the fertility life table analysis, the intrinsic rate of increase (*r*) was higher at the 30%-range fluctuation in humidity and control conditions as 0.218 and 0.205, respectively. At the 10 °C-range fluctuation in temperature conditions, *r* was significantly lower as 0.169. High fluctuations in temperature and low fluctuations in humidity appear to be the best conditions for controlling *F. occidentalis* populations in greenhouses.

2.1. Introduction

Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae) originates from North America, began spreading worldwide with the increasing international exchange of horticultural products in the late 1970s, and has become a major greenhouse pest worldwide (Kirk and Terry, 2003). In Korea, *F. occidentalis* was first found in 1993 on Jeju Island, a far southern island, and spread throughout the country within a few years (Han et al., 1998; Lee et al., 2001). *F. occidentalis* has host plants from over 13 families and is the vector of tomato spotted wilt virus and maize chlorotic mottle virus (Sakimura, 1962; Yudin et al., 1986; Kirk, 2002; Zhao et al., 2014; Szostek et al., 2017). *F. occidentalis* feeds by sapping leaves and flowers, resulting in decreased photosynthesis and malformed fruit loading (Kirk, 2002). *F. occidentalis* is very difficult to control because it has a high reproductive rate and develops insecticide resistance rapidly (Jensen, 2000). In addition, *F. occidentalis* is haplodiploid, in which fertilized eggs become females and unfertilized eggs become males, and has rather variable sex ratios (Higgins and Myers, 1992; Terry and Kelly, 1993; Ding et al., 2018).

The population dynamics of *F. occidentalis* are significantly affected

by temperature and humidity (Shipp and Gillespie, 1993). Many studies regarding life history characteristics of *F. occidentalis* have focused on the effects of constant and/or fluctuating temperature conditions (Gaum et al., 1994; van Rijin et al., 1995; McDonald et al., 1998; Hulshof et al., 2003; Kumm and Moritz, 2010; Nielsen et al., 2010; Wang et al., 2014; Ullah and Lim, 2015; Cao et al., 2019) because thermal condition is probably the major influential environmental factor for insects. However, determination of the effect of humidity would contribute to understanding of the life history characteristics of *F. occidentalis*, which would allow us to develop a better management strategy for *F. occidentalis*. Humidity can affect the development and survival of *F. occidentalis*. For example, *F. occidentalis* needs at least 80% relative humidity (RH) for successful pupation, and the optimal condition is 90% RH (Steiner et al., 2011). Larvae of *F. occidentalis* showed low survival below 80% RH, and adults suffered at low RH (Shipp and Gillespie, 1993). However, these studies were conducted at constant RH, and further studies are needed to examine the effects of fluctuating humidity conditions.

Greenhouse crop cultivation systems and techniques have become sophisticated as agricultural technology develops (Baek et al., 2013). For example, the automatic environmental control system has become essential for greenhouse farming. It enables fluctuating environmental conditions,

such as temperature and relative humidity, in greenhouses to be controlled effectively and automatically through a system setting. The primary purpose of this system is to maintain the health of greenhouse crops. However, crop growth under favorable conditions may also endow insect pests such as *F. occidentalis* to flourish. The objective of this study was to investigate effects of fluctuating temperature or relative humidity on the life history characteristics of *F. occidentalis.* This study would help us understand what extent fluctuating temperature or humidity conditions could affect the population dynamics of *F. occidentalis* and would provide basic information for proper environmental conditions for control of *F. occidentalis* in greenhouses.

2.2. Materials and Methods

2.2.1. Rearing of *F. occidentalis*

Frankliniella occidentalis was obtained from Gyeonggi-do Agricultural Research and Extension Service in Hwaseong-si, Korea. The insects were reared in petri dishes (100 diameter × 42 mm height) with a nylon-mesh-covered hole (40 mm diameter) in the lid (SPL Life Sciences, Pocheon-si, Korea) in an incubator at 27.0 ± 2.3 °C and 80.4 ± 8.4% RH (mean ± SD) with a photoperiod of 14:10 (L:D) h. A wet filter paper was laid on the bottom of the petri dish as a water supply, and cotyledons of kidney beans were supplied for food and oviposition.

2.2.2. Life table experiments

To obtain eggs from *F. occidentalis*, 100 female adults were randomly collected from the rearing colony and then evenly divided into four petri dishes (100 mm diameter × 42 mm height) with a nylon-mesh-covered hole (40 mm diameter) in the lid. This lid would allow ventilation. A water saturated cotton pad and kidney bean leaf disc (7 cm diameter) were placed in the petri dishes. These petri dishes were placed in an incubator at 27.0 ± 2.3 °C and 80.4 ± 8.4% RH (mean ± SD) with a photoperiod of 14:10 (L:D) h. *F. occidentalis* were allowed to lay eggs for 12 h and were then removed from the petri dishes. Then, each petri dish was placed randomly into incubators, and one of four environmental conditions was applied to each incubator. The baseline condition (control) was set at 27 °C and 80% RH because the optimal temperature and humidity for *F. occidentalis* appear to be ca. 27 °C (van Rijin et al., 1995) and over 80% RH (Steiner et al., 2011), respectively. The range settings were 22 ~ 32 °C (12:12 h), 80% RH; 17 ~ 37 °C (12:12 h), 80% RH; 27 °C, 70 ~ 90% RH (12:12 h), and 27 °C, 65 ~ 95% RH (12:12 h). The photoperiod condition for all experiments was 14:10 (L:D) h. The environmental conditions of each incubator were recorded by the data logger (HOBO, OnSet Computer, Pocasset, MA, USA) at 10 minute

intervals during the whole experimental period. The actual measured conditions are presented in Table 3, and these actual measured conditions were used as treatments.

Petri dishes were checked daily until the eggs hatched, and sixty newly hatched larvae were randomly selected and transferred individually onto 35 mm kidney bean leaf discs in sixty petri dishes (50 mm diameter × 15 mm height) with a nylon-mesh-covered hole (13.2 mm diameter) in the lid (i.e., one larva per petri dish). Since eggs cannot be counted because they were buried into plant tissues, egg mortality was not estimated. The egg developmental period was estimated as the time between when leaves with newly laid eggs were placed in incubators and larvae enclosed. Development of immature stages was checked every day. Larval instars, prepupa and pupa were determined according to methods of van Rijn et al. (1995) and Ullah and Lim (2015). Additionally, data for missing individuals, which cannot be identified for death or survival, during the experiment were excluded in the analysis. Newly emerged female and male adults from each treatment were paired and placed onto 35 mm kidney bean leaf discs in petri dishes (50 mm diameter × 15 mm height) with a nylon-mesh-covered hole (13.2 mm diameter) in the lid for oviposition in the same treatment, and these petri dishes with kidney bean leaf discs were replaced daily. The petri dishes were checked daily to count the hatched larvae. Then, by summing

the number of all the hatched larvae, fecundity was estimated. In addition, all the hatched larvae in the fecundity test for each treatment were placed onto kidney bean leaf discs in petri dishes (100 mm diameter x 42 mm height) with a nylon-mesh-covered hole (40 mm diameter) in the lid and were observed until they became adults to measure the sex ratio and survival rate of offspring. The developmental period, death, longevity, and fecundity were recorded for each individual.

Table 3. Experimental conditions (mean ± S.D.) in the life table experiments for *F. occidentalis* on kidney bean leaves under different environmental conditions.

Condition	Temperature (°C) (setting range)	Relative humidity (%) (setting range)
Control	27.3 ± 0.54 (27 °C)	79.9 ± 2.79 (80%)
10 °C-range fluctuation	27.1 ± 5.28 (22 – 32 °C)	81.5 ± 4.03 (80%)
20 °C-range fluctuation	26.5 ± 10.09 (17 – 37 °C)	80.4 ± 5.76 (80%)
20%-range fluctuation	26.8 ± 0.37 (27 °C)	80.7 ± 9.55 (70 – 90%)
30%-range fluctuation	27.3 ± 0.41 (27 °C)	76.3 ± 15.28 (65 – 95%)

2.2.3. Statistical analysis

By using PROC GLM in SAS (SAS Institute, 2013), the effect of fluctuating environmental conditions on the developmental period of immature stages, adult longevity, and fecundity was analyzed. For mean separation, Tukey's studentized range test was conducted. To analyze the effect of environmental conditions on survivorship and the sex ratio, a chi-square test was conducted by using the R program (R Core Team, 2019).

2.2.4. Life table analysis

For estimating life table parameters, two types of life table analysis were used: the fertility life table (Maia et al., 2000, 2014) and the age-stage, two-sex life table (Chi and Liu, 1985; Chi, 1988). The following population parameters, including the intrinsic rate of increase (*r*), finite rate of increase (*λ*), net reproductive rate (R_0) and mean generation time (*T*), were calculated.

The intrinsic rate of increase (*r*)

$$\sum_{x=0}^{\infty} e^{-rx} l_x m_x = 1$$

The finite rate of increase (*λ*)

$$\lambda = e^r$$

The net reproductive rate (R_0)

$$R_0 = \sum_{x=0}^{\infty} l_x m_x$$

The mean generation time (*T*)

$$T = \ln R_0 / r$$

The fertility life table analysis

The fertility life table analysis and jackknife estimation were conducted by using the R program (R Core Team, 2019) of Maia et al. (2014), which requires data for the parent and offspring: the number, longevity and daily fecundity of female adults from the parent, as well as the development period, immature stage survivorship and sex ratio of the offspring. In particular, the sex ratio of the offspring is used so that treatment effect on the sex ratio can be considered. To calculate age-specific survival rate (l_x) and fecundity (m_x), the following equations were used.

$$l_x = SURV \times \frac{NSF_x}{NF}$$

The *SURV* is survival rate of offspring to adult, *NSF*x is the number of surviving females at time x, and *NF* is the initial number of females at each test.

$$m_x = NEGG_x \times SR$$

The $NEGG_x$ is the total number of eggs laid at each pivotal age (x), and the *SR* is the sex ratio of offspring. More details for this life table analysis and jackknife estimation can be found in Maia et al. (2000, 2014).

The age-stage, two-sex life table analysis

The age-stage, two-sex life table analysis and bootstrap estimation were conducted by using TWOSEX MSChart (Chi, 2018), which requires data for the parent only: development period, survival rate, longevity of male and female adults, daily fecundity of female adults and sex ratio of the parent. To calculate age-specific survival rate (l_x) and fecundity (m_x), the age-stage specific survival rate (S_{xj}, j = stage) and fecundity (f_{xf}, f = female adult stage) were applied.

$$l_x = \sum_{j=1}^{n} S_{xj} \qquad m_x = \frac{\sum_{j=1}^{k} S_{xj} f_{xj}}{\sum_{j=1}^{k} S_{xj}}$$

where x is age and *k* is the number of stages. More details for this life table analysis and bootstrap estimation can be found in Chi and Liu (1985), Chi (1988) and Smucker et al. (2007).

2.3. Results

Overall, environmental fluctuation conditions significantly affected the biological traits of *F. occidentalis.* However, the effects of fluctuations in temperature and humidity appear to be different and differed according to the developmental stages of *F. occidentalis* (Egg, $F_{4,277}$=494.5, $P < 0.0001$; 1st larva, $F_{3,195}$=14.5, $P < 0.0001$; 2nd larva, $F_{3,184}$=6.2, P=0.0005; Prepupa, $F_{3,183}$=9.9, $P < 0.0001$; Pupa, $F_{3,181}$=2.0, P=0.1226; Female, egg to adult, $F_{3,74}$=10.1, $P < 0.0001$; Male, egg to adult, $F_{3,103}$=13.1, $P < 0.0001$; Preoviposition period, $F_{3,72}$=0.1, P=0.9336; Oviposition period, $F_{3,72}$=2.8, P=0.0470; Postoviposition period, $F_{3,72}$=1.6, P=0.1941; Daily fecundity, $F_{3,72}$=4.0, P=0.0103; Total fecundity, $F_{3,74}$=4.9, P=0.0035; Female adult longevity, $F_{3,74}$=3.0, P=0.0367; Male adult longevity, $F_{3,103}$=0.6, P=0.6183; Parent group's survivorship, χ^2_4=146.6, $P < 0.0001$; Offspring group's survivorship of immatures, χ^2_3=51.3, $P < 0.0001$; Parent group's sex ratio, χ^2_3=1.3258, P=0.7230; Offspring group's sex ratio, χ^2_3=50.5, $P < 0.0001$).

2.3.1. Immature development

The effects on eggs were not significantly different among conditions except for the 20 °C-range fluctuation in temperature in which eggs required 1 more day for hatching. Larval stages appeared to be vulnerable to fluctuating conditions, particularly temperature fluctuation. A very high fluctuation in temperature (20 °C range) was so fatal that any first instar larvae failed to develop to the next instar (Table 4).

Table 4. Development period (mean ± S.E.) of *F. occidentalis* on kidney bean leaves under different environmental conditions.

Condition	Egg (n)	1st larva (n)	2nd larva (n)	Prepupa (n)	Pupa (n)	Egg to adult	
						Female (n)	Male (n)
Control	3.0 ± 0.00b* (58)	1.4 ± 0.07b (52)	2.0 ± 0.09b (52)	0.9 ± 0.05a (51)	1.9 ± 0.04a (51)	9.3 ± 0.10b (23)	9.3 ± 0.12b (28)
10 °C-range fluctuation	3.0 ± 0.00b (57)	1.6 ± 0.10b (48)	2.5 ± 0.11a (45)	1.0 ± 0.00a (45)	2.0 ± 0.03a (45)	10.0 ± 0.21a (18)	10.2 ± 0.21a (27)
20 °C-range fluctuation	4.0 ± 0.04a (57)	-	-	-	-	-	-
20%-range fluctuation	3.0 ± 0.00b (55)	2.1 ± 0.06a (45)	2.0 ± 0.12b (40)	1.0 ± 0.04a (40)	2.1 ± 0.03a (40)	10.1 ± 0.15a (19)	10.1 ± 0.22a (21)
30%-range fluctuation	3.0 ± 0.00b (55)	1.5 ± 0.09b (54)	2.0 ± 0.09b (51)	0.7 ± 0.07b (51)	1.9 ± 0.03a (49)	9.2 ± 0.10b (18)	9.1 ± 0.08b (31)

*Means followed by the same letter within a column are not significantly different at α=0.05, Tukey's studentized range test.

2.3.2. Adult data

The total fecundity was similar between the 30%-range fluctuation in humidity (56.4 larvae) and control conditions (53.6 larvae), while a similar significantly lower fecundity was observed in the 10 °C-range fluctuation in temperature and 20%-range fluctuation in humidity conditions (36.7 and 35.1 larvae, respectively) (Table 5).

Table 5. Preoviposition, oviposition, postoviposition period, daily fecundity, total fecundity and adult longevity (mean ± S.E.) of *F. occidentalis* on kidney bean leaves under different environmental conditions.

Condition	Preoviposition period (n)	Oviposition period (n)	Postoviposition period (n)	Daily fecundity per female (n)	Total fecundity per female (n)	Adult longevity	
						Female (n)	Male (n)
Control	0.3 ± 0.12a* (23)	9.3 ± 0.72ab (23)	2.2 ± 0.32a (23)	7.7 ± 0.56ab (23)	53.6 ± 3.94ab (23)	11.8 ± 0.83ab (23)	11.5 ± 0.80a (28)
10 °C-range fluctuation	0.3 ± 0.11a (17)**	9.2 ± 1.00ab (17)	1.9 ± 0.32a (17)	5.5 ± 0.53ab (17)	36.7 ± 4.09bc (18)	11.1 ± 1.00ab (18)	10.1 ± 1.13a (27)
20%-range fluctuation	0.3 ± 0.14a (18)	7.2 ± 0.99b (18)	1.6 ± 0.23a (18)	5.3 ± 0.72b (18)	35.1 ± 5.12c (19)	8.8 ± 0.94b (19)	10.1 ± 1.05a (21)
30%-range fluctuation	0.4 ± 0.14a (18)	11.3 ± 1.20a (18)	1.4 ± 0.26a (18)	8.1 ± 0.95a (18)	56.4 ± 6.67a (18)	13.1 ± 1.28a (18)	9.8 ± 1.06a (31)

*Means followed by the same letter within a column are not significantly different at α=0.05, Tukey's studentized range test.

**No fecund females were excluded for calculating preoviposition, oviposition, postoviposition period and daily fecundity.

2.3.3. Survivorship and Sex ratio

The survivorship of juveniles of the parent group was similar between the control (0.88) and 30%-range fluctuation in humidity (0.89) (Table 6). For the offspring, the 10 °C-range fluctuation in temperature and 30%-range fluctuation in humidity showed similar survivorship of juveniles (0.84 and 0.83, respectively), while the 20%-range fluctuation in humidity caused lower survivorship (0.70) (Table 6). The offspring's sex ratio was significantly higher at the 20% and 30%-range fluctuations in humidity (0.47 and 0.49, respectively) than the control (0.35) and the 10 °C-range fluctuation in temperature (0.33) (Table 6). In contrast, the sex ratio was not different among treatments in the parent group.

Table 6. Survival rate of the immature stage and sex ratio in the parent and offspring groups of *F. occidentalis* on kidney bean leaves under different environmental conditions.

Condition	Survival rate of the immature stage		Sex ratio	
	Parent	Offspring	Parent	Offspring
Control	0.88ab* (51/58)**	0.79b (970/1232)	0.45a (23/51)***	0.35b (341/970)
10 °C-range fluctuation	0.79ab (45/57)	0.84a (554/660)	0.40a (18/45)	0.33b (185/554)
20 °C-range fluctuation	0c (0/57)	-	-	-
20%-range fluctuation	0.73b (40/55)	0.70c (475/676)	0.48a (19/40)	0.49a (231/475)
30%-range fluctuation	0.89a (49/55)	0.83a (835/1005)	0.37a (18/49)	0.47a (392/835)

*Means followed by the same letter within a column are not significantly different at α=0.05, Bonferroni correction after chi-square test.

**(survived number / initial number)

***(female number / total adult number)

2.3.4. Life table

The life history traits, age-specific development and fecundity of *F. occidentalis* estimated by the fertility life table and the age-stage, two-sex life table analyses are presented in Table 7, Figs. 20 and 21. In general, the effects of fluctuating environmental conditions on the life history parameters of *F. occidentalis* showed a similar pattern between the two analyses, although their respective estimates were somewhat different. Values of life history parameters such *as r,* λ and R_0 were higher under control and 30%-range fluctuation in humidity conditions than those under other conditions in both analyses. However, they were highest at the control condition in the age-stage, two-sex life table analysis, while they were highest at the 30%-range fluctuation in humidity condition in the fertility life table analysis. Overall, the life history traits of *F. occidentalis* were apparently more negatively affected under fluctuating environmental conditions, and the impact of temperature fluctuation was more severe than that of humidity fluctuation. Additionally, the degree of impact increased as the fluctuation range of temperature increased, while the reverse trend was observed under humidity conditions.

Table 7. Estimates (mean ± S.E.) of population parameters of *F. occidentalis* on kidney different environmental conditions.

Life table	Condition	*r*	*λ*	R_0
Fertility	Control	0.205 ± 0.0048ab*	1.228 ± 0.0059ab	14.826 ± 1.091
	10 ◦C-range fluctuation	0.169 ± 0.0080c	1.185 ± 0.0094c	10.278 ± 1.145
	20%-range fluctuation	0.184 ± 0.0102bc	1.202 ± 0.0122bc	11.996 ± 1.747
	30%-range fluctuation	0.218 ± 0.0078a	1.243 ± 0.0097a	22.016 ± 2.600
Age-stage, two-sex	Control	0.234 ± 0.0143a**	1.263 ± 0.0180a	21.241 ± 3.776
	10 ◦C-range fluctuation	0.178 ± 0.0175b	1.195 ± 0.0208b	11.579 ± 2.579
	20%-range fluctuation	0.185 ± 0.0182b	1.203 ± 0.0218b	12.109 ± 2.837
	30%-range fluctuation	0.204 ± 0.0171ab	1.227 ± 0.0209ab	18.473 ± 4.165

*Means followed by the same letter within a column in each life table are not significantly different at α=0. range test after jackknife estimates.

**Means followed by the same letter within a column in each life table are not significantly different at α=0.0 (B = 100,000).

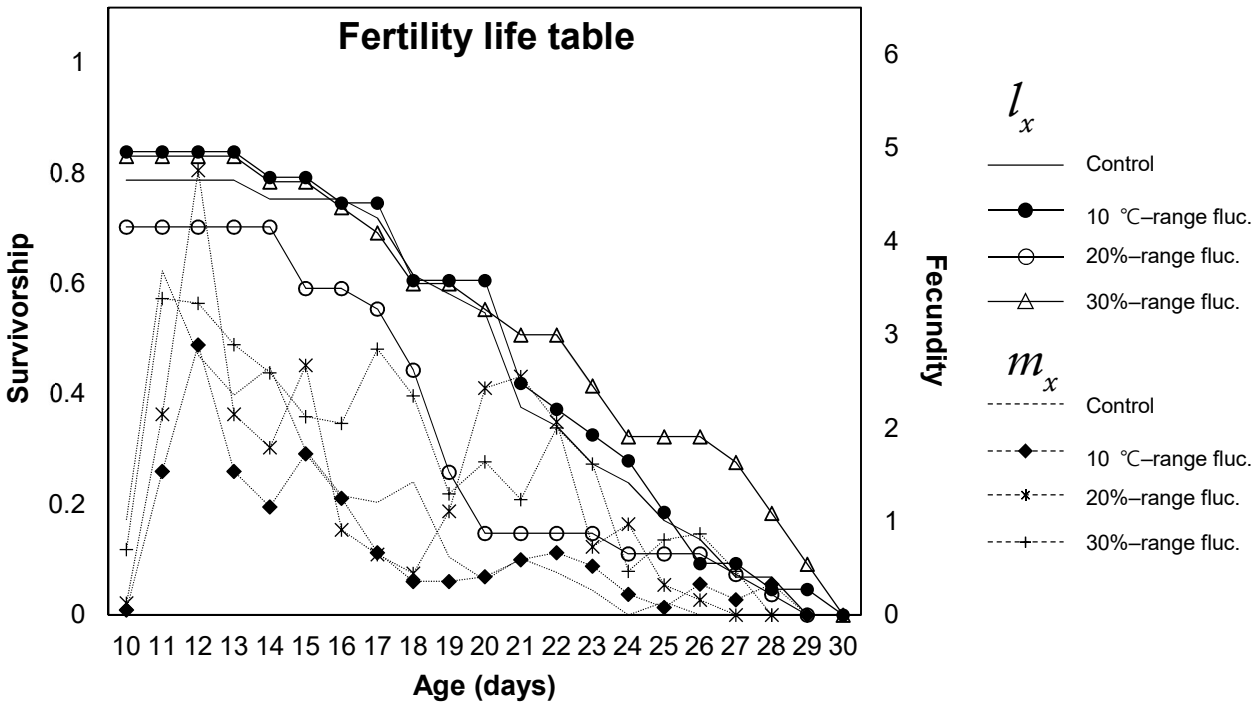


Figure 20. Age-specific survival rate and fecundity of *F. occidentalis* by fertility life table analysis.

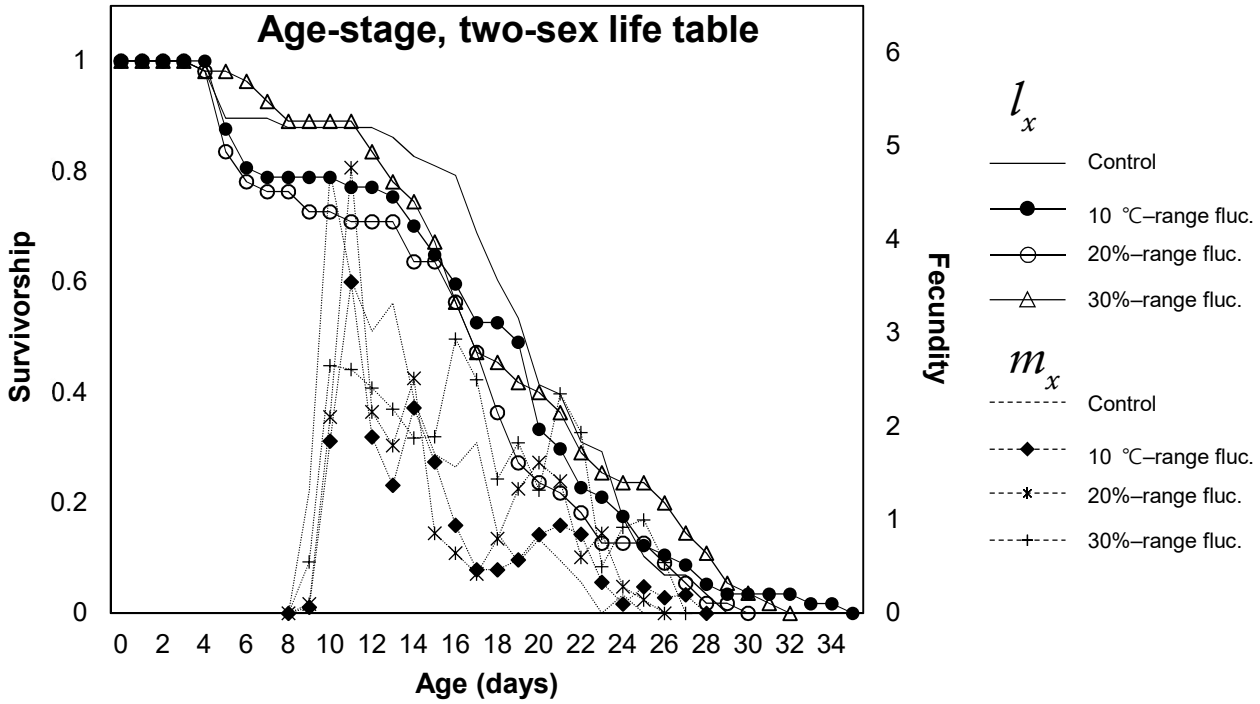


Figure 21. Age-specific survival rate and fecundity of *F. occidentalis* by age-stage, two-sex life table analysis.

2.4. Discussion

The life history characteristics of *F. occidentalis* have previously been compared under constant and fluctuating temperature conditions (Robb, 1989; Wang et al., 2014; Ullah and Lim, 2015; Cao et al., 2019). In most studies, fluctuating temperature conditions affected *F. occidentalis* more than constant temperature conditions.

In my study, *F. occidentalis* could not survive at very highly fluctuating temperature conditions (26.5 ± 10.09 °C) compared to other fluctuating or constant conditions (Table 4 and 6), in which the average temperature was approximately 27 °C (26.8 ~ 27.3 °C) (Table 3). This might occur since the upper threshold temperature in the development of *F. occidentalis* is 35.29 °C (van Rijin et al., 1995). At constant conditions of 27.3 °C and fluctuating conditions of 23.8 to 31.5 °C, with an average of 27.3 °C, the developmental period of *F. occidentalis* from egg to adult was 8.9 (♂) ~ 10.0 (♀) and 10.2 (♂) ~ 10.4 (♀) days, respectively (Ullah and Lim, 2015). These periods are comparable to the developmental periods at the 10 °C-range fluctuation, with an average of 27.1 °C in my study (Table 4). At these conditions, Ullah and Lim (2015) reported no significant difference in fecundity, as 58.7 and

60.5 larvae per female, which was somewhat different from findings of 53.6 and 36.7 larvae per female. These differences were not significantly different (Table 5). In contrast, at 26 °C and 20 ~ 32 °C, Wang et al. (2014) reported that the developmental period of *F. occidentalis* from egg to adult was approximately 0.5 days significantly shorter under fluctuating conditions, although fecundity was not different. At 27.2 °C and 18.5 ~ 36.0 °C with chrysanthemum as a food plant, the development period of egg to adult was 10.3 and 11.2 days, respectively (Robb, 1989), which was comparable to my results. However, fecundity was significantly higher than in my study. This difference might be partly due to different food or the strains of *F. occidentalis* used in the test (Hulshof et al., 2003; Nielsen et al., 2010).

Humidity is also an important factor for insect survival. Although relative humidity is important for the egg hatching rate, I could not examine egg hatching because *F. occidentalis* oviposit inside plant tissue. Steiner et al. (2011) reported that > 80% RH is suitable for pupation and that > 90% RH is the optimal condition for *F. occidentalis*. The *F. occidentalis* population decreased drastically at below 50% RH, and high mortality of the larval stage was observed below 80% RH (Shipp and Gillespie, 1993). In my study, low fluctuation in humidity (20%-range) led to a similar developmental period and fecundity compared to the 10 °C-range fluctuation in temperature

(Table 4 and 5). However, the results at high fluctuating humidity conditions (30%-range) were similar to those of the control (Tables 4 and 5). It appears that very high humidity conditions have a positive effect on *F. occidentalis*. Thus, the positive effect of high humidity might overcome the negative effect of lower humidity at very high fluctuating humidity condition.

The important finding in my study was the increase in the female proportions of offspring groups under fluctuating humidity conditions. Thrips are known to produce female-biased eggs under field conditions (Higgins and Myers, 1992; van Rijin et al., 1995; Vasiliu-Oromulu, 2002). The female-biased sex ratio of thrips observed in field research was due to the high dispersal ability and short longevity of males (Terry and Kelly, 1993). Sex allocation change of thrips by environmental conditions was reported by Kumm and Moritz (2010). The sex ratio changed from male-biased to female-biased with increasing temperature. Additionally, seasonal effects, fitness of the parent and food quality can change the sex allocation of thrips toward a female or male-biased ratio (Crespi, 1988; Terry and Kelly, 1993). Thus, test conditions or foods may affect sex ratio in laboratory tests. Ullah and Lim (2015), who conducted tests at 26 ~ 56% RH and used kidney beans with pine pollen as a food, and Cao et al. (2019), who conducted tests at 65 ± 5% RH and used rugosa rose as a food, found that the sex ratio was 0.57 ~ 0.59 and 0.56 ~ 0.61, respectively; these values were

higher than those found in my study. The sex ratios of the previous studies were higher than mine, which may be due to differences in feeding conditions and environmental conditions.

I used two types of life table analyses to analyze my data: fertility life table (Maia et al., 2000, 2014) and age-stage, two-sex life table (Chi and Liu, 1985; Chi, 1988). In the fertility life table analysis, the sex ratio and survivorship of the offspring are used in data analysis. In contrast, in the age-stage, two-sex life table analysis, the sex ratio and survivorship in the parent groups are used for data analysis. The parent group came from rearing colonies, and the colonies had less fluctuating temperature and humidity conditions. In my experiment, I found that the sex ratio changed significantly with a higher fluctuation in humidity, and in this case, the fertility life table analysis may be more appropriate because this method uses the sex ratio of the offspring.

Many of the aforementioned studies, except the study by Robb (1989), which used the sex ratio of the offspring group and survival rate of the parent group, used the sex ratio and survival rate of the parent group. In Ullah and Lim (2015), using the age-stage, two-sex life table, the *r* at constant (27.3 °C) and fluctuating temperatures (23.8 ~ 31.5 °C) was 0.181 and 0.173, respectively. In Cao et al. (2019), *r* was 0.173 at constant temperatures (23 °C) and 0.160 at fluctuating temperatures (19.5 ~ 27 °C).

In Robb (1989), *r* was 0.254 at constant temperatures (27.2 °C) and 0.249 at fluctuating temperatures (18.5 ~ 36 °C). However, in Wang et al. (2014), *r* was slightly higher at fluctuating temperatures (20 ~ 32 °C), 0.127, than at constant temperatures (26 °C), where *r* was 0.121. In general, as my study indicates that at the same mean temperature, *r* appears to be negatively affected by fluctuating conditions.

In conclusion, the life history traits of *F. occidentalis* were negatively affected under fluctuating environmental conditions. Of course, it is important to consider the optimal environment condition for crops in greenhouses. And, it needs to additional study against other greenhouse pests. However, considering only the *F. occidentalis*, my study is meaningful. The impact of temperature fluctuation was more severe than that of humidity fluctuation. Additionally, the degree of impact increased as the fluctuation range of the temperature increased, while the reverse trend was observed for humidity conditions. High fluctuations in temperature and low fluctuations in relative humidity appear to be the best conditions for controlling *F. occidentalis* populations in greenhouses.

Chapter III.

Biological control method – Whitefly

Increase of control efficacy of *Nesidiocoris tenuis* (Hemiptera: Miridae) in the greenhouse by enhancing its establishment using UV-LED

Abstract

Nesidiocoris tenuis is a biological control agent for controlling *Bemisia tabaci*, which is a major insect pest of greenhouse crops. Successful establishment of a biological control agent and its spatial coherence with pest in the target area is essential for effective biological control in greenhouses. In this study, I explored proper wavelengths of light that attracts both pest and natural enemy, *B. tabaci* and *N. tenuis*, and might enhance their spatial coherence so that biological control can be more effective. The 385 nm wavelength attracted *N. tenuis* most and highly attracted *B. tabaci* in the Y-tube test, and thus it was applied in greenhouse experiments. Lighting was implemented for 6 hours from the sunset every night. The 385 nm wavelength LED light significantly affected population dynamics of *N. tenuis* and *B. tabaci* in greenhouses. In the plots of 385 nm wavelength LED with release of *N. tenuis* and *B. tabaci*, the 385 nm wavelength appeared to enhance establishment of *N. tenuis* and control of *B. tabaci* was successful in comparison to plots of non-LED with release of both species. The 385 nm light appeared to attract both *B. tabaci* and *N. tenuis* to enhance early establishment of *N. tenuis* and spatial coherence of both species, resulting in proper control of *B. tabaci.*

3.1. Introduction

For success of biological control in greenhouses, successful settlement and persistence of natural enemy populations in the target area are indispensable, and proper environmental conditions and prey densities are important factors (Messelink et al., 2014). For enhancing settlement and persistence of natural enemies, escape rate of natural enemies should be reduced in the absence of food and aggregation of released natural enemies should be enhanced in the target area. Using nectar plants, supplementary food spray for natural enemies, pre-plant release of natural enemies, which is release of natural enemy to the transplant tray few days before planting, are some of attempt for accomplishing these requirements (Sanchez et al., 2003; Pineda and Marcos-García, 2008; Calvo et al., 2012a, 2012b; Messelink et al., 2014). Insects react differently to light, depending on the wavelength, by being attracted or repelled (Antignus, 2000; Johansen et al., 2011; Shimoda and Honda, 2013). The positive phototaxis behavior of some natural enemies have been explored for improving usability of natural enemies using lighting, mainly in Japan (Ogino et al., 2016; Tokushima et al., 2016; Uehara et al., 2019). For example, thrips control was successful by improving the establishment rate of *Orius sauteri*

(Hemiptera: Anthocoridae) in eggplant farmlands using violet LED (Ogino et al., 2016).

Generalist natural enemies have alternative food apart from the target pest, and thus they have more adaptability and can be easily sustained in the target area than specialist natural enemies (Symondson et al., 2002). *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) is a generalist predator, and has been used for biological control against mites, thrips, whiteflies, and aphids, and moths (Urbaneja et al., 2009; Pérez-Hedo and Urbaneja, 2015; Gavkare and Sharma, 2016; Yano et al., 2019). Especially, *N. tenuis* was effective for controlling major pest in tomato such as whiteflies and tomato borers (Calvo et al., 2012a, 2012b; Urbaneja et al., 2012). *N. tenuis* can eat more than 30 pupae of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and eat more than 50 eggs of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), tomato borer, in one day at 25 °C (Urbaneja et al., 2009; Madbouni et al., 2017). However, because of zoophytophagous feeding activity, *N. tenuis* could attack the plant at scarce prey condition (Calvo et al., 2009; Siscaro et al., 2019). For example, the threshold that could switch *N. tenuis* into a pest species was > 0.168 of *N. tenuis*:whitefly ratio (Sanchez, 2009). However, *N. tenuis* cannot complete its life cycle feeding only plants such as tomatoes, sweet peppers, and peppers (Urbaneja et al., 2005; Sanchez, 2008). They can cause damage

to plants such as necrotic rings on stems, flowers and fruits (Calvo et al., 2009). Nevertheless, *N. tenuis* is still considered an important biological control agent, and related studies have been underway to reduce its risk. Typical studies include reducing damage to plants by using with endophytic strain *Fusarium solanik*, and settlement of the proper density of *N. tenuis* (Calvo et al., 2009; Garantonakis et al., 2018). Also, plant resistances against mite, whitefly and tomato borer induced by the phytophagy of *N. tenuis* was reported (Perez-Hedo et al., 2015, 2018; Esmaeily et al., 2019). Punctured plant by *N. tenuis* significantly repelled *B. tabaci* and *T. absoluta* (Perez-Hedo et al., 2015). Thus, *N. tenuis* can be a valuable biological control agent if its settlement rate could be enhanced.

B. tabaci is one of the major insect pests in greenhouse crops such as tomato, sweet pepper, and flower, etc. (Oliveira et al., 2001; Simmons et al., 2008; Abd-Rabou and Simmons, 2010; Cloyd, 2016). *B. tabaci* causes significant damage on plants directly by feeding and indirectly by dropping honeydew which causes sooty mold and reduction of photosynthesis (Byrne and Miller, 1990; Buntin et al., 1993). More importantly, *B. tabaci* is a vector of plant viruses such as tomato yellow leaf curl virus, which causes serious economic loss in tomato production (Moriones and Navas-Castillo, 2000; Glick et al., 2009; Li et al., 2010). In general, chemical control has been most commonly used to control *B. tabaci*. However, despite of its popularity,

chemical control is being increasingly less effective because of development of insecticide resistance of *B. tabaci* (Prabhaker et al., 1985; Palumbo et al., 2001; Naveen et al., 2017; Basit, 2019). Thus, biological control has been increasingly promoted for control of *B. tabaci* (Gerling, 1986; Gerling et al., 2001; Stansly et al., 2004; Bale et al., 2008; Tan et al., 2016; Bouagga et al., 2018).

In this study, I explored the proper LED light to attract *B. tabaci* and its predator, *N. tenuis*, and tested enhancement of establishment of *N. tenuis* and successful control of *B. tabaci* in a tomato greenhouse using LED light.

3.2. Materials and methods

3.2.1. Test insects for Y-tube, life table and greenhouse experiments

N. tenuis was purchased from the Osang Kinsect System (Guri, Korea). After delivery, they were stored in a growth chamber at 25 °C at least for 1 h before used in the experiment. *B. tabaci* was obtained from the Gyeonggi-do Agricultural Research and Extension Service in Hwaseong, Korea, and was reared in cages (300 x 300 x 300 mm; width x length x height) in an insectary at 24 ~ 25 °C and 60 ~ 70% RH with a photoperiod of 16:8 (L:D) h. Tobacco plants (*Nicotiana tabacum*) were supplied for food and oviposition. The plants were replaced at intervals of one or two months.

3.2.2. Y-tube experiment

Y-tube (20cm length and 4cm diameter for each branch) tests were conducted to select wavelengths of LED which showed high attraction rate for *N. tenuis* and *B. tabaci*. One branch was for insect entrance and other two branches were lighting zones. At each lighting zone a transparent sticky trap was installed. In one lighting zone, a color temperature 5000K white LED was applied as a control, and in the other zone a test wavelength LED was applied. All LED chips were made by LG Innotek Co. Ltd. in Seoul, Korea, and all LED equipment used in this test were made by Skycares in Gimpo, Korea. During the test, each branch of Y-tube was capped to block the penetration of light from outside. Test for each wavelength was repeated five times, and the test time for each replication was one hour at 25 °C. For *N. tenuis*, total 10 wavelengths of LED (365, 385, 395, 405, 415, 425, 445, 495, 525 and 590 nm) were used in the Y-tube test and about 30 *N. tenuis* were used at each test. Five wavelengths of LED (365, 385, 395, 405, 445 nm), that attracted *N. tenuis,* were tested for *B. tabaci*. Approximately 50 *B. tabaci* were used for each test. After the test time elapsed, insects attached on the sticky trap and remained in the Y-tube were counted. These numbers were divided by the initial number of insects to calculate the attraction rate

for test and control LED, and non-response rate. The rate was transformed by the arcsine. Transformed data were analyzed by using PROC ANOVA in SAS (SAS institute, 2013). Mean separation was conducted by using Tukey's studentized range test.

3.2.3. Preliminary test in greenhouse

To determine the lighting time, preliminary test was conducted. The test was conducted in the greenhouse (14.5 x 7 m, width x length) of the National Institute of Agricultural Sciences in Wanju, Korea. The test was conducted during the fallow season of greenhouse. Thus, there were no plants in the greenhouse. 100 *N. tenuis* were released in the greenhouse, and 9-W 385 nm LED was turned on about 14 m far from the releasing point at 90 minutes before sunset. The transparent sticky trap (25.3 x 15 cm) was installed on LED light and was replaced every 30 minutes. Then, *N. tenuis* caught on the trap was counted.

3.2.4. Incubator experiment

To verify the effect of 385 nm, which was selected in Y-tube experiment, predation amount and establishment rate of *N. tenuis* were compared among different light condition in incubator. For this experiment, *N. tenuis* colony was reared by following method.

Insect culture

N. tenuis were purchased from the Osang Kinsect System (Guri, Korea), and they were reared about 4 months at the acryl cage (30 x 30 x 30 cm) in incubator (two cube and 50 cm^3 per one cube, HANBAEK Scientific Co., Suwon, Korea). The environmental conditions of incubator was 27.1 ± 0.22 °C and RH 68.9 ± 3.76% with a photoperiod of 14:10 (L:D) h. In acryl cage, 30 cm height of five to six tomato plants were provided for oviposition, and the eggs of *Cadra cautella* (Walker) (Lepidoptera: Pyralidae) were provided for food. The tomato plants were replaced about one-month interval, and newly laid *C. cautella* eggs were served twice in a week.

Experiment

The experiment was conducted at 27.0 ± 0.16 °C and RH 68.8 ± 1.82% with a photoperiod of 14:10 (L:D) h in 50 x 50 x 50 cm incubator (HANBAEK Scientific Co., Suwon, Korea). In the incubator, 30-W circular fluorescent light was installed on the ceiling.

The test was conducted for 24 hours per one replication, and each treatment of 385 nm LED, 5000K white LED and non-LED was repeated ten times. Thus, there were three treatment plots. At each corner of the cell of the incubator, total four acryl cages (13 x 12 x 20 cm, width x length x height) were set for the test. Side exposure surfaces of each acryl cage has a hole of 10 x 16.5 cm (width x length) for free movement of *N. tenuis*. In each acryl cage, 100 x 42 mm (width x height, SPL life sciences, Pochen, Korea) petri dish with water-saturated cotton was placed. In each petri dish, a tomato stem (9 cm length) with 3 leaves was laid on cotton and, a tomato stem (11 cm length) with 5 leaves was put into the cotton by standing for a refuge of *N. tenuis*. Also, 100 *C. cautella* eggs on petri dish (40 x 6 mm, width x height, SPL life sciences, Pochen, Korea) were provided for food in each 100 x 42 mm petri dish.

In the 385 nm and 5000K white LED treatment plot, one of the four cages had a hole on the roof. The 3-W 385 nm wavelength LED or 5000K

white LED was set at the hole, and the LED was lighted on for six hours; one hour was used with fluorescent light and five hours were 385 nm wavelength LED or 5000K white LED only The location of LED lighting cage was moved into other corner in every replication. Thus, I set the non-LED 1, 2 and 3 clockwise based on the location of LED lighting cage. In the non-LED treatment (control) plot, inner left corner was set as Cage 1, and other Cage 2, 3 and 4 were set clockwise.

Randomly selected 10 *N. tenuis* from the colony were put in the petri dish (100 x 42 mm, diameter x height) that had water-saturated cotton with 9 cm length of tomato stem with 3 leaves and no foods, and released in the center of incubator for each replication. After 24 hours, uneaten eggs of *C. cautella* and alive *N. tenuis* in each cage were counted. Also, *N. tenuis* remained in the center petri dish were counted.

Additional experiment was conducted under the condition of two LED lights (385 nm wavelength and 5000K white LED) and one non-LED. Thus, there were three treatment cages at the same time: 385 nm wavelength LED, 5000K white LED, and non-LED. The experiment was repeated 10 times, and 15 *N. tenuis* were used at each replication. The rest of the test conditions were the same as stated above. This experiment was

conducted to test if there was an effect of 385 nm wavelength even under conditions with other light.

The incubator was not closed space. It had some holes in inner side wall for environmental control, and there was water over the wall. Thus, *N. tenuis* that escaped from the experimental arena were drowned in the water. The hole that can make *N. tenuis* drowned in the incubator represent real condition because not all released natural enemies remain in the site. Thus, I did not seal the holes. The data of predation amount and remained number of *N. tenuis* among the cages and treatment were transformed to arcsine, and were analyzed by using PROC ANOVA in SAS (SAS institute, 2013). Mean separation was conducted by using Tukey' studentized range test.

3.2.5. Life table experiment

To test the effect of 385 nm wavelength on *N. tenuis* and *B. tabaci*, life table experiments on both species were conducted. For the life table experiment, there were two treatments for each species: 385 nm wavelength LED and non-LED (control) treatment. The experiments were conducted at 27 °C, RH 60~80% in the incubator (500 x 500 x 500 mm, width x length x height, HANBAEK Scientific Co., Suwon, Korea). There were 30-W fluorescent light on the ceiling of the incubator for photoperiod in both treatments, the photoperiod was 14:10 (L:D) h. In LED treatment, 3-W 385 nm wavelength LED was additionally installed on the ceiling of the incubator. The LED was lighted on for 6 hours, 1 hour was used with fluorescent light and 5 hours were 385 nm wavelength LED only.

Experiments for *N. tenuis*

To obtain eggs from *N. tenuis*, 200 *N. tenuis* adults were randomly collected from the two purchased packages (about 300 *N. tenuis* per package) (the Osang Kinsect System, Guri, Korea) and then evenly divided into two cages (300 x 300 x 300 mm; width x length x height). In each cage, there were five tomato plants (20 cm height) for oviposition. Each cage was laid in each incubator that set up for treatment. *N. tenuis* were allowed to lay eggs for 24 h and were then removed from the cages.

Tomato plants in each cage were checked daily until eggs hatched, and 55 newly hatched larvae were randomly selected and transferred individually by considering the proportion of daily hatched eggs and total hatched eggs onto the tomato leaf (3 cm^2) with 200 *C. cautella* eggs in 55 petri dishes (50 mm diameter x 15 mm height, SPL life science, Pocheon, Korea) with a nylon-mesh-covered hole (13.2 mm diameter) on the lid (i.e., one larva per petri dish). The tomato leaves and *C. cautella* eggs were replaced in two-days interval. Since eggs cannot be counted because they were buried into plant tissues, egg mortality was not estimated. The egg developmental period was estimated as the time between removal of female adults from the cage and larval eclosion. Development of immature stages was checked every day. Missing individuals, which cannot be identified for

death or survival, during the experiment were excluded. Newly emerged female and male adults from each treatment were paired and placed onto the tomato leaf ($3cm^2$) with 3 cm stem on petri dishes (50 mm diameter x 15 mm height) with no lid, and each petri-dish was placed into breeding dishes (70 x 70 x 70 mm; width x length x height) with a nylon-mesh-covered hole (40 mm diameter) on the lid. In each breeding dish, 300 *C. cautella* eggs were provided as a food for adult pair. These petri dishes with tomato leaf and *C. cautella* eggs were replaced daily and placed in the incubator. The petri dishes were checked daily to count the hatched larvae. Then, by summing the number of all the hatched larvae, fecundity was estimated. The developmental period, death, longevity, and fecundity were recorded for each individual.

Experiments for *B. tabaci*

To obtain eggs from *B. tabaci*, 200 *B. tabaci* adults were randomly collected from the rearing colony and then evenly divided into 10 petri dishes (100 mm diameter x 42 mm height) with a nylon-mesh-covered hole (40 mm diameter) on the lid for ventilation. Three tomato leaves (10 cm^2 per leaf) and 1.5% agarose gel were placed in the petri dishes. Five petri dishes were placed in the incubator for each treatment. *B. tabaci* were allowed to lay eggs for 24 h and were then removed from the petri dishes. Then, eggs on the leaves were removed so that total 60 eggs are left for each treatment.

Development of immature stages was checked every day. Missing individuals, which cannot be identified for death or survival, during the experiment were excluded. Newly emerged female and male adults from each treatment were paired and placed onto the tomato leaf (9 cm^2) with 1.5 % agarose in petri dishes (50 mm diameter x 15 mm height) with a nylon-mesh-covered hole (13.2 mm diameter) on the lid, and these petri dishes with tomato leaves were replaced daily and placed in the incubator. Then eggs on the tomato leaves were counted. The developmental period, death, longevity, and fecundity were recorded for each individual.

Data and life table analysis

By using PROC TTEST in SAS (SAS Institute, 2013), the effect of 385 nm wavelength on the developmental period of immature stages, adult longevity, and fecundity was analyzed.

For estimating life table parameters, two-sex life table analysis (Chi and Liu, 1985; Chi, 1988) was used. The age-stage, two-sex life table analysis and bootstrap estimation were conducted by using the TWOSEX MSChart (Chi, 2018), which requires data: development period, survival rate, longevity of male and female adults, daily fecundity of female adults and sex ratio of the parent. To calculate age-specific survival rate (l_x) and fecundity (m_x), the age-stage specific survival rate (S_{xj}, j = stage) and fecundity (f_{xf}, f = female adult stage) were applied.

$$l_x = \sum_{j=1}^{n} S_{xj} \qquad m_x = \frac{\sum_{j=1}^{k} S_{xj} f_{xj}}{\sum_{j=1}^{k} S_{xj}}$$

where x is age and *k* is the number of stages. More details for this life table analysis and bootstrap estimation can be found in Chi and Liu (1985), Chi (1988) and Smucker et al. (2007).

The following population parameters, including the intrinsic rate of increase (*r*), finite rate of increase (*λ*), net reproductive rate (R_0) and mean generation time (*T*), were calculated.

The intrinsic rate of increase (*r*)

$$\sum_{x=0}^{\infty} e^{-rx} l_x m_x = 1$$

The finite rate of increase (*λ*)

$$\lambda = e^r$$

The net reproductive rate (R_0)

$$R_0 = \sum_{x=0}^{\infty} l_x m_x$$

The mean generation time (*T*)

$$T = \ln R_0 / r$$

3.2.6. Greenhouse experiment

Experimental design and sampling method

The greenhouse experiment was conducted to verify the effects of 385 nm LED, which was selected as the best in the Y-tube test, on population dynamics of *B. tabaci* and *N. tenuis*. Commonly, the UV means 100 nm to 400 nm wavelength, and it divided into three types such as UV-A (320 to 400 nm), UV-B (280 to 320 nm) and UV-C (100 to 280 nm). Most UV-C that radiated by sun is absorbed into the ozone layer and UV-A is known that has non-harmful effect to crop (Kovacs and Keresztes, 2002). I used 385 nm wavelength, thus, it would be not necessary to take into account the adverse effects of commonly known UV. The greenhouses were located at the experimental farm of Seoul National University in Suwon, Korea. The greenhouse experiment was conducted two times. I used three greenhouses for each experiment, and each greenhouse served as a replication. The greenhouse (16 x 4 m) was divided into four plots. Each plot was blocked using a nylon screen (156 holes per 2.54cm^2) and a shading net for prohibiting insects and light from the neighbor plots. Each plot (4 x 4 m) contained two rows each of seven tomato plants. Two plots in each greenhouse were installed with the 385 nm LED Bars as a lighting plot, while

the other two plots were non-lighting plots. In a lighting plot, three LED Bars were installed in a row and the LED light was turned on for six hours from the sunset time. There were four 3-W LED of 385 nm at 25 cm interval in one LED bar. The windows of greenhouses were closed from the lighting time until sunrise for blocking infestation of *B. tabaci* from outside. Three plants in each row, thus total six plants in each plot, were randomly selected for visual counting of adult and nymphal *N. tenuis*, and adult *B. tabaci*. My test plot size was not so big like greenhouse that managed by farmer. And, using sticky trap for sampling of target insects in my test plot would be destructive sampling method, thus, I did not use it. And also, for counting nymphal stage of *B. tabaci*, stem sampling or using loupe with frequent touch to plant could be occur the damage to plant. Thus, I only counted adult *B. tabaci*, nymph and adult *N. tenuis* by visually. All target insects on all leaves, stems and trunk of the tomato plant were counted. When counting the insects on lower side of leaves, the plants were examined looking up from below to prevent injury of plant and flying of adult *B. tabaci*, and if the leaves had to be turned over, carefully flipped over. The releasing date and number of *N. tenuis* and *B. tabaci* for each experiment was presented in Table 8. In the laboratory, *N. tenuis* from the Osang Kinsect System (Guri, Korea) were put in the 100 x 42 mm petri dish (SPL life science, Pochen, Korea) with some tomato stems for each plot. And, it moved into

greenhouse and released in the center of each target plot. *B. tabaci* has a soft body, thus if put it in a small bottle or petri dish and moved by vehicle for release into greenhouse, so many individuals could die before the release. Thus, I moved the rearing cage to farmland. And, *B. tabaci* was aspirated at the outside 20 m away from the greenhouses. And, it was blown away in the center of each target plot. The environmental conditions were recorded by the data logger (HOBO, OnSet Computer, Pocasset, MA, USA) during the whole experimental period. All LED chips were made by LG Innotek Co. Ltd (Seoul, Korea). and All LED equipment used in this test were made by Skycares (Gimpo, Korea).

Experiment 1

The experiment 1 was conducted during 13 weeks from April 23 to July 23 in 2019. The tomato seedlings (30 cm height) were planted on April 23. There were four treatments: non-LED (*B. tabaci*) plot (*B. tabaci* only), LED (*B. tabaci*) plot (LED + *B. tabaci*), non-LED plot (*B. tabaci* + *N. tenuis*) and LED plot (LED + *B. tabaci* + *N. tenuis*). To facilitate the experiment, test insects were released three times, because densities of both test insects were consistently low (Table 8). Adult *B. tabaci* was released to all plots and 2~3 weeks later adult *N. tenuis* was released to plots for which efficacy of *N. tenuis* was tested. Although optimal release number of *N. tenuis* was 1 individual per plant (Calvo et al., 2009), I released 0.5 *N. tenuis* per plant by considering the density of *B. tabaci* in my experimental plots. Then, at the 3rd release100 *B. tabaci* and 14 *N. tenuis* were released. For comparison of control efficacy between non-LED and LED treatments, the control value was calculated from June 19 when the natural enemy effects were revealed by using the following equation:

$$\text{Control value } (\%) = \left[\frac{a - b}{a}\right] \times 100$$

where a was the *B. tabaci* density of non-LED (*B. tabaci*) plot and b was the *B. tabaci* density of non-LED and LED plots. Weekly mean density and total mean density in June 19 to July 23 of adult *B. tabaci* and *N. tenuis* per plant

was compared between non-LED and LED treatments. Statistical analysis of weekly density was performed from June 19, because density of *B. tabaci* and *N. tenuis* before June 19 was too low to get the significant results.

Experiment 2

In experiment 1, the effect of 385 nm wavelength to *N. tenuis* was not significant. It might be later colonization of *N. tenuis*. Thus, in experiment 2, I concentrated on the *N. tenuis* and its control efficacy for *B. tabaci*. The experiment 2 was conducted during 10 weeks from August 28 to November 5 in 2019. All plots (LED or non-LED) received both adult *B. tabaci* and *N. tenuis*. Thus, each treatment had 6 replications with two replicated plots in three greenhouses. Tomato seedlings (30 cm height) were planted on August 28, and 100 *B. tabaci* were released to each plots on Aug 29. Before release of *B. tabaci*, 5 to 10 *B. tabaci* per plant were already present in each plant. Thus, I released 20 *N. tenuis* to each plots on September 3. Weekly mean density of adult *B. tabaci* and *N. tenuis* were compared between non-LED and LED treatments.

Data analysis

The effect of 385 nm wavelength LED on *B. tabaci* in experiment 1 was analyzed by using PROC ANOVA in SAS (SAS institute, 2013), and mean separation was conducted by using Tukey's studentized range test. The effect of 385 nm wavelength LED on *N. tenuis* in experiment 1 and, *B. tabaci* and *N. tenuis* in experiment 2 was analyzed by using PROC TTEST in SAS (SAS institute, 2013).

Table 8. Test insects released date and number in experiment 1 and 2.

Experiment	Species	Date and released number (Date, No.)		
1	*B. tabaci*	Apr. 24, 100	Apr. 30, 150	May 30, 100
	N. tenuis	May 9, 7	May 21, 7	Jun. 14, 14
2	*B. tabaci*		Aug. 29, 100	
	N. tenuis		Sep. 3, 20	

3.3. Results

3.3.1. Y-tube experiment

Results of the Y-tube test for *N. tenuis* were presented in Table 9. *N. tenuis* was differently attracted to wavelengths (Test LED, $F_{9, 40}$=31.02, P<0.0001; Non-response, $F_{9, 40}$=7.82, P<0.0001; Control LED, $F_{9, 40}$=24.11, P<0.0001). The attraction rate of *N. tenuis* was highest at 385 nm wavelength (73.5%), followed by 365 nm wavelength (62.6%). At 395 to 425 nm wavelengths attraction rate was 34 to 45.1%. The attraction rate to control LED of *N. tenuis* was over 50% at 495, 525 and 595 nm wavelengths. Top five attraction rate for *N. tenuis* occurred at 385, 365, 445, 395 and 405 nm wavelength in order, and they were tested for *B. tabaci*. The attraction rate to test LED of *B. tabaci* decreased as the wavelength increased (Table 10). No significantly different attraction rate and non-response rate were shown from 365 to 405 nm wavelengths (Test LED, $F_{4, 20}$=4.40, P=0.0103; Non-response, $F_{4, 20}$=2.27, P=0.0977; Control LED, $F_{4, 20}$=3.49, P=0.0256). Thus, the 385 nm wavelength was selected for the greenhouse experiment.

Table 9. Attraction rate (mean ± S.E.) of *N. tenuis* to test wavelength LED and white LED in Y-tube selection test.

Wavelength (nm)	Test LED	Non-response	Control (5000K white)
365	62.6 ± 4.09ab*	25.5 ± 1.53bc	11.9 ± 2.83bc
385	73.5 ± 3.85a	16.8 ± 1.86c	9.7 ± 3.08bc
395	45.1 ± 7.39bc	48.9 ± 8.96ab	6.1 ± 2.80cB
405	41.6 ± 3.27cd	46.8 ± 4.39ab	11.6 ± 2.87bc
415	40.5 ± 3.43cd	51.3 ± 2.72a	8.2 ± 2.63c
425	34.0 ± 2.80cd	50.7 ± 3.06a	15.4 ± 1.56bc
445	52.1 ± 3.23bc	20.6 ± 2.32c	27.3 ± 3.56b
495	23.6 ± 2.35de	21.8 ± 4.26c	54.6 ± 4.40a
525	10.8 ± 2.38ef	34.1 ± 8.85abc	55.0 ± 7.97a
595	7.0 ± 1.96f	35.9 ± 4.42abc	57.2 ± 3.07a

*Means followed by the same letter within a column are not significantly different at α=0.05, Tukey's studentized range test.

Table 10. Attraction rate (mean ± S.E.) of *B. tabaci* to test wavelength LED and white LED in Y-tube selection test.

Wavelength (nm)	Test LED	Non-response	Control (5000K white)
365	75.6 ± 4.55a*	18.1 ± 4.52a	6.3 ± 1.47ab
385	67.9 ± 6.26a	27.8 ± 6.78a	4.3 ± 1.99ab
395	63.3 ± 3.59ab	34.3 ± 4.39a	2.4 ± 0.98b
405	60.7 ± 4.89ab	35.1 ± 4.86a	4.2 ± 0.10ab
445	37.6 ± 6.94b	43.5 ± 7.89a	18.8 ± 1.98a

*Means followed by the same letter within a column are not significantly different at α=0.05, Tukey's studentized range test.

3.3.2. Preliminary test in greenhouse

The result of preliminary test in greenhouse was presented in Fig. 22. Before sunset, *N. tenuis* did not respond to 385 nm wavelength LED. After sunset, *N. tenuis* started to be attracted to 385 nm wavelength. The last investigation time was 270 minutes after sunset, and *N. tenuis* was consistently attracted. Thus, in the greenhouse test, the 385 nm wavelength LED was used for 6 hours from one hour before the sunset time.

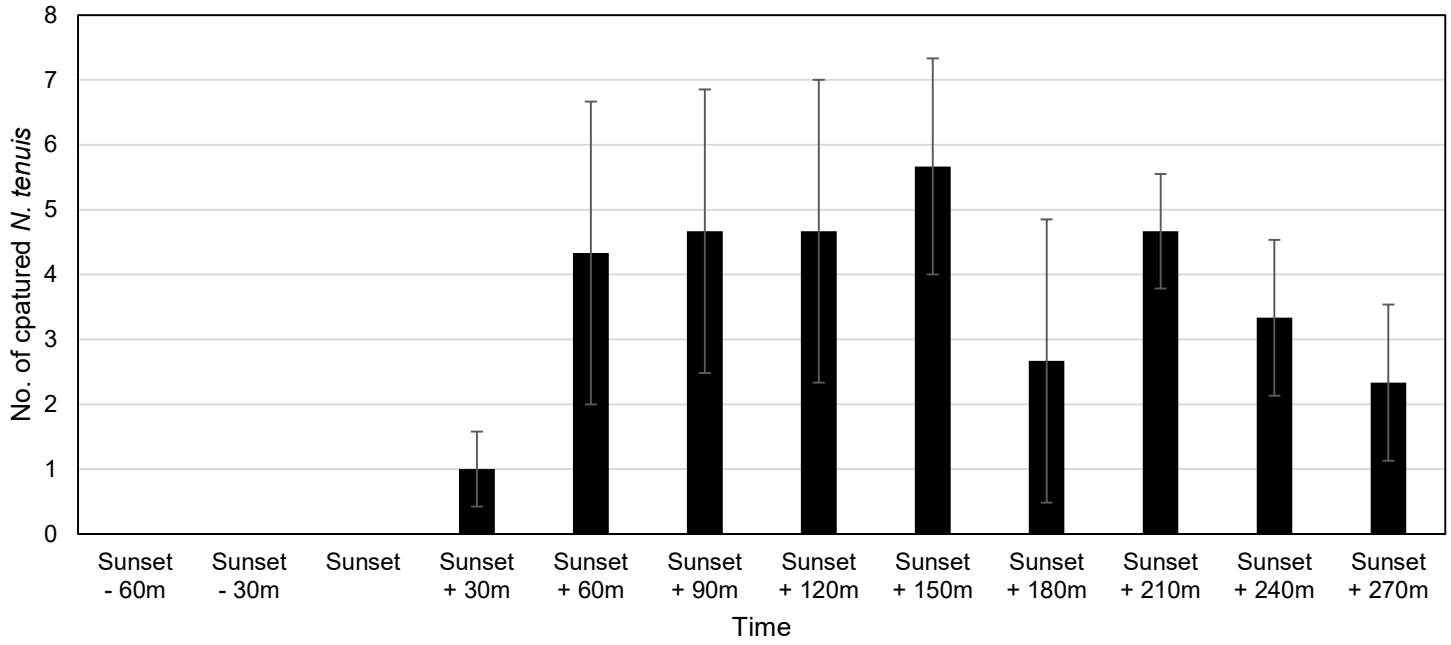


Figure. 22. Time-dependent attracted number (± S.E.) of *N. tenuis* in no planting greenhouse.

3.3.3. Incubator experiment

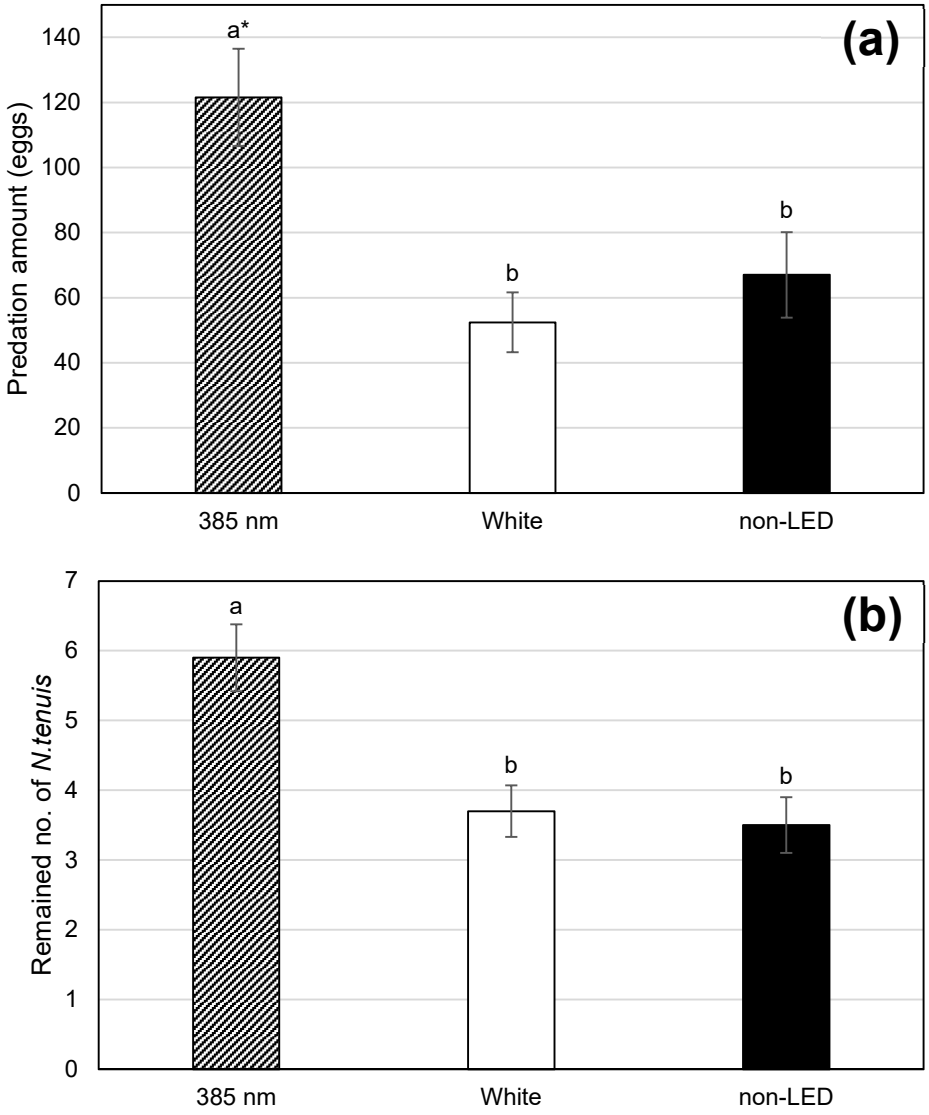
LED treatment significantly affected predation amount and remained number of *N. tenuis*. Comparison among the cages in each treatment was presented in Table 11 (Predation amount in 385 nm wavelength LED, $F_{3, 36}$=9.20, P<0.0001; Remained number of *N. tenuis* in 385 nm wavelength LED, $F_{4, 45}$=8.89, P<0.0001; Predation amount in 5000K white LED, $F_{3, 36}$=3.41, P=0.0278; Remained number of *N. tenuis* in 5000K white LED, $F_{4, 45}$=2.05, P=0.1039; Predation amount in non-LED, $F_{3, 36}$=1.20, P=0.3244; Remained number of *N. tenuis* in non-LED, $F_{4, 45}$=1.86, P=0.1333). In the 385 nm wavelength LED treatment plot, the predation amount (57.4 eggs) and remained number of *N. tenuis* (3.0 *N. tenuis*) were highest in the LED lighting cage. In the 5000K white LED treatment plot, the predation amount (23.6 eggs) was highest in the LED lighting cage, however, it was not significant with cage 2 and 3, and remained number of *N. tenuis* was not significantly different among the cages. There were no significant difference in the predation amount and remained number of *N. tenuis* among the cages in the non-LED treatment (control) plot.

Table 11. Predation amount and remained *N. tenuis* at each cage in 385 nm wavelength LED, 5000K white LED and non-LED treatment (mean ± S.E.).

Treatment		Predation amount	Remained *N. tenuis*
385 nm	LED	57.4 ± 6.10a*	3.0 ± 0.49a
	1	16.6 ± 5.53b	0.7 ± 0.26b
	2	24.0 ± 3.41b	0.6 ± 0.16b
	3	23.5 ± 7.24b	0.4 ± 0.16b
	Center	-	1.2 ± 0.29b
White	LED	23.6 ± 6.20a	1.1 ± 0.23a
	1	4.4 ± 1.90b	0.4 ± 0.31a
	2	11.5 ± 3.57ab	0.7 ± 0.26a
	3	12.9 ± 3.52ab	0.9 ± 0.18a
	Center	-	0.6 ± 0.22a
non-LED	1	20.4 ± 7.91a	0.6 ± 0.22a
	2	9.2 ± 4.00a	0.2 ± 0.13a
	3	12.3 ± 3.27a	0.8 ± 0.25a
	4	25.1 ± 8.97a	1.2 ± 0.33a
	Center	-	0.7 ± 0.30a

*Means followed by the same letter within a column in each treatment are not significantly different at α=0.05, Tukey's studentized range test.

Comparisons of total predation amount and remained *N. tenuis* number among three treatments were presented in Fig. 23. Total predation amount of 385 nm wavelength LED, 5000K white LED and non-LED treatment were 121.5, 52.4 and 67.0 eggs, respectively, and predation amount 385 nm wavelength treatment was significantly higher ($F_{2, 27}$=8.17, P=0.0017). Total number of remained *N. tenuis* in 385 nm wavelength LED, 5000K white LED and non-LED treatment were 5.9, 3.7 and 3.5 *N. tenuis*, respectively. Total number of remained *N. tenuis* was significantly higher in the 385 nm wavelength treatment ($F_{2, 27}$=9.64, P=0.0007). It appears that *N. tenuis* stay more longer in the 385 nm wavelength treatment in incubator than other treatments, and thus predation amount was higher. In the same time treatment of two LED, predation amount and remained *N. tenuis* number was also significantly higher in the 385 nm wavelength LED treatment (Table 12) (Predation amount, $F_{2, 27}$=3.90, P=0.0325; remained number of *N. tenuis*, $F_{3, 36}$=3.61, P=0.0224).



*Means followed by the same letter in each graph are not significantly different at α=0.05, Tukey's studentized range test.

Figure 23. Total egg predation amount (a) and remained *N. tenuis* number (b) in 385 nm wavelength LED, 5000K white LED and non-LED treatment (mean ± S.E.).

Table 12. Predation amount and remained *N. tenuis* in the condition of same time treatment of 385 nm wavelength LED, 5000K white LED and non-LED (mean ± S.E.).

Treatment cage	Predation amount	Remained *N. tenuis*
385 nm LED	41.6 ± 10.84a*	2.9 ± 0.53a
White	22.5 ± 7.42ab	2.2 ± 0.53ab
non-LED	11.7 ± 3.44b	0.8 ± 0.25b
Center	-	1.6 ± 0.37ab

*Means followed by the same letter in each column are not significantly different at α=0.05, Tukey's studentized range test.

3.3.4. Life table

Overall, 385 nm wavelength did not significantly affect the biological traits of *N. tenuis* and *B. tabaci* (Table 13 to 16). Only preoviposition period of *N. tenuis* was significantly affected by 385 nm wavelength LED. However, other biological trait of *N. tenuis* and *B. tabaci* were not affected by the treatment (*N. tenuis*: Egg, T_{94}=-1.10, P=0.2741; 1st larva,T_{90}=1.05, P=0.2976; 2nd larva, T_{90}=0.00, P=1.0000; 3rd larva, T_{90}=0.84, P=0.4011; 4th larva, T_{88}=0.00, P=1.0000; 5th larva, T_{85}=1.15, P=0.2527; Female, egg to adult, T_{38}=1.99, P=0.0534; Male, egg to adult, T_{45}=-0.35, P=0.7031; Preoviposition period, $T_{17.20}$=2.39, P=0.0284; Oviposition period, T_{33}=0.22, P=0.8255; Postoviposition period, $T_{24.18}$=1.94, P=0.0679; Daily fecundity per female, T_{33}=-0.82, P=0.4179; Total fecundity per female, T_{33}=0.58, P=0.5643; Female, adult longevity, T_{33}=1.87, P=0.0706; Male, adult longevity, T_{45}=0.71, P=0.4817; *B. tabaci*: Egg, T_{103}=0.80, P=0.4262; 1st larva,T_{94}=0.96, P=0.3404; 2nd larva, T_{91}=1.48, P=0.1412; 3rd larva, T_{90}=-0.99, P=0.3232; 4th larva, T_{89}=-1.24, P=0.2165; Pupa, T_{79}=0.71, P=0.4809; Female, egg to adult, T_{37}=-0.04, P=0.9690; Male, egg to adult, T_{40}=-0.39, P=0.6994; Preoviposition period, T_{35}=0.07, P=0.9418; Oviposition period, T_{35}=0.07, P=0.9411; Postoviposition period, T_{35}=0.32, P=0.7500; Daily fecundity per female, T_{35}=-1.86, P=0.0713; Total fecundity per female, T_{37}=-

1.39, P=0.1731; Female, adult longevity, T_{37}=-0.38, P=0.7077; Male, adult longevity, T_{40}=-0.04, P=0.9692).

The population parameters of *N. tenuis* and *B. tabaci* were presented in Table 17, and also age specific survivorship and fecundity of each species were presented in Figs. 24 and 25. Population parameters were not significantly different except for mean generation time (T) of *N. tenuis*. Overall, 385 nm wavelength seems to have no effect on life history characteristics of *N. tenuis* and *B. tabaci*.

Table 13. Development period (mean ± S.E.) of *N. tenuis* under 385 nm wavelength LED and non-LED treatment.

Treatment	Egg (n)	1st larva (n)	2nd larva (n)	3rd larva (n)	4th larva (n)	5th larva (n)	Egg to adult	
							Female (n)	Male (n)
non-LED (Control)	7.3 ± 0.07a* (48)	2.6 ± 0.07a (46)	1.8 ± 0.08a (46)	1.9 ± 0.06a (46)	2.0 ± 0.07a (44)	3.3 ± 0.07a (42)	18.6 ± 0.21a (20)	19.0 ± 0.20a (22)
385 nm LED	7.2 ± 0.06a (48)	2.7 ± 0.07a (46)	1.8 ± 0.06a (46)	2.0 ± 0.05a (46)	2.0 ± 0.07a (46)	3.4 ± 0.07a (45)	19.1 ± 0.14a (20)	19.0 ± 0.15a (25)

*Means followed by the same letter within a column are not significantly different at α=0.05, *T*-test.

Table 14. Preoviposition, oviposition, postoviposition period, daily fecundity, total fecundity and adult longevity (mean ± S.E.) of *N. tenuis* under 385 nm wavelength LED and non-LED treatment.

Treatment	Preoviposition period (n)	Oviposition period (n)	Post oviposition period (n)	Daily fecundity per female (n)	Total fecundity per female (n)	Adult longevity	
						Female (n)	Male (n)
non-LED (Control)	1.3 ± 0.11b* (18)**	12.5 ± 1.54a (18)	1.2 ± 0.51a (18)	7.7 ± 0.86a (18)	106.8 ± 19.22a (20)	13.7 ± 1.54a (20)	20.5 ± 3.40a (22)
385 nm LED	2.6 ± 0.56a (17)	13.0 ± 1.64a (17)	3.4 ± 0.99a (17)	6.7 ± 0.87a (17)	115.3 ± 21.59a (20)	16.9 ± 1.87a (20)	24.1 ± 3.59a (25)

*Means followed by the same letter within a column are not significantly different at α=0.05, *T*-test.

**No fecund females were excluded for calculating preoviposition, oviposition, postoviposition period and daily fecundity.

Table 15. Development period (mean ± S.E.) of *B. tabaci* under 385 nm wavelength LED and non-LED treatment.

Treatment	Egg (n)	1st larva (n)	2nd larva (n)	3rd larva (n)	4th larva (n)	Pupa (n)	Egg to adult	
							Female (n)	Male (n)
non-LED (Control)	6.8 ± 0.10a* (49/54)	4.3 ± 0.21a (44)	2.5 ± 0.13a (43)	2.9 ± 0.16a (42)	4.9 ± 0.50a (42)	2.3 ± 0.09a (37)	24.6 ± 1.04a (17)	23.4 ± 1.26a (20)
385 nm LED	6.9 ± 0.08a (56/57)	4.6 ± 0.26a (52)	2.8 ± 0.16a (50)	2.7 ± 0.15a (50)	4.2 ± 0.20a (49)	2.4 ± 0.08a (44)	24.6 ± 0.97a (22)	22.8 ± 0.85a (22)

*Means followed by the same letter within a column are not significantly different at α=0.05, *T*-test.

Table 16. Preoviposition, oviposition, postoviposition period, daily fecundity, total fecundity and adult longevity (mean ± S.E.) of *B. tabaci* under 385 nm wavelength LED and non-LED treatment.

Treatment	Preoviposition period (n)	Oviposition period (n)	Post oviposition period (n)	Daily fecundity per female (n)	Total fecundity per female (n)	Adult longevity	
						Female (n)	Male (n)
non-LED (Control)	1.5 ± 0.19a* (17)	24.8 ± 3.80a (17)	2.1 ± 0.57a (17)	5.7 ± 0.55a (17)	153.9 ± 21.60a (17)	28.4 ± 4.13a (17)	17.6 ± 4.27a (20)
385 nm LED	1.6 ± 0.20a (20)	25.1 ± 2.62a (20)	2.4 ± 0.45a (20)	4.4 ± 0.43a (20)	115.8 ± 17.43a (22)	26.5 ± 2.95a (22)	17.4 ± 2.62a (22)

*Means followed by the same letter within a column are not significantly different at α=0.05, *T*-test.

Table 17. Estimates (mean ± S.E.) of population parameters of *N. tenuis* and *B. tabaci* under 385 nm wavelength LED and non-LED treatment.

Species	Treatment	*r*	*Λ*	R_0	*T*
N. tenuis	non-LED (Control)	0.145 ± 0.0097a*	1.156 ± 0.0107a	44.664 ± 10.9510a	25.997 ± 0.3763b
	385 nm LED	0.136 ± 0.0094a	1.145 ± 0.0111a	48.003 ± 11.9930a	28.284 ± 0.4087a
B. tabaci	non-LED (Control)	0.107 ± 0.0079a	1.113 ± 0.0088a	48.480 ± 11.7640a	35.968 ± 1.5055a
	385 nm LED	0.108 ± 0.0074a	1.114 ± 0.0083a	44.727 ± 9.9649a	35.117 ± 1.3045a

*Means followed by the same letter within a column in each species are not significantly different at α=0.05, Paired bootstrap test (B = 100,000).

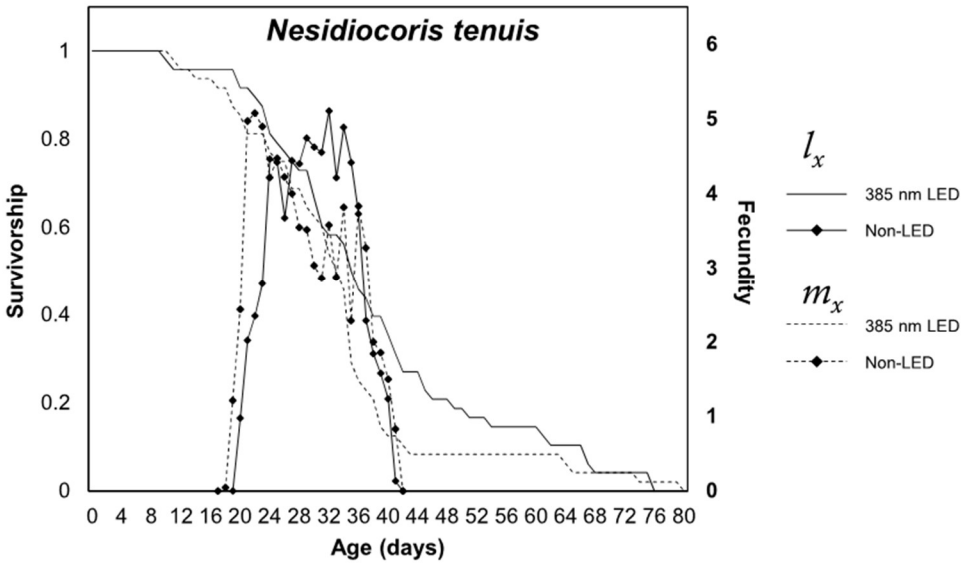


Figure 24. Age-specific survival rate and fecundity of *N. tenuis*.

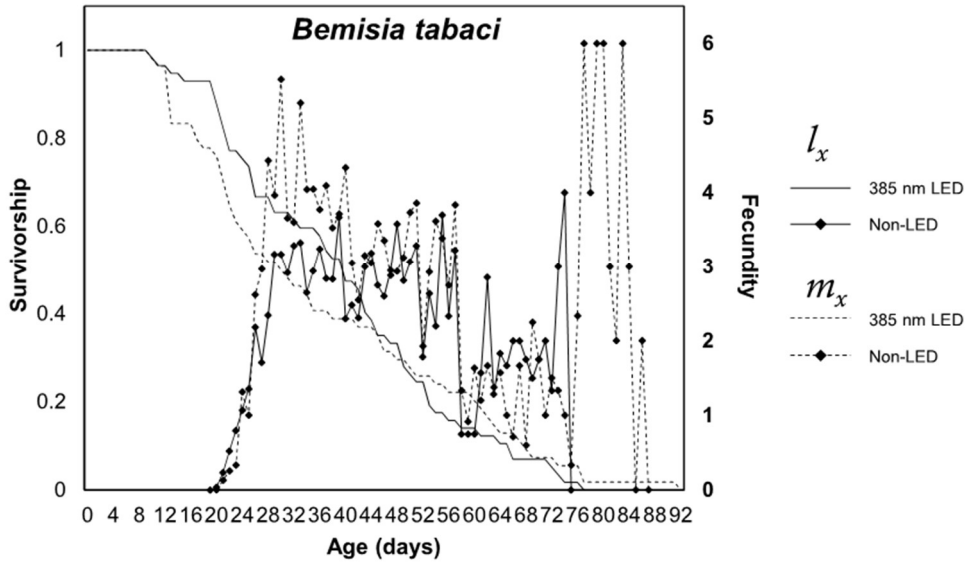


Figure 25. Age-specific survival rate and fecundity of *B. tabaci*.

3.3.5. Greenhouse experiment

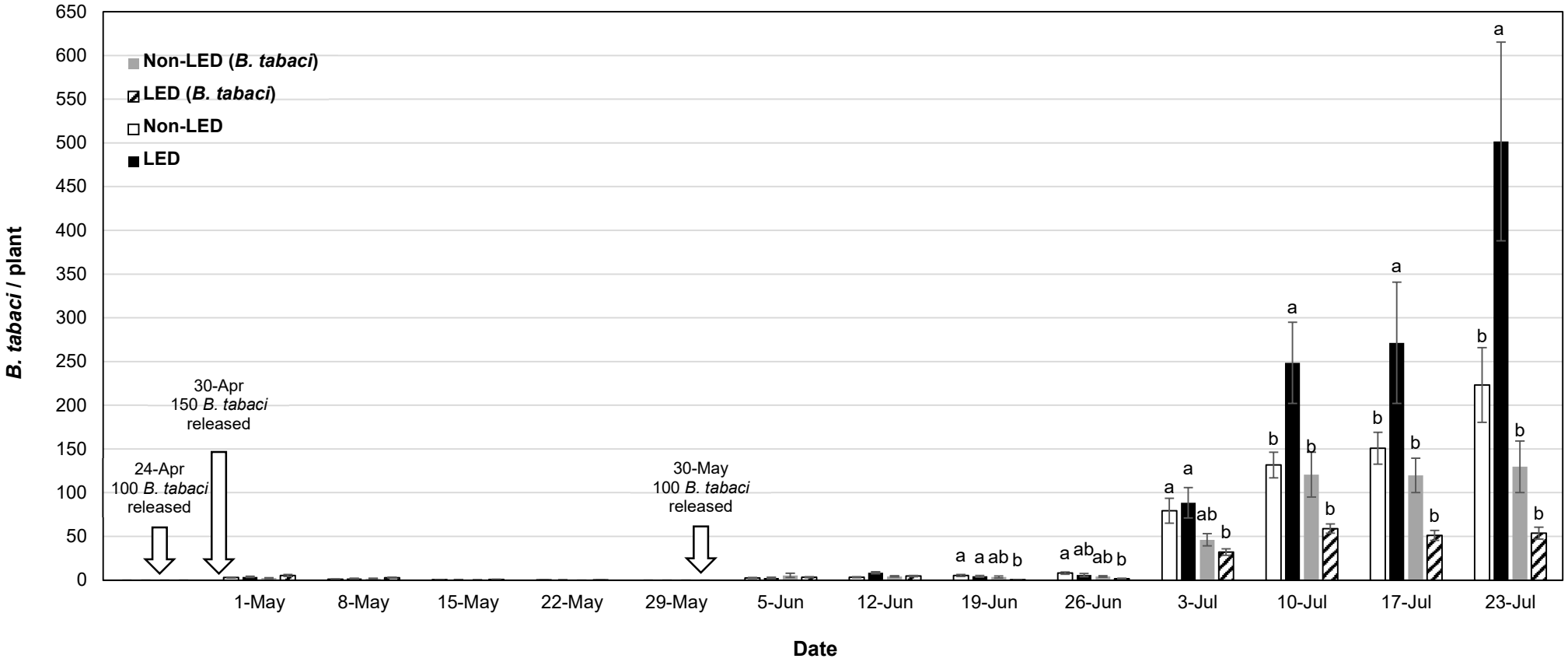
Experiment 1

The 385 nm wavelength LED light significantly affected population dynamics of *N. tenuis* and *B. tabaci*. The population dynamics of adult *B. tabaci* in 4 treatment plots are shown in Fig. 26. After last release of *N. tenuis* on June 14, density of *B. tabaci* at LED plot was consistently lower than other plots. However, it was not significantly different with non-LED plot or non-LED (*B. tabaci*) plot (19-Jun, $F_{3, 68}$=4.77, P=0.0045; 26-Jun, $F_{3, 68}$=4.79, P=0.0043; 3-Jul, $F_{3, 68}$=5.04, P=0.0032; 10-Jul, $F_{3, 68}$=8.25, P<0.0001; 17-Jul, $F_{3, 68}$=6.09, P=0.0010; 23-Jul, $F_{3, 68}$=9.81, P<0.0001). In LED (*B. tabaci*) plot, density of *B. tabaci* was significantly higher than other plots from July 10. On July 23, the density of *B. tabaci* was the highest during test period: 501.6 *B. tabaci* per plant in LED (*B. tabaci*) plot, 223.1 *B. tabaci* per plant in non-LED (*B. tabaci*) plot, 129.6 *B. tabaci* per plant in non-LED plot and 53.7 *B. tabaci* per plant in LED plot. In this results, *B. tabaci* seems to have a different trend of population dynamics to LED depending on the presence or absence of natural enemies.

The population dynamics of *N. tenuis* in experiment 1 are shown in Fig. 27. From July 3, more individuals of *N. tenuis* observed in LED plot than non-LED plot, however, it was not statistically different until July 17 (19-Jun,

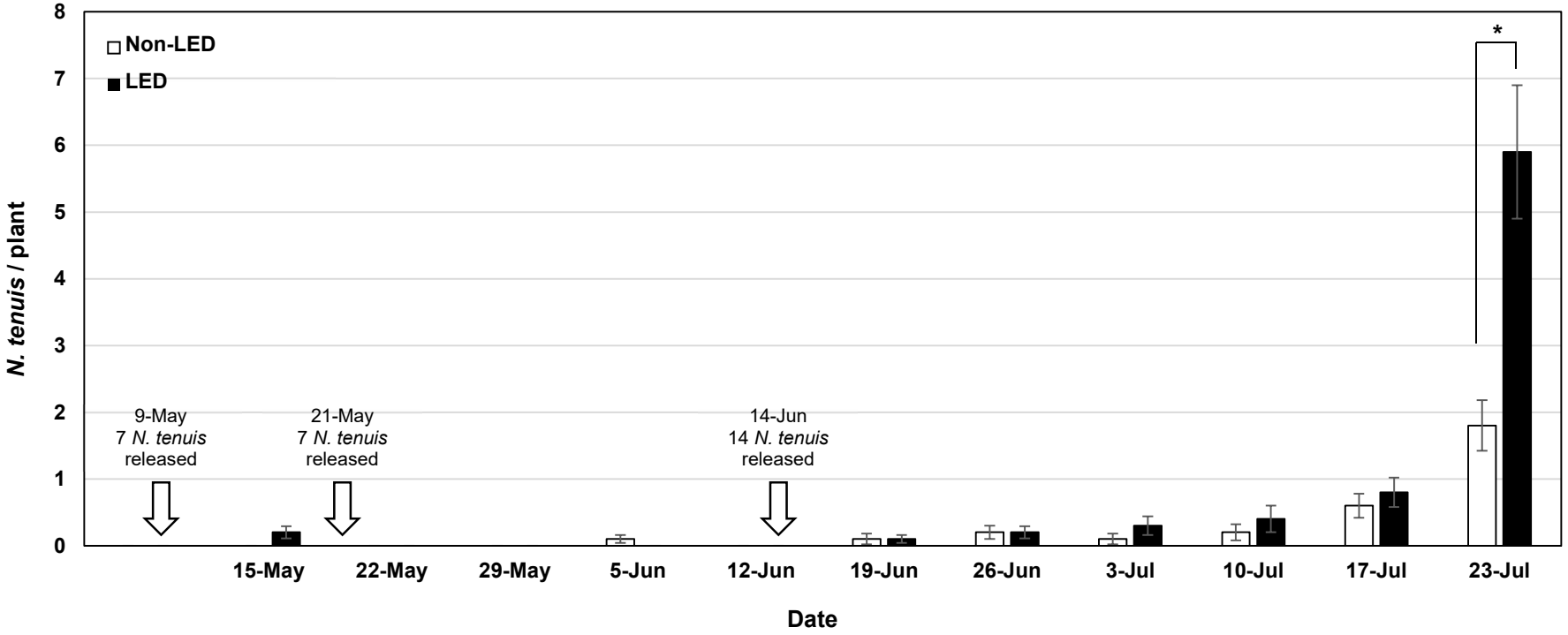
T_{34}=0.59, P=0.5597; 26-Jun, T_{34}=0.41, P=0.6842; 3-Jul, $T_{26.79}$=-1.07, P=0.2930; 10-Jul, $T_{27.87}$=-1.18, P=0.2478; 17-Jul, T_{34}=-0.97, P=0.3375). On July 23 last observation day of experiment 1, 5.9 *N. tenuis* per plant were observed in LED plot, and 1.8 *N. tenuis* per plant were observed in non-LED plot. It was significantly different result ($T_{21.88}$=-3.83, P=0.0009). In *N. tenuis* non-released plots, the 385 nm wavelength LED was significantly accelerated the population increase of *B. tabaci*. In *N. tenuis* released plots, 385 nm wavelength LED seems to help the control activity of *N. tenuis* for *B. tabaci*. However, the densities of *B. tabaci* in non-LED and LED were not significantly different. It would be due to the later colonization of *N. tenuis* by an indeterminate reason that might be temperature.

The control value of LED plot was consistently higher than non-LED plot (Fig. 28). The maximum control value of LED plot was 90.6% at June 19. During period from June 19 to July 23, the total control value of non-LED and LED plots was 28.1 and 64.7%, respectively.



*Means followed by the same letter in each date are not significantly different at α=0.05, Tukey's studentized range test.

Figure 26. Weekly densities (mean ± S.E.) of *B. tabaci* in experiment 1.



*Statistically significant in each date at α=0.05, *T*-test

Figure 27. Weekly densities (mean ± S.E.) of *N. tenuis* in experiment 1.

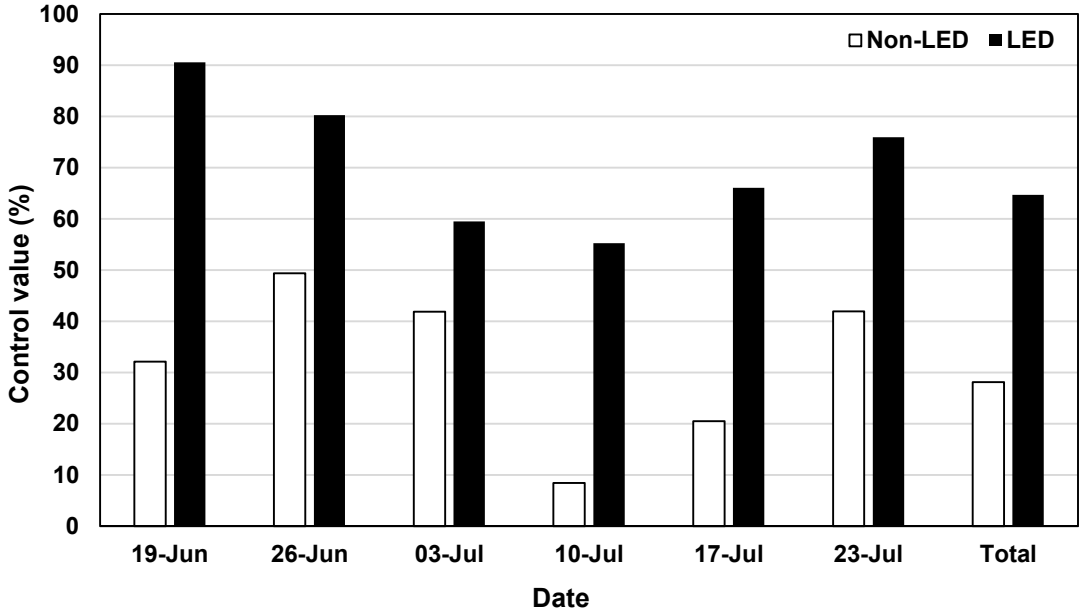
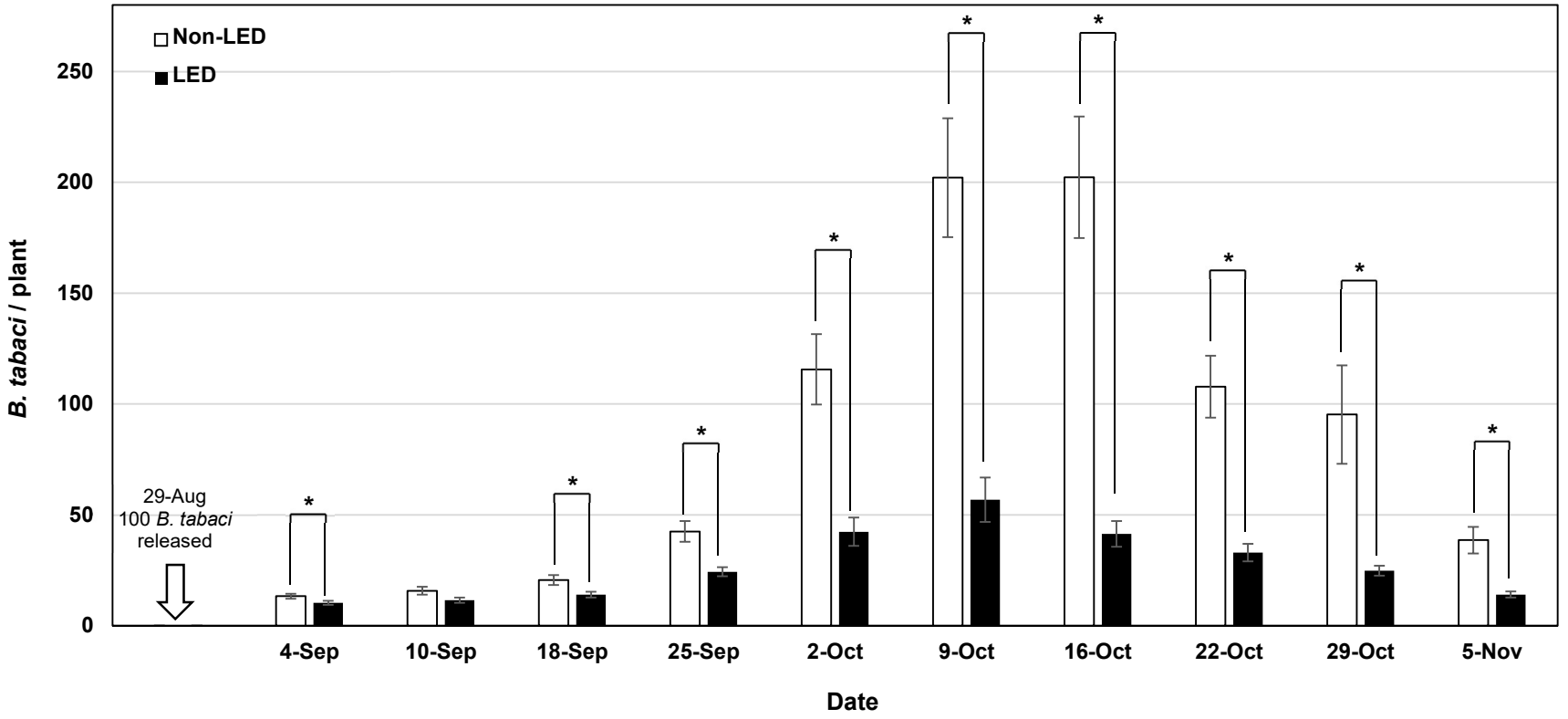


Figure 28. Control values of LED and Non-LED plot in experiment 1.

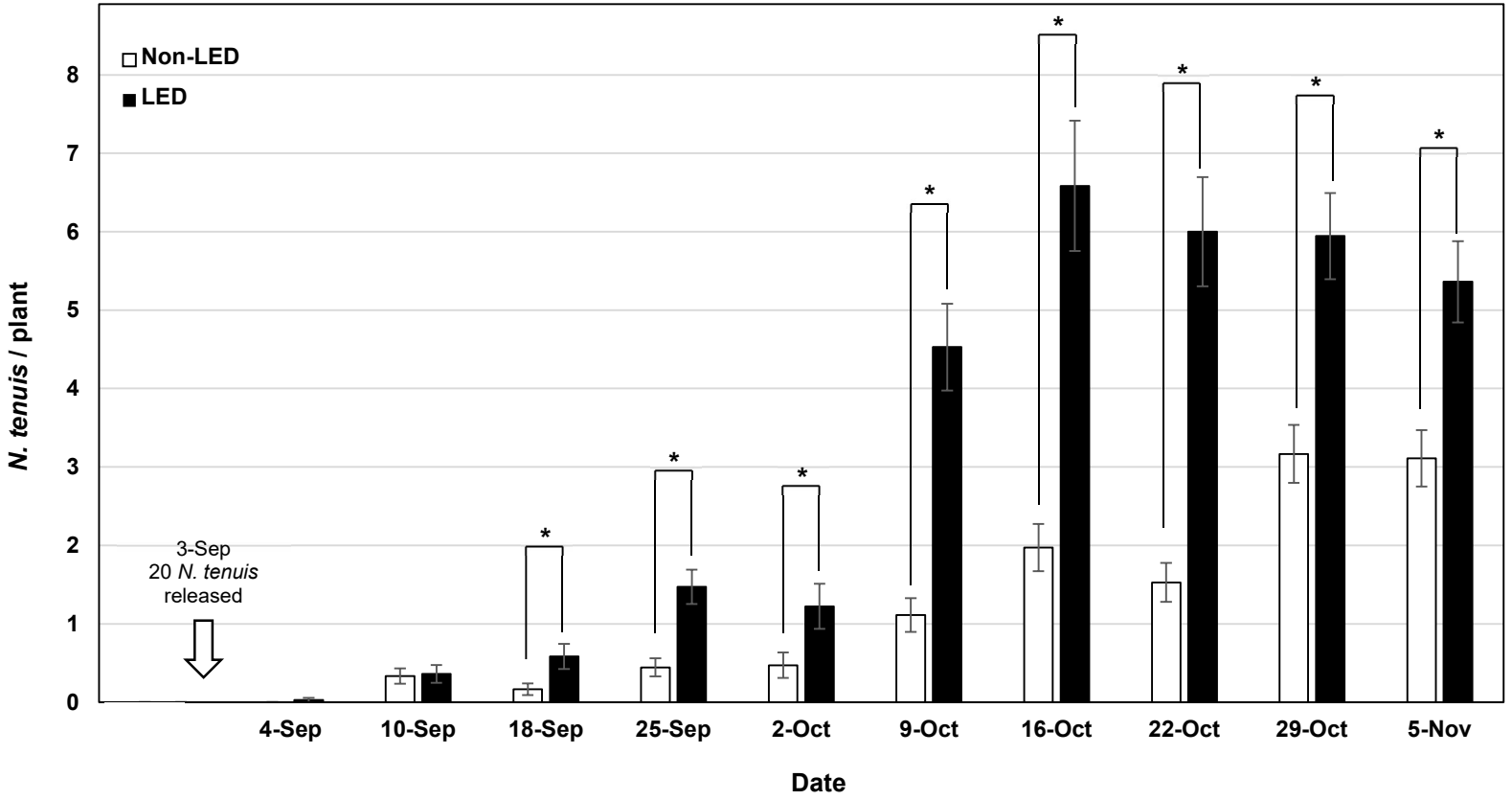
Experiment 2

Overall, densities both *N. tenuis* and B. *tabaci* were higher in the experiment 2 than the experiment 1. The density of *B. tabaci* was significantly lower while density of *N. tenuis* was significantly higher in the 385 nm wavelength LED plot than in the non-LED plot during the most of experimental period (Figs. 29 and 30) (*B. tabaci*: 4-Sep, T_{70}=2.15, P=0.0352; 10-Sep, $T_{60.75}$=1.98, P=0.0524; 18-Sep, $T_{55.43}$=2.54, P=0.0141; 25-Sep, $T_{47.55}$=3.57, P=0.0008; 2-Oct, $T_{45.94}$=4.27, P<0.0001; 9-Oct, $T_{44.55}$=5.07, P<0.0001; 16-Oct, $T_{38.18}$=5.74, P<0.0001; 22-Oct, $T_{40.51}$=5.15, P<0.0001; 29-Oct, $T_{35.71}$=3.15, P=0.0033; 5-Nov, $T_{38.71}$=4.00, P=0.0003; *N. tenuis*: 4-Sep, $T_{35.00}$=-1.00, P=0.3242; 10-Sep, T_{70}=-0.19, P=0.8535; 18-Sep, $T_{49.31}$=-2.35, P=0.0230; 25-Sep, $T_{53.01}$=-4.13, P=0.0001; 2-Oct, $T_{55.13}$=-2.27, P=0.0270; 9-Oct, $T_{45.24}$=-5.77, P<0.0001; 16-Oct, $T_{43.93}$=-5.22, P<0.0001; 22-Oct, $T_{43.72}$=-6.07, P<0.0001; 29-Oct, $T_{61.40}$=-4.19, P<0.0001; 5-Nov, $T_{62.43}$=-3.57, P=0.0007). The density of *B. tabaci* was significantly lower from the first observation time except September 10. And, the density difference of *N. tenuis* appeared from September 18. In this results, the 385 nm wavelength LED was affected to the density of *B. tabaci* in *N. tenuis* released condition, and it could help the control activity of *N. tenuis* for *B. tabaci*.



*Statistically different in each date at α=0.05, *T*-test.

Figure 29. Weekly densities (mean ± S.E.) of *B. tabaci* in experiment 2.



*Statistically different in each date at α=0.05. *T*-test

Figure 30. Weekly densities (mean ± S.E.) of *N. tenuis* in experiment 2.

3.4. Discussion

Many insects have a phototaxis, and thus are often attracted to UV light sources (Goldsmith and Bemard, 1974; Scherer and Kolb, 1987; Coombe, 1982; Raviv and Antignus, 2007; Johansen et al., 2011). For example, a positive phototaxis is known to insects like thrips, whiteflies and aphids (Kennedy, 1961; Coombe, 1982; Matteson et al., 1992; Antignus et al., 1996, 2001; Chyzik et al., 2003; Raviv and Antignus, 2007). Table 18 shows the list of wavelengths that show high attractiveness to natural enemies and insect pests. Table 19 is the list of wavelength range attractive to them, which was extracted from Johansen et al. (2011) and reorganized. All the selected wavelengths were UV (> 400 nm) or near UV (405 nm) (Table 18). Most of listed species in Table 19 showed attraction response to UV. Because UV often plays an important role in insect movement such as navigation, and orientation (Scherer and Kolb, 1987; Coombe, 1982; Raviv and Antignus, 2007), the use of UV light has been explored for greenhouse insect pest control (Chu et al., 2005; Kim and Lee, 2012; Shimoda and Honda, 2013). Using these UV characteristics, there were some studies related to light traps that attract and capture pests directly or UV absorbing films that interrupt the dispersal of pest species (Chu et al., 2003b, 2005; Antignus, 2009; Kim and Lee, 2012; Shimoda and Honda, 2013). And, UV

LED can enhance the attractiveness of western flower thrips to sticky trap (Chu et al., 2003b). Also, like my study, some studies on the enhancement of the establishment of natural enemies using lighting were also reported (Ogino et al., 2016; Tokushima et al., 2016; Uehara et al., 2019).

Table 18. High attractiveness wavelengths to referenced insects.

Species	Order: Family (type)	Prey	Most attracted wavelength (nm)	References
Exorista japonica	Diptera: Tachinidae (parasitoid)	moth	405	Tokushima et al., 2016
Nesidiocoris tenuis	Hemiptera: Miridae (predator, zoophytophagous)	whitefly, thrips, aphid, mite, moth, plant, etc.	365, 385	This study
Nesidiocoris tenuis	Hemiptera: Miridae (predator, zoophytophagous)	whitefly, thrips, aphid, mite, moth, plant, etc.	405	Uehara et al., 2019
Orius laevigatus	Hemiptera: Anthocoridae (predator)	thrips, whitefly, etc.	365, 385	Park et al., 2018
Orius sauteri	Hemiptera: Anthocoridae (predator)	thrips, whitefly, etc.	405	Ogino et al., 2016
Bemisia tabaci	Hemiptera: Aleyrodidae (plant feeder)	numerous horticultural crops	365, 385, 395, 405	This study

Table 19. Wavelength range that showed attraction response to referenced insects.

Species	Order: Family (type)	Prey	Attracted wavelength range (nm)	References
Aphidius ervi	Hymenoptera: Braconidae (parasitoid)	aphid	500-550, 560-590	Goff and Nault, 1984
Aphidius colemani	Hymenoptera: Braconidae (parasitoid)	aphid	< 400	Chiel et al., 2006
Eretmocerus mundus	Hymenoptera: Aphelinidae (parasitoid)	whitefly	< 400	Chiel et al., 2006
Aphis gossypii	Hemiptera: Aphididae (plant feeder)	numerous horticultural crops	520-530	Chu et al., 2003
Bemisia tabaci	Hemiptera: Aleyrodidae (plant feeder)	numerous horticultural crops	510[3], 520-530[3, 4], 560-590[1, 2]	[1]Mound, 1962; [2]El-Helaly et al., 1981; [3]Chu et al., 2000; [4]Chu et al., 2003a

(continued)

Species	Order: Family (type)	Prey	Attracted wavelength range (nm)	References
Frankliniella occidentalis	Thysanoptera: Thripidae (plant feeder)	numerous horticultural crops	< 400[10], 400-440[1, 4, 5, 6], 440-490[3, 4, 5, 6, 7, 8, 9, 11], 560-590[2, 3, 4, 6, 9, 10]	[1]Coombe, 1982; [2]Yudin et al., 1987; [3]Brødsgaard, 1989; [4]Gillespie and Vernon, 1990; [5]Matteson and Terry, 1992; [6]Vernon and Gillespie, 1995; [7]Chu et al., 2000; [8]Roditakis et al., 2001; [9]Chen et al., 2004; [10]Chu et al., 2005; [11]Chu et al., 2006
Trialeurodes vaporariorum	Hemiptera: Aleyrodidae (plant feeder)	numerous horticultural crops	< 400[1], 520-530[3], 560-590[2]	[1]Vaishampayan et al., 1975; [2]Webb et al., 1985; [3]Chu et al., 2003a

In this study, *B. tabaci* and *N. tenuis* showed slightly different wavelength preference. *N. tenuis* appeared to be most attracted to the 385 nm wavelength while *B. tabaci* was most attracted to the 365 nm wavelength (Table 9 and 10). The main objective of this study was to determine the proper wavelength that could enhance establishment of natural enemy, *N. tenuis*. Since there was no significant difference in *B. tabaci* attraction rate between 365 and 385 nm wavelength, thus, 385 nm wavelength was finally chosen, and tested in this study. Uehara et al. (2019) reported highest attraction rate of *N. tenuis* at 405 nm wavelength although it was not significantly different from 385 nm except for mated males. On the contrary, the attraction rate of 385 and 405 nm wavelength to *N. tenuis* in this study was 73.5% and 41.6%, respectively, with statistically difference ($P < 0.05$). In general, blue (near 450 nm) and red (near 650 nm) lights are often used for a supplementary light for promoting plant growth (Hernández and Kubota, 2012; Lu et al., 2012; Nanya et al., 2012; Samuolienė et al., 2012; Olle and Viršile, 2013). When considering plant growth, 445 nm wavelength which was also tested in this study might be considered. However, this wavelength may have limitation in pest control because it attracted insects significantly less than the 385 nm wavelength. In addition, attraction of *N. tenuis* was drastically decreased in the longer wavelength, indicating that red light wavelengths are not proper for use of pest control. In the treatment of 495,

525 and 595 nm wavelengths of Y-tube test, unlike other test wavelengths, attraction rates of *N. tenuis* to 5000K white LED were over 50%, it was significantly higher than others. These reactions might be occurred by avoidance behavior to those wavelength range. Those wavelength range including 505 and 530 nm wavelength is used for growth and photosynthesis promotion of tomato and cucumber. Thus, it would refrain from using *N. tenuis* and foregoing wavelength range together (Samuolienė et al., 2012). Supplementary light for promoting plant growth is often used by combination of red and blue (Hernández and Kubota, 2012; Nanya et al., 2012; Olle and Viršile, 2013). Thus, when using supplementary light for promoting plant growth, interference effect of red light on insect pest and natural enemies might occur. Further study is required to elucidate the potential effect of the wavelength that are used for supplementary light for plant growth.

Population dynamics of insects is affected by environmental condition as well as initial density (Mueller, 1988; Drost et al., 1998; Naranjo et al., 2009). Although both experiments showed positive results in effects of shortwave light, there were differences in the density of *N. tenuis* and *B. tabaci* between greenhouse experiment 1 and 2. *B. tabaci* explosively increased from June 26 in the experiment 1. In the experiment 2, *B. tabaci* increased until October 9 and 16, and declined since then. These results

would be caused by external inflow and temperature. External population of *B. tabaci* is developed from spring season, and it is maximized at late summer to fall season in temperate regions (Naranjo et al., 2009). *N. tenuis* and *B. tabaci* were known to be thermophilic species, and the early season temperature of the experiment 1 might be low to colonize in greenhouse (Drost et al., 1998; Hughes et al., 2009; Naranjo et al., 2009; Sanchez et al. 2009) (Fig. 31). The population of *B. tabaci* was low until June 26, it would affect *N. tenuis* population (Figs. 26 and 27).

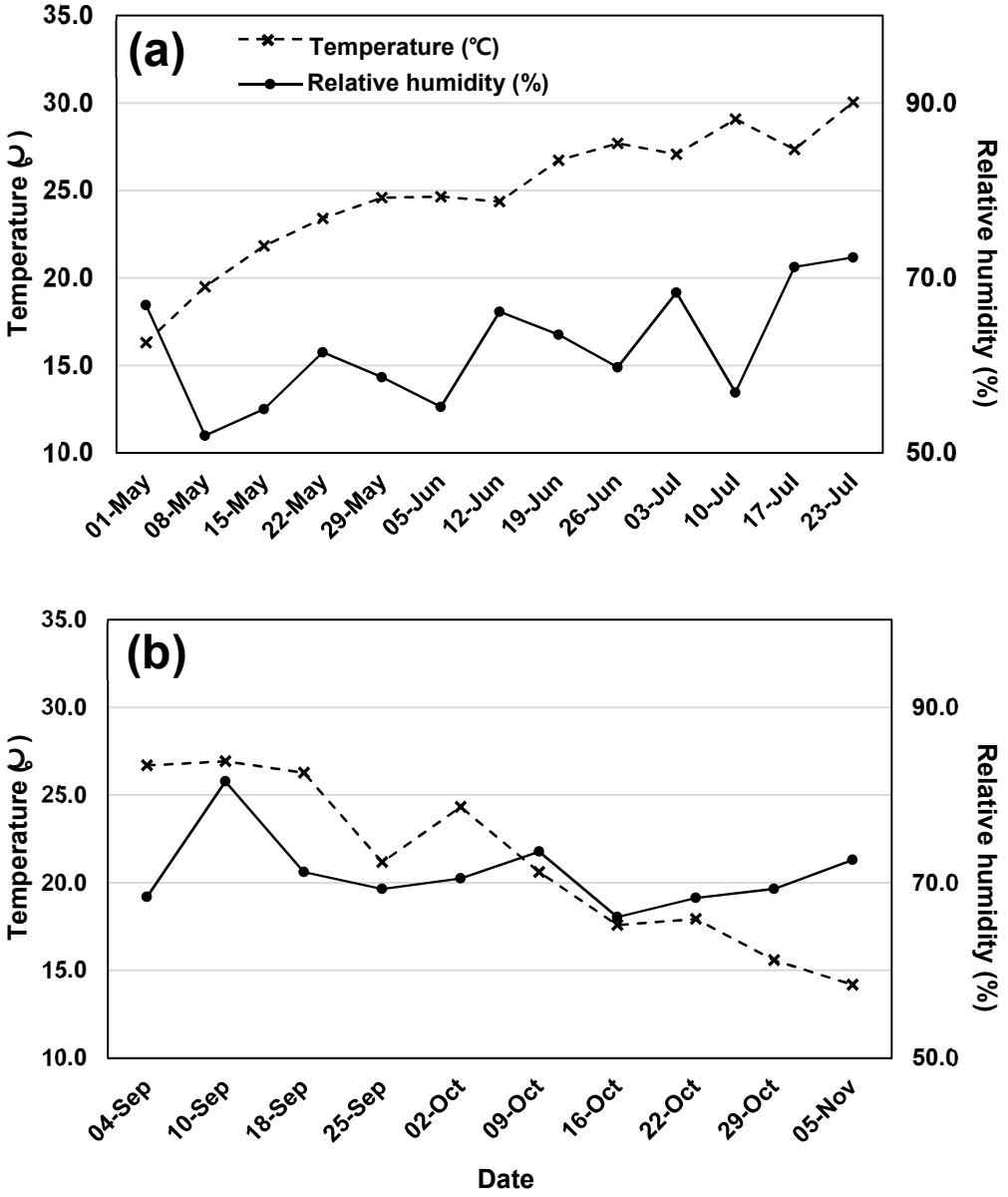


Figure 31. Weekly mean environmental conditions in experiment 1 (a) and 2 (b).

In the plots of 385 nm wavelength LED with release of *N. tenuis* and *B. tabaci*, the 385 nm wavelength appeared to enhance establishment of *N. tenuis* and control of *B. tabaci* was successful in comparison to plots of non-LED with release of both insect species. Ogino et al. (2016) reported that establishment of *Orius sauteri* (Hemiptera: Anthocoridae), for controlling thrips, was enhanced using 405 nm wavelength in eggplant fields. Although short wavelength light should be useful for biological control in greenhouses or open fields, some caution should be made because these light also may attract target insect pests. For example, when abundance of natural enemies is low due to chemical spray or poor supply of natural enemies in the crop systems, this lighting condition may accelerate population increase of target insect pest species by external inflow. Therefore, further studies are needed to elaborate the methods of applying short wavelength lights in IPM.

The LED test in incubator for verifying the 385 nm wavelength LED effect on *N. tenuis* also supported results of the greenhouse experiment Total predation amount of 385 nm wavelength LED treatment was twice as much as non-LED and 5000K white treatment, and also remained number of *N. tenuis* was the highest in 385 nm wavelength LED treatment. *N. tenuis* seems to stay longer in space affected by 385 nm wavelength, and finally it can increase the predation amount. This result would support my results of

greenhouse test. The lighting time was set based on the preliminary test (Fig. 22), and thus there was no interference light in the greenhouse experiment. However, the effect of 385 nm wavelength was weakened when there was the interference light that has some attractive effect (Table 12). Thus, it would be better to avoid same time use of wavelengths other than UV.

In the life table study, *r* values of *B. tabaci* (0.107 and 0.108) was lower than rhose of *N. tenuis* (0.145 and 0.136) (Table 17). In general, the *r* value of predator population can vary depending on prey species. The *r* value of *B. tabaci* would also vary depending on the food or environmental conditions (Costa et al., 1991; Musa and Ren, 2005; Bonato et al., 2007; Curnutte et al., 2014). In Musa and Ren (2005), the *r* value of *B. tabaci* varied from 0.110 to 0.186 depending on the food. *B. tabaci* that used in this study was reared on tobacco plants, and life table study was conducted by using tomato leaves. As purpose of life table study was verifying the effect of 385 nm LED, the food effect was not concerned. Since experimental condition was same for both treatments (LED and non-LED), the result cannot be flawed.

The results of this study are meaningful in that the 385 nm wavelength LED can improve the utilization of *N. tenuis* in the greenhouse. The 385 nm light appeared to attract *N. tenuis* to enhance early

establishment of *N. tenuis*, resulting in proper control of *B. tabaci*. For success of biological control in greenhouses, proper establishment of a released biological control agent and its spatial coherence with target insect pests is absolutely important. In this regard, using certain short wavelength light like 385 nm would be useful to increase utilization of biological agents. Use of light would be more effective in combination with other control tactics such as banker plant for habitat and alternative food, and method of reducing feeding damage that use endophytic strain *F. solani* K. (Garantonakis et al., 2018). Further studies are needed to elaborate use of short wave length lights such as lighting time, wattage of light, and applicability to other greenhouse crops.

General conclusion

This study was conducted for developing eco-friendly pest management strategies, based on survey data on smart greenhouses in Korea (Park et al., 2020). Tomato was selected as the target crop because it accounted for the highest percentage of smart greenhouses. The target pest was selected as *F. occidentalis* and *B. tabaci*, which were major insect pests in tomato smart greenhouses. The biggest feature and advantage of smart greenhouses is automatic environmental control system. At present, there is a limit to the precision of environmental control, but the precision of environmental management would be improved by constant research and development. Therefore, I wanted to find proper ways to manage important insect pests in tomato smart greenhouses.

The smart greenhouse refers to a greenhouse in which the crop growth environment can be managed remotely by incorporating ICT, and is a system that enables labor reduction and high efficiency production through automatic environmental control and environmental optimization by computers. In Korea, tomato is a major plant in smart greenhouses, and *Frankliniella occidentalis* and *Bemisia tabaci* are major insect pests in tomato greenhouses (MAFRA, 2016; MAFRA et al., 2016; Park et al., 2020). Chemical control has been the most frequently used method for insect pest control in greenhouses. However, in addition to environmental and health

problems due to excessive use of chemicals, its control efficacy has been also hampered by insecticide resistance development in insect pests including *F. occidentalis* and *B. tabaci* (Prabhaker et al., 1985; Zhao et al., 1995; Denholm et al., 1998; Jensen, 2000; Bielza, 2008). Thus, strategies enhancing eco-friendly pest management such as cultural and biological control methods have been increasingly considered.

To explore the eco-friendly management strategy for *F. occidentalis* and *B. tabaci* in tomato smart greenhouses, following studies were conducted. I examined relationship between occurrence of thrips and whitefly and environmental conditions in tomato smart greenhouses to determine which factors should be considered to manage populations of these two pests. *F. occidentalis* was the dominant thrips species, and *B. tabaci* was the dominant whitefly species in investigated greenhouses. For thrips, its population density in the greenhouse was highly related with its outside population, indicating prohibition of inflow of thrips from outside of the greenhouse is important. Also, its population was correlated with variation of temperature and humidity in greenhouses. On the contrary, whitefly density in the greenhouse was not significantly correlated with greenhouse environmental conditions, but was also related with its outside population.

The life history characteristics of *F. occidentalis* were investigated at control temperature and humidity (27.3 ± 0.54 °C, 79.9 ± 2.79% RH) (mean ± SD), a 10 °C-range fluctuation in temperature (27.1 ± 5.28 °C, 81.5 ± 4.03% RH), a 20 °C-range fluctuation in temperature (26.5 ± 10.09 °C, 80.4 ± 5.76% RH), a 20%-range fluctuation in humidity (26.8 ± 0.37 °C, 80.7 ± 9.55% RH) and a 30%-range fluctuation in humidity (27.3 ± 0.41 °C, 76.3 ± 15.28% RH). Overall, the life history traits of *F. occidentalis* were more negatively affected by fluctuating environmental conditions. The impact of temperature fluctuation was more severe than that of humidity fluctuation. Additionally, the degree of impact increased as the fluctuation range of the temperature increased, while the reverse trend was observed with humidity fluctuations. With the 20 °C-range fluctuation in temperature, *F. occidentalis* died at the 1st instar larval stage. The offspring's sex ratio was significantly higher at the 20%- and 30%-range fluctuations in humidity (0.47 and 0.49, respectively). From the fertility life table analysis, the intrinsic rate of increase (*r*) was higher at the 30%-range fluctuation in humidity and control conditions as 0.218 and 0.205, respectively. At the 10 °C-range fluctuation in temperature conditions, *r* was significantly lower as 0.169 than other conditions. High fluctuations in temperature and low fluctuations in humidity appear to be the best conditions for controlling *F. occidentalis* populations in greenhouses.

Nesidiocoris tenuis is a biological control agent for controlling *B. tabaci* (Calvo et al., 2012a, 2012b; Urbaneja et al., 2012). Successful establishment of a biological control agent and its spatial coherence with pest in the target area is essential for effective biological control. To explore effective wavelength which can be used for enhancing spatial coherence of *B. tabaci* and *N. tenuis*, Y-tube test was conducted for various wavelengths. The 385 nm wavelength was found to be best. The incubator test was conducted to verify effect of 385 nm wavelength on *N. tenuis*, and enhanced establishment rate of *N. tenuis* was observed at 385 nm wavelength treatment. The 385 nm wavelength LED light significantly affected population dynamics of *N. tenuis* and *B. tabaci* in greenhouses. In the plots of 385 nm wavelength LED with release of *N. tenuis* and *B. tabaci*, the 385 nm wavelength appeared to enhance establishment of *N. tenuis* and control of *B. tabaci*.

In conclusion, control of *F. occidentalis* might be enhanced by humidity control in smart greenhouses. Enhanced establishment rate of *N. tenuis* by 385 nm wavelength would help to control the *B. tabaci* population in smart greenhouses (Fig. 32).

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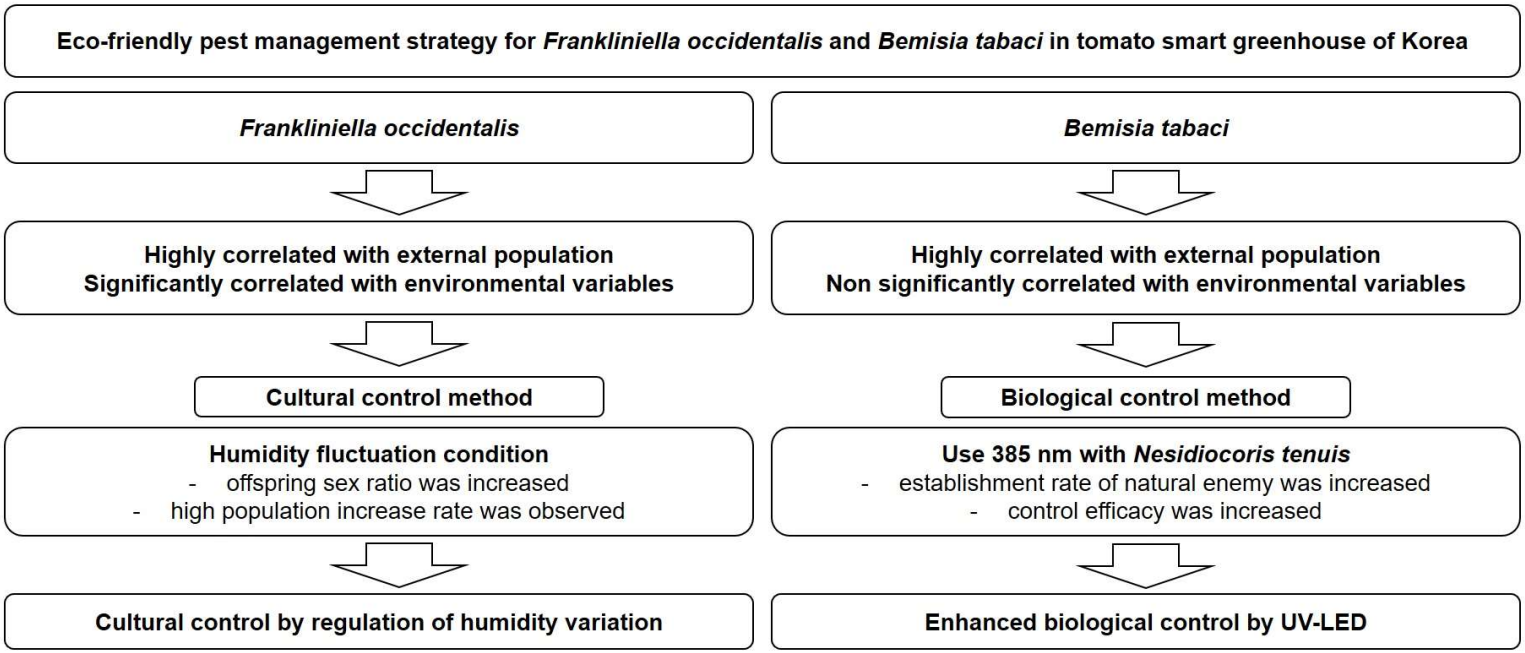

Figure 32. Suggested eco-friendly management strategy for *F. occidentalis* and *B. tabaci* in tomato greenhouses of Korea .

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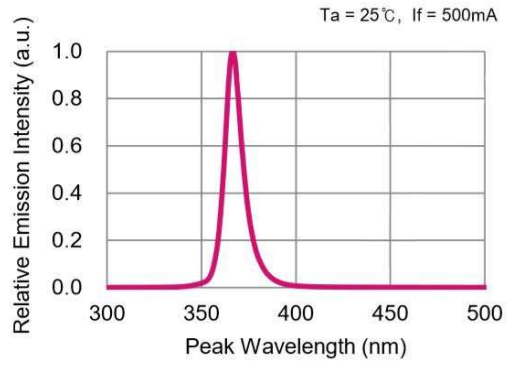
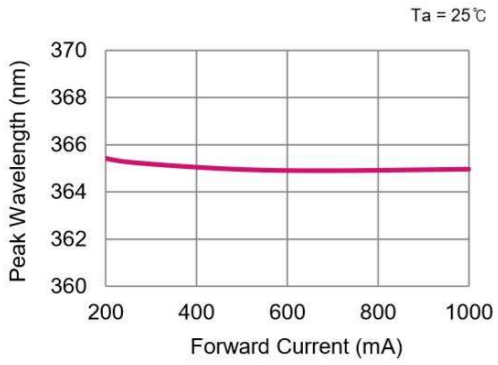
Appendix

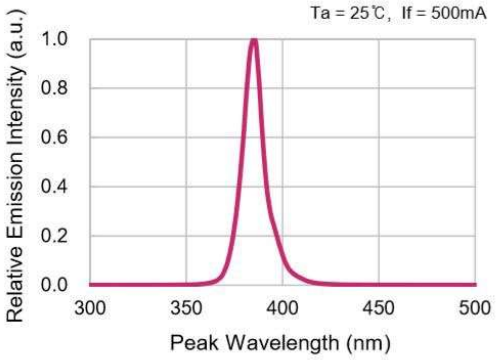
Appendix 1. Spectrum and forward current dependent peak wavelength of UV-LED that used in chapter III.

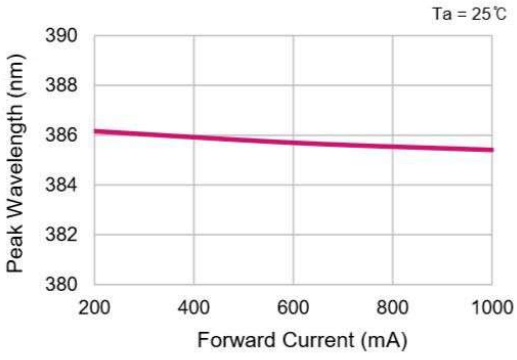
365 nm wavelength LED





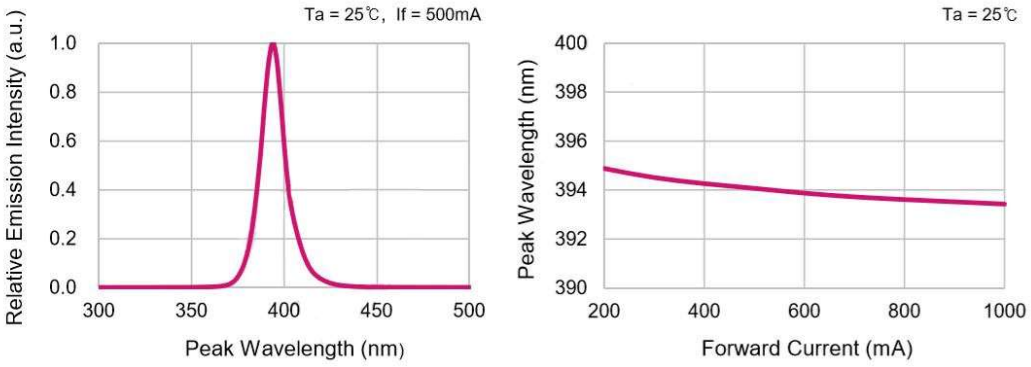
385 nm wavelength LED



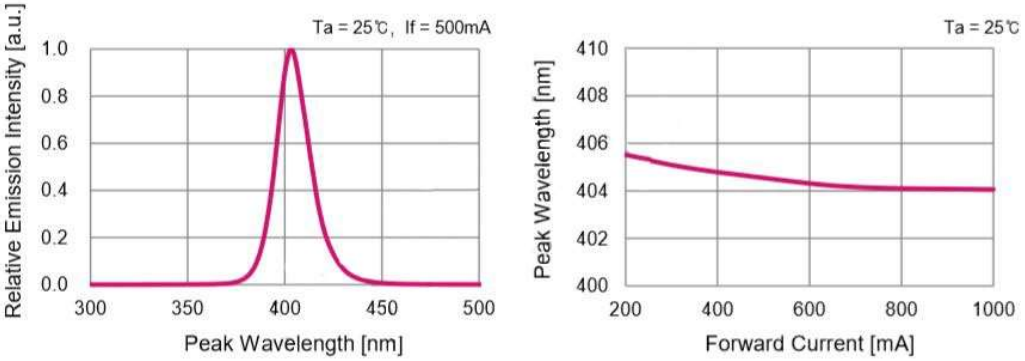


Continued Appendix 1.

395 nm wavelength LED



405 nm wavelength LED



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국문 초록

토마토 스마트 온실에서 꽃노랑총채벌레와 담배가루이의 친환경 관리 전략

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스마트온실이란 ICT를 농가에 접목해 농작물의 성장환경을 원격으로 유지·관리할 수 있는 농장을 말하며, 컴퓨터에 의한 자동 환경 제어와 환경 최적화를 통해 노동력 감소와 고효율 생산이 가능한 시스템이다. 갈수록 커지는 농경지와 노동 고령화 문제를 해결할 대안으로 정밀농업

과 자동생산 등이 떠오르고 있다. 토마토는 한국의 스마트 온실의 주요 작물이며, 토마토의 주요 해충으로는 담배가루이와 꽃노랑총채벌레가 있다. 화학 살충제를 사용한 해충 방제는 매우 유용한 방법이지만, 과사용시 환경 오염이나 농약 중독과 같은 문제를 일으킬 수 있다. 또한, 화학살충제의 과사용은 총채벌레나 가루이류와 같은 온실 해충들에게 살충제 저항성을 유발시킬 수 있다. 그러므로, 경종적 방제법이나 생물학적 방제법 같은 친환경 해충 관리 방법에 대한 연구는 필요하다.

꽃노랑총채벌레와 담배가루이의 스마트 온실에서의 친환경 방제 전략을 수립하기 위해 연구들이 수행되었다. 토마토 스마트 온실에서 어떤 환경 변수가 총채벌레류와 가루이류의 발생에 유의미한 영향을 미치는지 확인하기 위해, 온실 내부 환경조건들과 해충 발생에 대한 상관관계를 조사했다. 조사된 온실에서 총채벌레류는 꽃노랑총채벌레가 우점종이었고, 가루이류는 담배가루이가 우점종이었다. 총채벌레류는 온실 외부 밀도와 높은 상관관계를 보여 외부로부터 유입을 줄이는 것이 중

요해 보였다. 또한, 총채벌레류는 온실 내부 온도와 습도와 상관관계를 보였다. 이와 반대로, 가루이류는 온실 내부 환경변수들과 유의미한 상관관계를 보이지 않았고, 외부 밀도와 높은 상관성을 보였다.

정온/정습 조건과 변온, 변습 조건에서 꽃노랑총채벌레의 생활사적 특징이 연구되었다. 전반적으로, 꽃노랑총채벌레의 생활사적 특징은 변동하는 환경조건에서 부정적인 영향을 받는 것으로 나타났다. 온도의 편차가 큰 조건이 습도의 편차가 큰 조건보다 더 큰 영향을 미쳤다. 그리고, 온도의 편차가 커질 수록 더 심한 영향을 미쳤고, 습도는 이와 반대의 경향을 보였다. 자식 세대의 성비는 20% 범위(0.47), 30% 범위(0.49)의 습도 편차 조건에서 유의미하게 높게 나타났다. Fertility life table 분석 결과, 30% 범위의 습도 편차 조건에서 0.218의 내적 자연증가율을 보였고, 정온/정습 조건에서 0.205로 뒤를 이었다. 스마트온실내에서 꽃노랑총채벌레의 밀도 조절을 위해서는 온도 편차가 크고, 습도 편차가 작은 조건이 유리할 것이라 생각된다.

담배장님노린재는 담배가루이의 천적이다. 방제 대상지역 에서 천적의 성공적인 정착과 대상 해충과의 공간적 일관성은 효과적인 생물학적 방제를 위해 필수적이다. Y-tube 실험을 통해 385 nm LED가 선발되었고, 이 파장을 온실 내에서 검증하기 전에 항온기 내에서 실험을 진행하였다. 그 결과 385 nm LED 처리구에서 담배장님노린재의 정착률이 높아지는 것을 확인하였다. 385 nm LED는 온실내 담배장님노린재와 담배가루이의 개체군 동태에 유의미한 영향을 미쳤다. 385 nm LED와 함께 담배장님노린재, 담배가루이를 방사한 실험구에서 385 nm LED는 성공적으로 담배장님노린재의 정착률을 높였고, 이는 성공적으로 담배가루이의 밀도를 낮췄다. 이 결과는 대조구와 비교했을 때 유의미한 결과였다. 385 nm LED는 담배가루이와 담배장님노린재 모두를 유인했다. 그리고, 담배장님노린재의 정착률을 향상 시킴과 함께, 담배가루이와의 공간적 일관성을 통해 방제율을 높일 수 있었다.

결론적으로, 온실내 총채벌레류 밀도조절을 위한 경종적방제

법은 습도 편차의 조절을 통해 가능할 것으로 보인다. 또한, 385 nm LED를 사용하면 담배가루이의 천적인 담배장님노린재의 정착률을 높일 수 있고, 이를 통해 담배가루이의 효과적인 방제가 가능할 것이다.

주요어: 경종적방제, 꽃노랑총채벌레, 담배가루이, 담배장님노린재, 상대습도, 생명표, 생물학적방제, 자외선, 주광성, 환경조절

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