MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE

SUMY NATIONAL AGRARIAN UNIVERSITY

Qualified scientific work (manuscript)

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UDC: 631.524.633.111

Thesis

MOLECULAR MECHANISM OF REPRODUCTIVE ISOLATION OF *BEMISIA TABACI* SPECIES COMPLEX IS FOR PLANT PROTECTION AND QUARANTINE SERVICES

Specialty : 202 -Plants Protection and Quarantine

Field of study: 20 –Agricultural sciences and food

Submitted for a scientific degree of a Doctor of philosophy

The dissertation contains the results of own research. The use of other authors' ideas, results and texts link to the source

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SUMY – 2023

ANNOTATION

Liu Shunxiao. Molecular mechanism of reproductive isolation of *Bemisia tabaci* species complex is for plant protection and quarantine services. – Manuscript.

Dissertation for a Doctor of Philosophy degree (PhD): Specialty 202 -Plants Protection and Quarantine– Sumy National Agrarian University, Ministry of Education and Science of Ukraine – Sumy, 2023.

Bemisia tabaci (Gennadius) intensively inhabits tropical, subtropical and adjacent temperate regions, distributed in different countries and regions such as South America, Europe, Africa, Asia and Oceania. This species is a serious invasive pest, causing significant damage to plants, reducing crop yields during its infestations. It is an important object of research in modern agriculture.

Studying the heat resistance of *B. tabaci* is important because air temperature has a significant impact on the morphology and stressors that affect this pest. Biologically active substances, such as heat stress proteins (HSPs), play an important function in the adaptation of organisms to changes in temperature. High temperature can cause a stress response and affect the rate of development and reproduction of *B. tabaci*, and therefore understanding the role of HSPs becomes critical.

In this study, RACE technology and insect ecology technology were used to reveal the effects of different temperatures on the expression of heat shock protein and the growth and development characteristics of *B. Tabaci*. The control effects of different chemical treatments on *B. tabaci* were clarified. The results are as follows:

1. Through RACE technology, a part of the hsf1 gene sequence (about 900bp) of *B. tabaci* was amplified. The DNAMAN software analysis and verification showed that the amplified gene sequence conforms to the relevant characteristics of hsf1, and the similarity with the conserved gene sequence of hsf1 reaches 66.55%, which can be used for fluorescence quantitative verification. The Blast function was performed on the

sequenced results. After the alignment, the homologous genes with high similarity were searched and downloaded, and then the full-length sequence was assembled using the Sequence Assembly function of the DNAMAN software, and the full-length hsp60 was electronically cloned to about 2349 bp. The full-length cDNA of *B. tabaci* hsp60 was obtained by homologous cloning and electronic splicing clone verification technology. The full-length cDNA of Hsp60 gene of *B. tabaci* and its predicted amino acid sequence has a 1372 bp open reading frame (ORF), which can encode 608 amino acids. The isoelectric point is predicted to be 9.28 and the protein molecular weight is about 49446 Da. The 5'UTR of the gene contains 145 bp, and the 3'UTR contains 840 bp.

2. The effects of different temperatures on the growth and development of *B. tabaci*. The temperature has a significant effect on the development process of each insect state of *B. tabaci*. The experiment shows that the treatment of *B. tabaci* in the range of 7-39℃ has an effect on the hatching rate of eggs and adult reproduction. There were significant differences in the mortality rate of *B. tabaci* in different temperature conditions. The test showed that *B. tabaci* had nearly zero death when the optimum temperature was 26℃. There was no significant difference between male and female adults at 37℃and 39℃. Temperature has a great influence on the reproduction of *B. tabaci* adults, and the sex ratio of male and female adults is different at different temperatures. The research results show that high temperature leads to a decrease in the reproductive fitness of *B. tabaci* (reduced egg production), which will affect the survival of the offspring of the population scale. High temperature may shorten the lifespan of *B. tabaci* female adults and reduce the number of matings, thereby reducing the fertilization rate of female adult eggs and increasing the proportion of male offspring.

3. The expression levels of Hsf1 regulatory factors in *B. tabaci* were significantly different at 9℃($P<0.05$), while the expression differences at other different temperatures were not significant. Overall, different high and low temperature treatments had no significant effect on the expression of Hsf1. The highest expression

temperature of Hsp60 in *B. tabaci* is around 25℃. There was no significant difference in the expression of Hsp60 in *B. tabaci* at different temperatures, and only significant difference was found in the expression at 7℃and 41℃ ($P<0.05$), and at 13℃compared with other temperatures ($P<0.05$). Overall, different high and low temperature had no obvious effect on the expression of Hsp60.

4. According to the comparison of the expression levels of Hsf1 regulator and Hsp 60 gene in *B. tabaci* at different temperatures, it can be seen that the two genes have obvious expression at low temperature, but there is no significant expression at high temperature. *B. tabaci* biotype B has a wide temperature ecological range. At the same time, it can be seen that the expression of Hsf1 is positively correlated with the expression of Hsp 60, and they are induced at low temperature at the same time, This can clearly show that Hsp 60 gene plays an induced protective role through Hsf1 regulatory factors. It provides a reference for studying the population expansion of other invasive species.

5. Generally, after the outbreak of *B. tabaci*, the virus transmitted by it will occur greatly. These viruses can cause severe damage by causing leaf curling, dwarfing plants and fruit abortion. In order to screen out high-efficiency pesticides for controlling *B. tabaci* on tomato, a single foliar spray of 10 pesticides was used to carry out control experiments at the initial stage of the occurrence of *B. tabaci*, and a survey of the control effect was carried out 1, 3, and 7 days after the treatment. The results showed that the best effect of treatment F on *B. tabaci* 1 day after treatment was 41%, which was significantly higher than that of other test reagents, and none of the treatment reagents showed good quick-acting effects. Three days after treatment, treatment C had the best control effect on *B. tabaci* at 72%, and treatments I and G had the worst effect at 62%. The best control effect of J treatment 7 days after the drug is 86%, which is significantly higher than other test reagents. In the three field surveys conducted on October 13 to 20, 2020, there was no phytotoxicity, which is safe for tomatoes. In the field control process, we can choose 20% Mevirpirazone suspension concentrate, which

can be used in combination with 22.4% Spirotetramat suspension concentrate and 5% Diprofen dispersible concentrate to achieve better control effect. We can select to use in facility vegetables.

Keywords: *Bemisia tabaci,* heat stress protein, fluorescent quantification, infestation, damaged plants, yield, air temperature, productivity, biologically active substances, pests, morphology, stress factors, morphological factors, weather conditions.

АНОТАЦІЯ

Лю Шуньсяо. Молекулярний механізм репродуктивної ізоляції комплекс у видів *Bemisia tabaci* призначений для служб з захисту та карантину рослин. – Рукопис.

Дисертація на здобуття наукового ступеня доктора філософії (PhD): Спеціальність 202 – Захист та карантин рослин – Сумський національний аграрний університет, Міністерство освіти і науки України – Суми, 2023.

Bemisia tabaci (Gennadius) інтенсивно поширений у тропічних, субтропічних та прилеглих до них регіонах. Окрім того, шкідник розповсюджений в різних країнах, таких як Південна Америка, Європа, Африка, Азія тощо. Цей вид є серйозним інвазійним шкідником, що завдає значної шкоди рослинам, знижуючи врожайність сільськогосподарських культур. Ентомофаг є важливим об'єктом досліджень у сучасному сільському господарстві.

Вивчення стресостійкості *B. tabaci* є важливим, оскільки температура повітря має значний вплив на морфологію та стресові фактори, які впливають на цього шкідника. Біологічно активні речовини, такі як білки теплового стресу (HSP), відіграють важливу функцію в адаптації організмів до змін температури. Висока температура може викликати стресову реакцію і впливати на швидкість розвитку та розмноження *B. tabaci*, а тому визначення ролі HSPs стає нині критично важливим.

У нашому дослідженні було використано технологію RACE та технологію екології комах для виявлення впливу різних температур на експресію білка теплового шоку та характеристики росту і розвитку *B. tabaci*. Було з'ясовано вплив ефектів різних хімічних обробок на *B. tabaci*. У результаті проведених досліджень було отримано наступні результати:

1. За допомогою технології RACE було ампліфіковано частину послідовності гена hsf1 (близько 900 п.н.) *B. tabaci*. Аналіз та верифікація за допомогою програмного забезпечення DNAMAN показали, що ампліфікована послідовність гена відповідає характеристикам hsf1, а схожість з природною послідовністю гена hsf1 досягає 66,55%, що може бути використано для кількісної флуоресцентної верифікації. До секвенованих результатів було застосовано функцію Blast. Після вирівнювання було здійснено пошук гомологічних генів з високою подібністю, а потім повнорозмірну послідовність було зібрано за допомогою функції Sequence Assembly програмного забезпечення DNAMAN, і повнорозмірний hsp60 було електронно клоновано до довжини приблизно 2349 п.н. Повнорозмірну кДНК hsp60 *B. tabaci* було отримано за допомогою технології гомологічного клонування та верифікації клонів електронним сплайсингом. Повнорозмірна кДНК гена Hsp60 *B. tabaci* та її передбачена амінокислотна послідовність має відкриту ділянку зчитування (ORF) довжиною 1372 п.н., яка може кодувати 608 амінокислот. Ізоелектрична точка прогнозується на рівні 9,28, а молекулярна маса білка становить близько 49446 Da. 5'UTR гена містить 145 п.н., а 3'UTR – 840 п.н.

2. Вплив різних температур на ріст і розвиток *B. tabaci.* Температура має значний вплив на процес розвитку кожної стадії *B. tabaci*. Експеримент показав, що обробка *B. tabaci* в діапазоні 7-39℃ впливає на швидкість вилуплення яєць та розмноження імаго. Були виявлені значні відмінності за рівнем смертності *B. tabaci* в різних температурних умовах. Тест показав, що *B. tabaci* мав майже нульову смертність при оптимальній температурі 26℃. Не було суттєвої різниці між дорослими самцями та самками при температурі 37℃ та 39℃. Температура має великий вплив на розмноження імаго *B. tabaci*, а співвідношення статі самців і самок відрізняється при різних температурах. Результати дослідження показують, що висока температура призводить до зниження репродуктивної здатності *B. tabaci* (зменшення кількості яєць), що вплине на виживання потомства в

масштабах популяції. Висока температура може скоротити тривалість життя імаго *B. tabaci* і зменшити кількість спарювань, тим самим знижуючи рівень запліднення яєць імаго і збільшуючи частку нащадків чоловічої статі.

3. Показники експресії регуляторних факторів Hsf1 у *B. tabaci* суттєво відрізнялися при 9℃ (P<0,05), тоді як відмінності в експресії при інших температурах були несуттєвими. Загалом, різні високотемпературні та низькотемпературні обробки не мали суттєвого впливу на експресію Hsf1. Найвища температура експресії Hsp60 у *B. tabaci* становить близько 25 ℃. Не було виявлено суттєвої різниці в експресії Hsp60 у *B. tabaci* при різних температурах, і лише достовірну різницю було виявлено в експресії при 7℃ і 41℃ (P<0,05), а також при 13℃ порівняно з іншими температурами (P<0,05). Загалом, різні високі та низькі температури не мали очевидного впливу на експресію Hsp60.

4. Порівняння рівнів експресії регулятора Hsf1 та гена Hsp 60 у *B. tabaci* за різних температур показує, що обидва гени мають очевидну експресію за низьких температур, але не мають значної експресії за високих температур. Біотип B у *B. tabaci* має широкий температурний екологічний діапазон. У той же час видно, що експресія Hsf1 позитивно корелює з експресією Hsp 60, і вони індукуються при низькій температурі одночасно, Це може чітко показати, що ген Hsp 60 відіграє індуковану захисну роль через регуляторні фактори Hsf1. Це дає підстави для вивчення розширення популяцій інших інвазивних видів.

5. Як правило, після зараження *B. tabaci* вірус, що переноситься ним, набуває значного поширення. Ці віруси можуть завдати серйозної шкоди, викликаючи скручування листя, карликовість рослин і відмирання плодів. З метою підбору високоефективних пестицидів для боротьби з *B. tabaci* на томаті, проведено контрольні досліди на початковій стадії появи *B. tabaci.* У досліді використовували одноразове позакореневе обприскування 10 пестицидами, а через 1, 3 і 7 днів після обробки проводили контрольне обстеження. Результати

показали, що найкращий ефект від препарату F на *B. tabaci* через 1 день після обробки становив 41%, що було значно вище, ніж у інших тестових препаратів, і жоден з них не показав хороших швидкодіючих ефектів. Через три дні після обробки препарат C мав найкращий контрольний ефект щодо *B. tabaci* – 72%, а препарати I та G мали найгірший ефект – 62%. Найкращий контрольний ефект препарату J через 7 днів після обробки становить 86%, що значно вище, ніж у інших тест-препаратів. У трьох польових дослідженнях, проведених з 13 по 20 жовтня 2020 року, не було виявлено фітотоксичності, що є безпечним для томатів. У процесі польового контролю ми можемо вибрати 20% концентрату суспензії *Mevirpirazone*, який можна використовувати в поєднанні з 22,4% концентратом суспензії *Spirotetramat* та 5% дисперсного концентрату *Diprofen* для досягнення кращого контрольного ефекту. Ми можемо рекомендувати для використання таку схему застосування препаратів на овочевих культурах.

Ключові слова: *Bemisia tabaci*, білок теплового стресу, флуоресцентна кількісність, заселення, пошкоджені рослини, врожайність, температура повітря, урожайність, біологічно активні речовини, шкідники, морфологія, стресогенні фактори, морфологічні фактори, погодні умови.

LIST PAPERS PUBLISHED ON THE TOPICS OF THESIS

Articles in scientific professional journals Ukraine

1. Shunxiao Liu, Hao Yu, Vlasenko Volodymyr. (2020). Quantitative analysis of the regulatory gene hsf1 of *Bemisia tabaci* under different temperatures. Bulletin of Sumy National Agrarian University.The Series: Agronomy and Biology, 41(3),49-56. DOI: https://doi.org/10.32845/agrobio.2020.3.6

2. Shunxiao Liu, Vlasenko Volodymyr. (2022). Field control effect of 10 insecticides on *Bemisia tabaci* in greenhouse vegetables. Bulletin of Sumy National Agrarian University. The Series: Agronomy and Biology, 46(4), 83-88. DOI: https://doi.org/10.32845/agrobio.2021.4.12

Articles in professional scientific publications of other countries

3. Shunxiao Liu, Hao Yu, Vlasenko V.A. (2020). Effect of different temperature and humidity on *Bemisia tabaci*. Journal of Botanical Research. 2(2),21-24. DOI: http://doi.org/10.30564/jrb.v2i2.1987

4. Shunxiao Liu, Hao Yu, Vlasenko V.A. (2021). Overview of the Occurrence and Prevention of *Bemisia tabaci* Invasion in China. NASS Journal of Agricultural Sciences. 3(1),18-20. DOI: https://doi.org/10.36956/njas.v3i1.142

5. Shunxiao Liu, Zhifang Yang, Vlasenko Volodymyr. (2022). Importance of *Bemisia tabaci* Forecasting Technology: a Review. Advances in Entomology. 10(2),149-158. DOI: https://doi.org/10.4236/ae.2022.102011

6. Shunxiao Liu, Kui Wang, Vlasenko Volodymyr. (2022). A Review of Heat Shock Proteins Research on *Bemisia tabaci*. Agricultural Sciences. 13, 393-403. DOI: https://doi.org/10.4236/as.2022.133027

Articles in scientific journals with an impact factor (Scopus and WoS)

7. Kui Wang, Zhifang Yang, Xiaohui Chen, Shunxiao Liu, Xiang Li, Liuhao Wang, Hongwei Zhang, Hao Yu*. (2022). Characterization and analysis of myosin gene family in the whitefly (*Bemisia tabaci*). AIMS Molecular Science. 9(2),91-106. DOI: https://doi.org/10.3934/molsci.2022006

8. Kui Wang, Zhifang Yang, Xiang Li, Shunxiao Liu, Liuhao Wang, Hongwei Zhang, Hao Yu*. (2022). A Hepatocyte Nuclear Factor BtabHNF4 Mediates Desiccation Tolerance and Fecundity in *whitefly* (*Bemisia tabaci*). Journal of Environmental Entomology. 52(1)138-147. DOI: https://doi.org/10.1093/ee/nvac103

9. Zhifang Yang, Kui Wang, Shunxiao Liu, Xiang Li, Hongliang Wang, Liuhao Wang, Hongwei Zhang, Hao Yu *. (2023). Identification and functional analysis of isopentenyl pyrophosphate isomerase genes in the *whiteflies* (*Bemisia tabaci*). Journal of Insect Science. 23(3),1-9. DOI: https://doi.org/10.1093/jisesa/iead041

10. Hao Yu, Kui Wang, Zhifang Yang, Xiang Li, Shunxiao Liu, Liuhao Wang, Hongwei Zhang. (2023). A ferritin protein is involved in the development and reproduction of the *whitefly*, *Bemisia tabaci*. Journal of Environmental Entomology. 52(4),750-758. DOI: https://doi.org/10.1093/ee/nvad056

11. Hao Yu, Liuhao Wang, Shunxiao Liu, Xiaohui Chen, Xiaoyu Wei, Chenxi Niu, Vlasenko Volodymyr, Qisheng Song and Hongwei Zhang. (2023). Functional analysis of a NPC1 gene from the whitefly, Bemisia tabaci (Hemiptera: Aleyrodidae). Archives of Insect Biochemistry and Physiology. e22048. DOI: https://doi.org/10.1002/arch.22048

Abstracts of conferences

12. Liu Shunxiao, Yu Hao, Vlasenko V.A. The development and innovation of plant protection in agricultural production. Ternopol National Economical University, the work of the XI International Scientific and Practical Conference "Innovative processes of economic and socio-cultural development: domestic and foreign experience". (on April 10-11st, Ternopol, Ukraine). Ternopol, 2019.

13. Liu Shunxiao, Vlasenko V.A. Study on the complex species of *Bemisia Tabaci* and modern quarantine technology. Sumy National Agrarian University, International Scientific-Practical Conference «HONCHAROV'S READINGS». (on May 24-25th, Sumy, Ukraine). Sumy, 2019. P.182.

14. Liu Shunxiao, Yu Hao, Vlasenko V.A. Occurrence and harm of Bemisia tabaci in China. Sumy National Agrarian University, International Peer Reviewed Conference "MODERN AGRARIAN MANAGEMENT OF THE XXI CENTURY :AGRICUTURE OF THE FUTURE". (on May 27-29th, Sumy, Ukraine). Sumy, 2020. P.157.

15. Liu Shunxiao, Vlasenko V.A. Integrated control technology of Bemisia tabaci. Sumy National Agrarian University, International Scientific-Practical Conference «HONCHARIVSKI CHYTANNYA». (on May 25th, Sumy, Ukraine). Sumy, 2021. P.168.

16. Liu Shunxiao, Vlasenko V.A. The selectivity of Bemisia tabaci to host plants. National Academy of Agricultural Sciences, National Science Center of Ukraine, Science Internet Conference «Institute of Agriculture of NAAS». (on October 5th, Sumy, Ukraine). Sumy, 2021.P.101.

CONTENT

ABBREVIATIONS

HSE-Heat shock element

HSPs-Heat shock proteins

HSF-Heat shock factor

HSR-Heat shock response

DMF-Dimethylformamide

EB-Ethidium bromide

TAE-Tris acetate buffer

PCR-Polymerase chain reaction

RT-PCR-Real time PCR

RACE-Rapid-amplification of cDNA ends

D-Day

H-Hour

Min-minute

RH-Relative Humidity

bp-base pair

Da-Dalton

ml-milliliter

G-Gram

µl -microliter

INTRODUCTION

Justification of the choice of research topic. *B. tabaci*, also known as cotton whitefly and sweet potato whitefly, belonging to *Homoptera*, *Aleyrodidae*, and *Bemisia*, is one of the major pests in tropical and subtropical regions. In 1889, it was first discovered on Greek tobacco, hence the name Aleyrodes tabaci (*Gennadius*). Invasive *B. tabaci* is a major agricultural pest that is closely concerned around the world today. In 1991, the American "Science" magazine called *B. tabaci* biotype B a "super pest"; currently, *B. tabaci* biotype Q is due to its stronger drug resistance. also received extensive attention. At present, *B. tabaci* is widely distributed in more than 90 countries and regions around the world, and is the main pest of cotton, vegetables and garden flowers in many countries. In recent years, with the rapid development of greenhouses and facility horticulture in China, *B. tabaci* has occurred in large numbers in some areas.

In China, the earliest record of *B. tabaci* appeared in 1949, and it is mainly distributed in Hainan, Guangxi, Guangdong, Yunnan, Fujian, Hubei, Sichuan, Shaanxi, Zhejiang, Jiangxi, Beijing, Shanghai, Taiwan and other regions south of the Yangtze River Basin. However, in the past ten years, with the wide application of agricultural vegetable greenhouses and the frequent transportation of vegetables and flowers, more favorable conditions have been created for the occurrence and spread of *B. tabaci*. Occurred and it is now one of the major agricultural pests and invasive organisms in China. In Beijing, *B. tabaci* caused serious damage to tomatoes, eggplants, cucumbers, melons and zucchini, resulting in more than 70% of the yield loss.

Relationship with academic programs, plans, themes. This paper is a scientific plan of research work on the comprehensive management of harmful organisms in Henan Institute of Science and Technology.

Also, the dissertation was carried out within the framework of the research work of the Department "Department of Plant Protection named after Associate Professor

Mishnev A.K." on the topic: "Phytosanitary monitoring and regulation of pests in agriculture" (State registration number: 0123U104019).

With the development of modern agriculture, we must understand the occurrence law of *B. tabaci* and predict the damage degree of *B. tabaci* to ensure high yield, high quality and high efficiency of agriculture. Develop scientific and effective control measures, and carry out the prevention and control of *B. tabaci* in a timely manner.

The purpose and objectives of the study. The purpose of this study was to understand the expression of heat shock protein and heat transcription factor in *B. tabaci*, observe the expression of this regulatory factor under different temperature conditions, and evaluate the molecular mechanism of *B. tabaci*.

For the purpose were assigned the following tasks:

1. Determine the host plant cotton and tomato greenhouse cultivation and greenhouse cultivation.

2. Determine the indoor feeding of *B. tabaci*, design degenerate primers according to the conservation of heat shock protein, PCR amplify the HSP gene cDNA fragment of *B. tabaci*, and use RACE technology to amplify the full length of the gene.

3. The expression levels of heat shock protein transcription factors HSF and Hsp genes were quantified by fluorescence quantitative PCR at different temperatures.

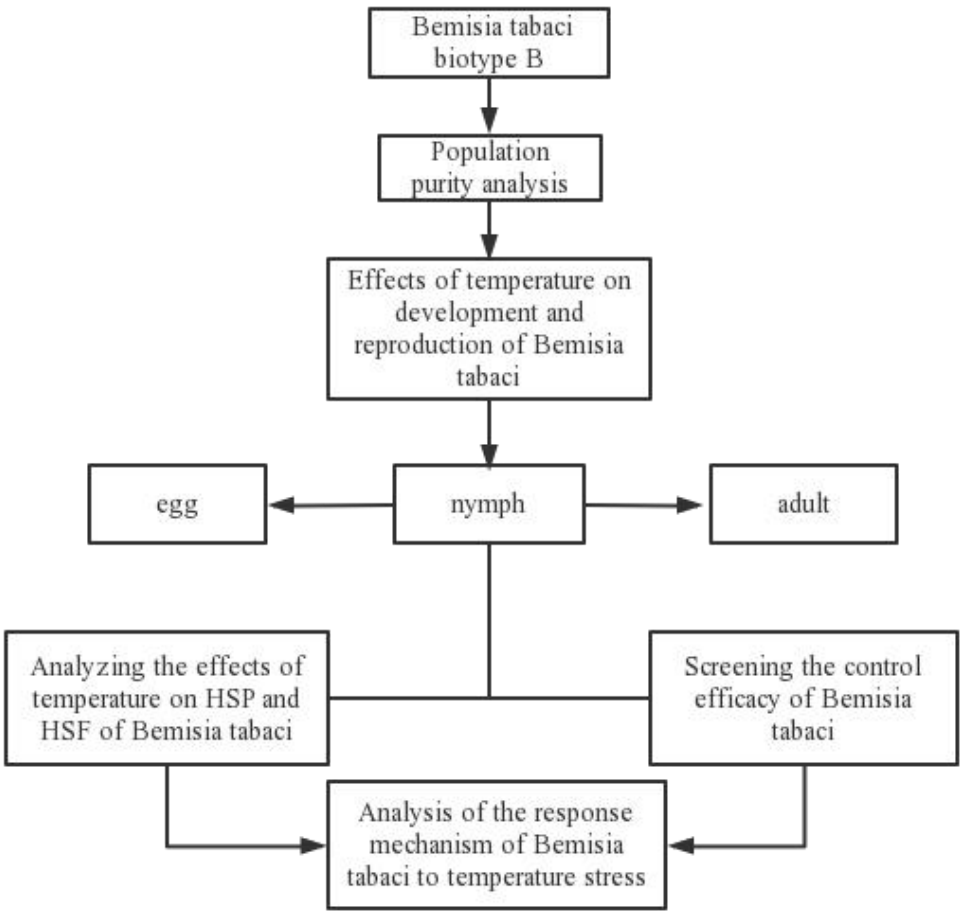
4. Design the control effects of different insecticides against *B. tabaci*, and screen the ideal pesticides for agricultural production to improve the economic benefits of growers.

Object of study. Study on the difference in expression of HSF1 regulators in *B. tabaci* under high temperature and low temperature stress.

Subject of study. To understand the regulation and functional reflection of HSF1 on HSPs in the invasive *B. tabaci*, and to further reveal the internal mechanism of *B. tabaci* adaptation to temperature stress.

Research methods. Insect taxonomy, insect physiology, insect ecology and forecasting, insect toxicology, agricultural entomology, biological control, phytochemical protection, phytosanitary science and statistics.

Technology roadmap:



Scientific novelty of the obtained results. This study suggests that there may be a positive correlation between hsf1 in *B. tabaci* and its heat tolerance. In the process of adapting to the new environment, the invading *B. tabaci* with stronger heat tolerance is selected by environmental pressure to survive in harsh environments. Under stress, more heat shock protein gene expression can be induced, so as to obtain stronger stress resistance.

The practical significance of the results. The results of this study provide a basis for the study of *B. tabaci* stress resistance adaptation, and further verify that the conserved functional gene heat shock protein can be used as one of the means to study the development of biological systems. Studying the production and changing laws of HSP in *B. tabaci* can understand the relationship between its growth and development and the changes of various influencing factors, and provide new ideas for comprehensive control in agricultural production. China has established a quantitative PCR method, and quantitative detection of imported products has been carried out at some ports.

Personal contribution of PhD. Statements of goals and objectives, discussion of results, and formation of conclusions are carried out with supervisors, the authors use modern methods for experimental research, and co-authorship of scientific papers is carried out with teachers, in published scientific works, the dissertation is based on factual material and mainly creative work.

Publications. According to the results of research, 16 scientific papers were published, including: 2 articles in professional editions of Ukraine, 5 conferences; 4 articles - in foreign editions, included in the international scientometric citation databases Scopus and WoS - 5.

Structure and scope of the thesis.The dissertation structure. The dissertation structure contains an annotation, a list of symbols, introduction, six chapters,

conclusions, chemical prevention effect practice, a list of references, appendexes.The work is illustrated with 6 tables, 22 figures.

CHAPTER 1

LITERATURE REVIEW

1.1 Occurrence and distribution of *B. Tabaci*

B. tabaci was first discovered on Greek tobacco in 1889 and was named Aleyrodes tabaci Gennadius [1]. *B. tabaci*, discovered early, are distributed in tropical, subtropical and some temperate regions, and are important pests in these regions [1,2]. Before the 1980s, *B. tabaci* mainly harmed cotton, and *B. tabaci* caused certain damage to cotton production in some cotton-producing countries such as the United States, Brazil, India, Turkey, and Iran [3,4]. Since 1985, it has gradually spread to vegetables and garden flowers and plants. In particular, unprecedented whitefly damage has occurred on greenhouse flowers in the United States and Europe [5,6]. In 1991, *B. tabaci* occurred in California and Arizona, and caused serious losses [6]. At present, *B. tabaci* is widely distributed in more than 90 countries and regions in Europe, America, Africa, Asia and Oceania except Antarctica. It is one of the main pests of agricultural and garden plants in the United States, France, India, Brazil and other countries. Now the distribution and damage of *B. tabaci* have surpassed that of the greenhouse whitefly and become the main pest of seedlings and flowers worldwide [7].

The earliest record of *B. tabaci* in China was in 1949, mainly distributed in Guangdong, Guangxi, Hainan, Fujian, Zhejiang, Shanghai and other provinces in the south of the Yangtze River Basin. The frequent transportation of vegetables and flowers has created more favorable conditions for the occurrence and spread of *B. tabaci*, making it an important agricultural pest in China [4,11]. In Beijing, the damage caused by *B. tabaci* on tomatoes, eggplants, cucumbers, melons, and zucchini caused severe losses, exceeding 70% at the worst [12,13]. From 2005 to 2007, the cumulative annual occurrence area of *B. tabaci* on various crops has reached about 100,000 hectare (667 m^2) [14,15].

B. tabaci (*Gennadius*) belongs to *Homoptera*: *Aleyrodidae*, and is a kind of tiny piercing and sucking pests, mainly concentrated in tropical and subtropical regions[16-19]. Since the successful invasion of whiteflies in the mid-to-late 1990s, they have successively broke out in vegetable areas in China, not only in greenhouses, but also in large numbers in fields [20,21]. Because the invasive whitefly is harmful to agricultural production, it has become one of the typical invasive alien species that researchers at home and abroad are more interested in in recent years [22-27].

At present, *B. tabaci* biotype B is the most serious pest in the world. Since its emergence in the early 1990s, *B. tabaci* biotype B has rapidly spread to all parts of the world and caused outbreaks, becoming a global invasive pest, which has attracted the attention of all countries in the world [8]. American researchers have shown that when *B. tabaci* biotype B arrived in Florida, its poinsettia production was hit hard, followed by vegetable production. During 1991-1992, *B. tabaci* outbreaks occurred in Arizona, Florida, California, Texas and other states, causing 200 million and 500 million dollars of damage respectively [8], and the cost of controlling *B. tabaci* damage reached 160 million dollars. *B. tabaci* biotype B has become the main pest on vegetables, flowers and cotton in the United States [8]. In addition, the damage caused by *B. tabaci* has reduced the area planted with cotton in Mexico to 35% [9]. The same situation occurred in Italy, France, Russia, Azerbaijan, Cuba, El Salvador, Haiti, Honduras, Nicaragua, Guatemala, Jamaica and other countries, resulting in a large reduction in the production of cantaloupe, pepper, poinsettia, tomato, cotton, okra and tobacco crops, resulting in serious economic losses [4]. Ramos et al. mapped global risk levels for *B. tabaci* under current and future climate conditions in 2018 [28].

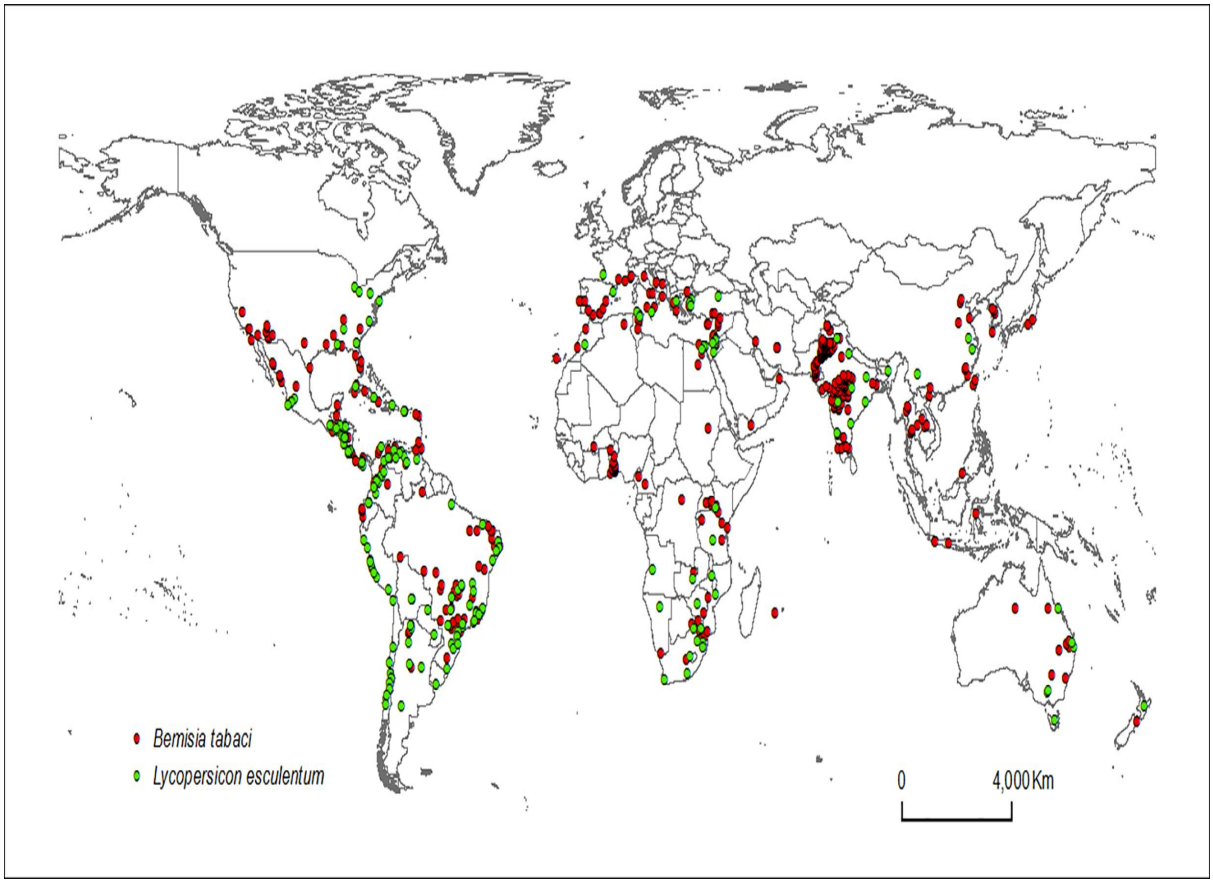


Figure 1.1 Global known occurrences of *B. tabaci* (Biotype B and Q) in open field (red dots), and S. lycopersicum in open field (green dots).

During 1994–2003, MEAM1 was identified in 23 provinces and MED in 3 provinces (Figure 2.2 a). During 2004–2007, MEAM1 was recorded in 28 provinces and MED in 27 provinces (Figure 2.3 b). During 2008–2017, MEAM1 was recorded in 26 provinces and MED in 30 provinces (Figure 2.4 c). Guo et al. believe that the spread of both alien *B. tabaci* cryptic species was very rapid through these data [28].

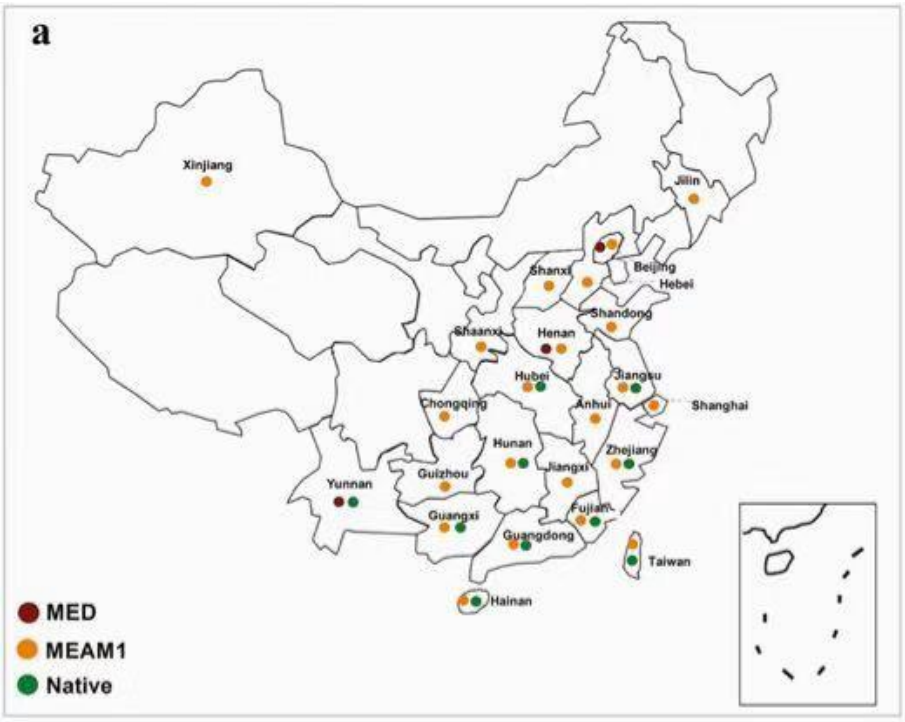


Figure 1.2 Distribution of *Bemisia tabaci* cryptic species in China during (a) 1994–2003.

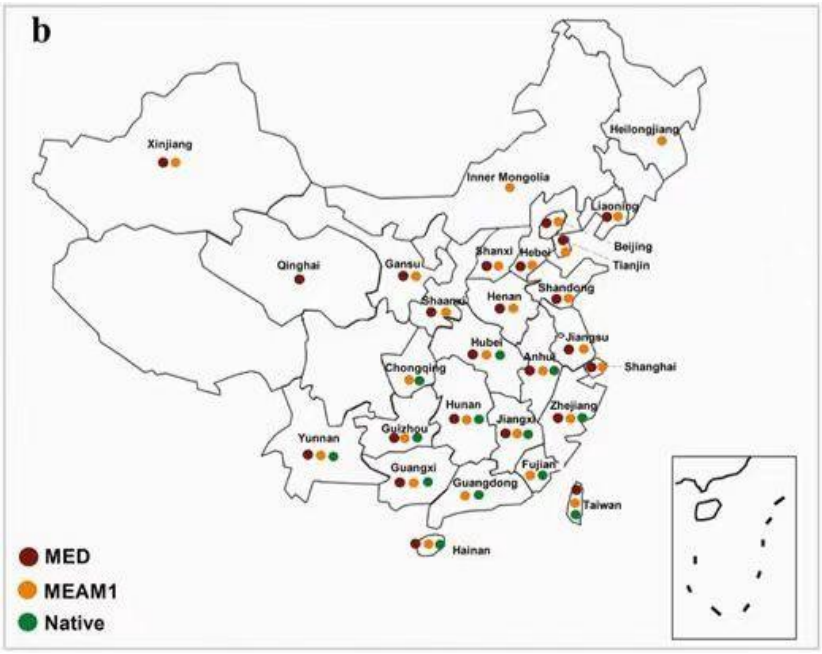


Figure 1.3 Distribution of *Bemisia tabaci* cryptic species in China during(b) 2004–2007.

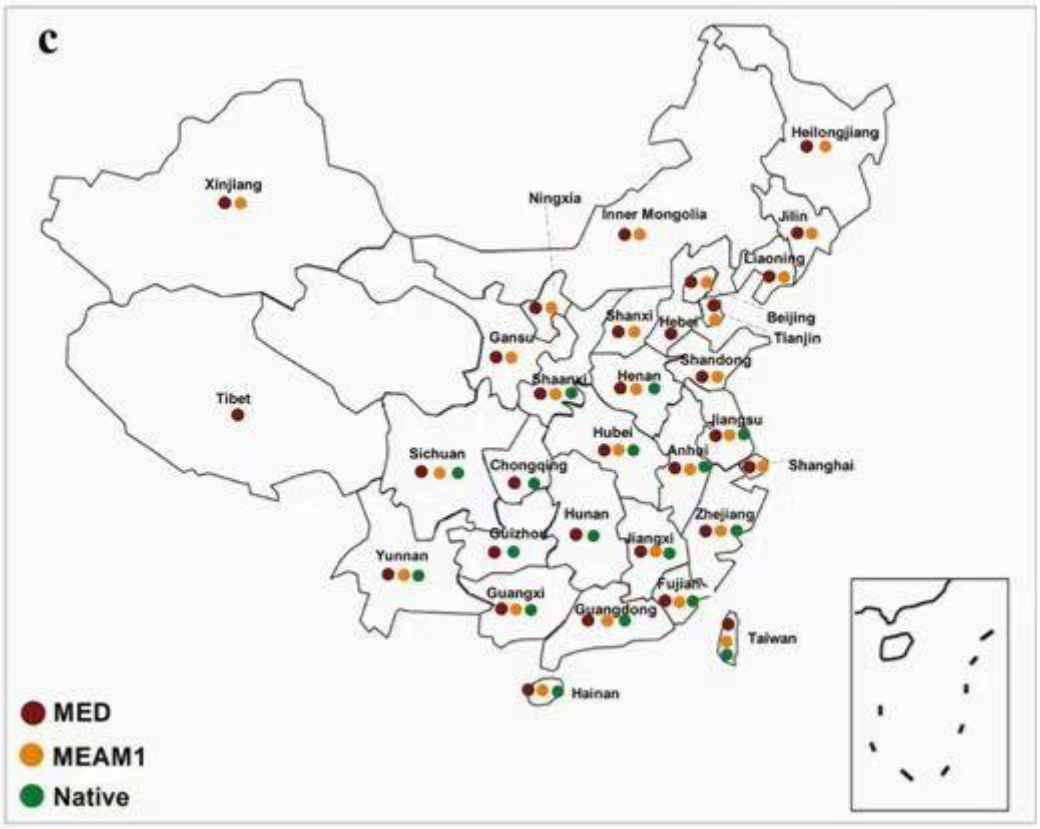


Figure 1.4 Distribution of *Bemisia tabaci* cryptic species in China during (c) 2008–2017.

1.2 Morphological characteristics of B. tabaci

The individual development of *B. tabaci* is divided into three stages: egg, nymph and adult. Nymphs are 3rd instar, and the pupae formed after the 3rd instar nymphs molt are usually called pseudopupa [29-34].

B. tabaci eggs are oval in shape, about 0.2 mm long, shiny, with an egg stalk at the base, pale yellow-green at first birth, and slowly turn dark brown before hatching.

The nymphs are light green to yellow, the first instar nymphs are 0.2-0.4 mm long, oval in shape, gray-white, with legs and antennae, two yellow spots can be seen through the epidermis from the abdomen, and they can move. The body length of the 2nd instar nymph is 0.36 mm, and the body length of the 3rd instar nymph is 0.5 mm. The metamorphosis of *B. tabaci* is different from other Homopteran insects, similar to

holometabolism. The 4th instar nymph is called pseudopupa. The pseudopupa is about 0.7 mm long, with yellow-brown stripes with thin or naturally drooping edges, and a pair of bristles on the tail. There are 1-7 pairs of stout long setae on the back.

Adults are mainly parasitic on the underside of leaves, with yellowish body color, white wings without spots, and white wax powder. The female body is about 0.91 mm long, and the male body is about 0.85 mm long. Smaller than the greenhouse whitefly. It has 7 antennae, 2 longitudinal veins on forewing and 1 longitudinal vein on hind wing. The tarsus has 2 claws, the tail of the female is pointed, and the tail of the male is pincer-shaped. At rest, the left and right wings are closed together to form a roof ridge, and there is an obvious slit on the back of the ridge [7,29-34].

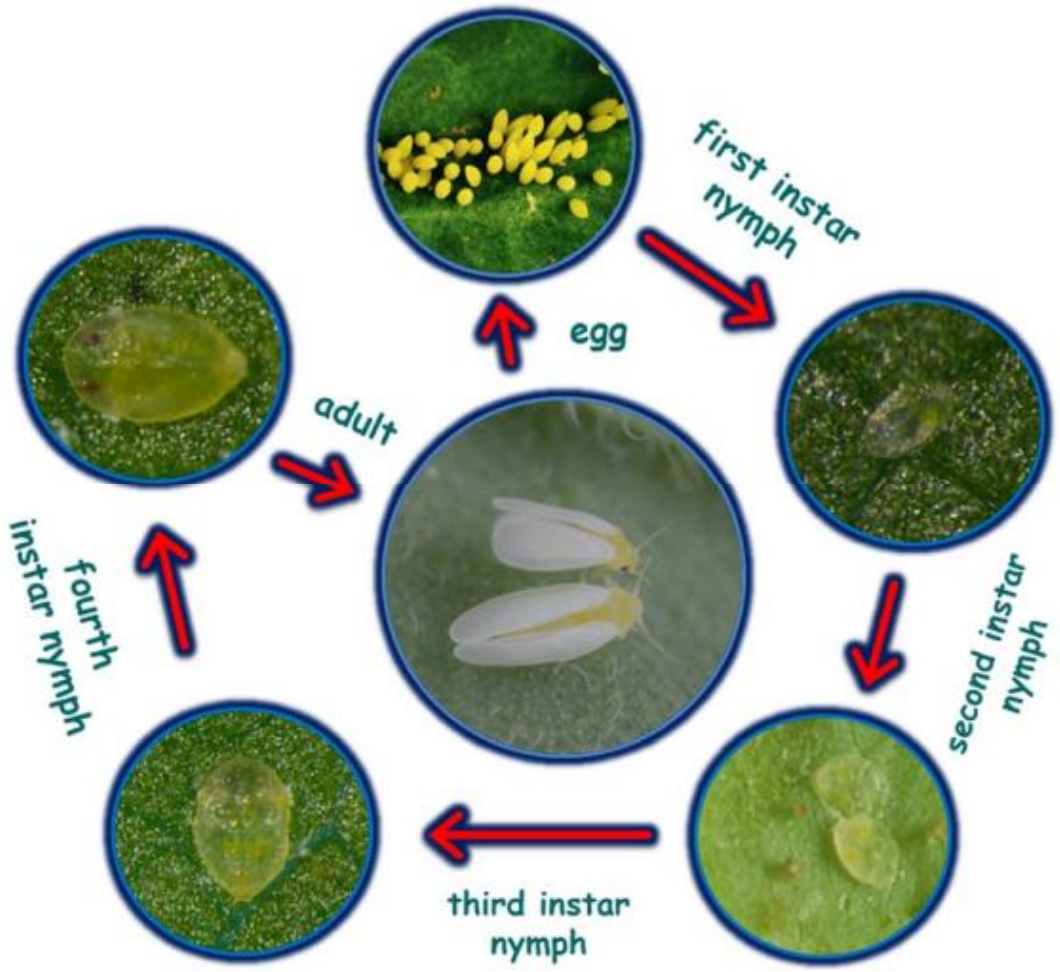


Figure 1.5 The figure of B. tabaci life history (Gong Chen).

1.3 Harm of *B. tabaci*

The damage of *B. tabaci* to crops is mainly manifested in direct feeding of plant juice, affecting plant nutrient metabolism, resulting in yellow spots on plant leaves, yellowing and falling off in severe cases, and abnormal or irregular fruit structure. The adults and nymphs of *B. tabaci* can secrete honeydew to pollute plant organs and induce the occurrence of sooty disease. When the density is high, the leaves will turn black, which will seriously affect the photosynthesis of plants and reduce the quality of crops [8]. Another important damage mode of *B. tabaci* is the transmission of plant viruses. Generally, after the outbreak of *B. tabaci*, the virus transmitted by it will occur greatly [31]. These viruses cause severe damage by causing leaf curling, dwarfing plants and fruit abortion [35-38].

The tone of *B. tabaci* is piercing-sucking. When feeding, the nymphs and adults pierce the phloem of the host plant to suck the sap. *B. tabaci* develops rapidly, reproduces strongly, and the population density is high, which leads to the weakening of plants. For example, after tomato infestation, the fruit ripens irregularly, showing a faded skin color, some with normal skin color but white, hardened or immature internal tissue. Typically, a plant will exhibit physiological abnormalities if it is fed by 5-10 nymphs, but these physiological abnormalities are often absent from feeding by adults [39,40].

B. tabaci nymphs and adults will secrete honeydew while feeding on plant sap, polluting plant organs and products, inducing the occurrence of sooty disease, and seriously affecting the appearance quality of crops. When the *B. tabaci* density is high, crop leaves turn black, and the photosynthesis of crops is severely hindered [41,42].

B. tabaci is an important transmission vector for many viral diseases, which generally occur shortly after an outbreak of *B. tabaci* [43,44]. *B. tabaci* most easily transmit geminiviruses on cucurbit, mallow and nightshade plants. After plants are infected with geminiviruses, the plants show symptoms of dwarfing, yellowing,

chlorotic mottled and leaf curling, some leaves become smaller, the leaf surface is shriveled, the edges of the leaves are curled downwards or upwards, and some leaves are shrunk into a ball shape, etc. *B. tabaci* can also transmit a variety of viruses such as the long-line virome, carnation virome, and potato virome on crops [45,46]. These viruses can cause leaf curling, yellowing or dwarfing of plants, and can also cause fruit abortion, resulting in severe losses [47,48].

Among the many plant viruses transmitted by *B. tabaci*, there is a special type of virus, namely the twinviridae virus, which can only be transmitted by *B. tabaci*. Current reports show that most of the virus species transmitted by *B. tabaci* belong to the twinviridae virus family, and there are about more than 111 kinds [36]. Tomato etiolated leaf curl virus is one of the most serious viruses transmitted by *B. tabaci* [43]. This virus can be transmitted by *B. tabaci* for a long time, infecting a large number of tomato plants, resulting in a large reduction in the production of tomatoes in the field, resulting in serious economic losses [36].

B. tabaci can transmit approximately 270 viruses belonging to the genus Begomoviru in the family Geminivirida [43]. In addition to Tomato etiolated leaf curl virus, *B. tabaci* also transmitted two viruses in China, Tomato chlorosisvirus (ToCV) and Cucurbit chlorotic yellow virus (CCYV). Melons faded green yellowing virus found time later than faded green tomato virus, its main infection of cucumber, watermelon and melon, melon crops, such as now in China, Zhejiang, Heilongjiang, Fujian, Hebei, Shanxi, Hunan, Hubei, Shandong, Jiangsu, Hainan, Anhui, and Beijing and other provinces to carry the virus detected whiteflies species [40]. Guo et al. reported the distribution of *B.tabaci* tomato chlorosis virus in provinces of China and the year of first detection [28].

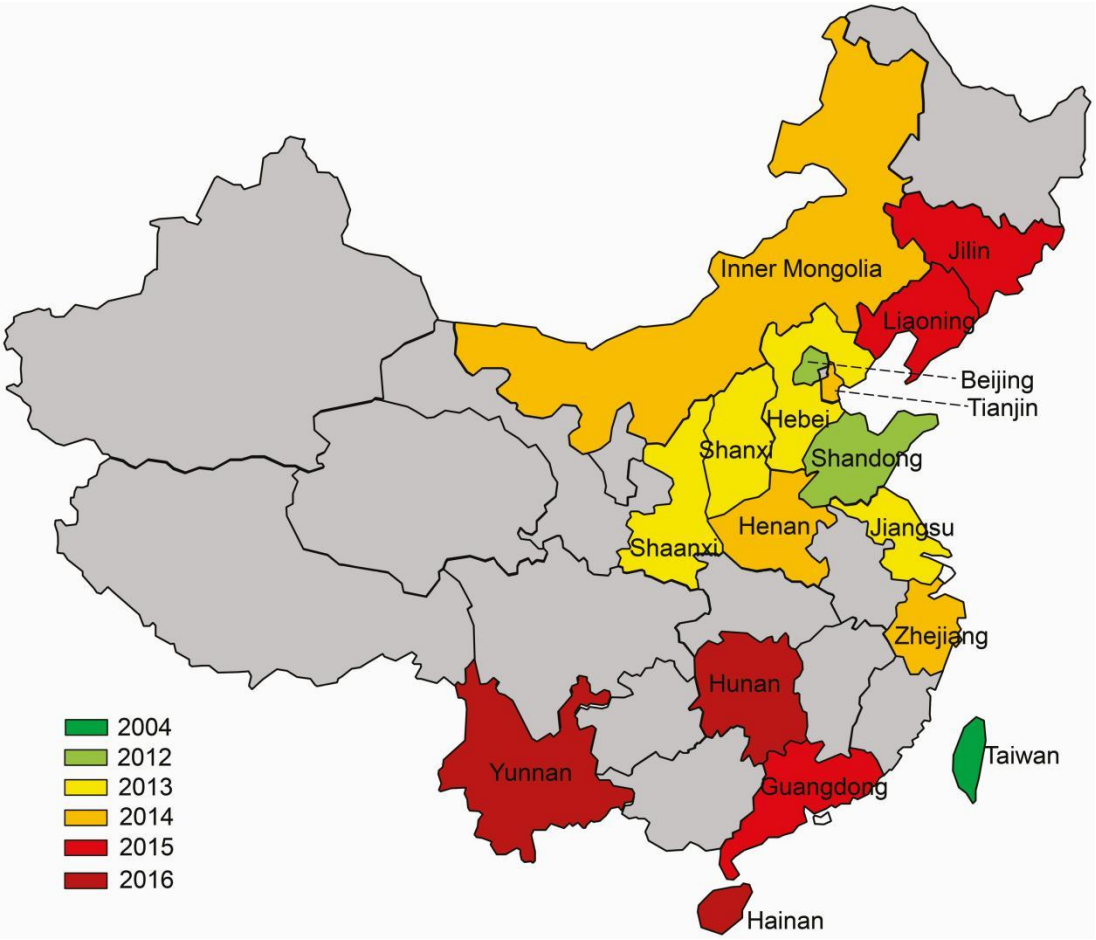


Figure 1.6 Distribution and year of first detection of Tomato chlorosis virus in provinces of China.

1.4 *B. tabaci* host plant

B. tabaci is a polyphagous pest with a wide range of hosts, and its host range is increasing year by year. In 1970, Azab et al. reported that the host of *B. tabaci* in Egypt had at least 155 species of plants [49]. In 1978, Mound and Halsey listed more than 300 host plants in the book "whitefly of the world" [2]. In the 1980s, Greathed et al. reported that the host plants of *B. tabaci* include 420 species of 74 families [50]. At present, the host of *B. tabaci* is estimated to have reached more than 600 species. *B. tabaci* mainly damage economic crops such as Cruciferae, Compositae, Solanaceae, Cucurbitaceae, Leguminosae and Euphorbiaceae, including vegetables, fruit trees, flowers, ornamental plants, economic plants and wild weeds [51].

Although its host range is very wide, the growth rate, mortality, and fecundity of *B. tabaci* differ on different host plants [52]. For example, *B. tabaci* had higher survival rates of immature nymphs on tomato, eggplant, cucumber, and cabbage, while the mortality of young nymphs was higher on pepper, kidney bean and tobacco. At the same time, the difference of host plants will also affect the growth and development of *B. tabaci* population, and *B. tabaci* also has certain selectivity to host plants, and different biotypes of *B. tabaci* also have a certain degree of host for feeding and laying eggs [53,54]. In an environment with a moderate population density, *B. tabaci* has strong selectivity to host plants such as cauliflower, cucumber, eggplant, green beans, and squash, but poor selectivity to host plants such as spinach, amaranth, and carrot [55,56].

1.5 Biological characteristics of *B. tabaci*

1.5.1 Life history of *B. tabaci*

Under suitable climatic conditions, *B. tabaci* can have 11-15 generations in one year, and some can have 19-21 generations. *B. tabaci* cannot overwinter on open field crops in northern China, but can reproduce for many years in protected areas, with a population peak almost once a month. *B. tabaci* generally have an egg period of 5 days and a nymph period of 15 days, and it takes 19-27 days to complete a generation [57,58].

The development time of *B. tabaci* on different host plants varies, and it takes 18-30 days to develop from egg to adult at 25℃. *B. tabaci* adults usually lay their eggs on the underside of leaves of host plants after feeding on leaf sap. Under suitable conditions, they hatch into nymphs after 4-5 days, and molt into the adult stage in about 7 days [59,60]. *B. tabaci* adults mostly emerge under light conditions. Adults mate within 1-8 hours after eclosion in summer, and within 3 days after eclosion in spring and autumn. *B. tabaci* adults can spread within or between plants within a short distance, while large-scale seedlings and seed transportation can spread them over long distances,

and they can also migrate long distances with the help of wind or airflow [61,62]. *B. tabaci* adults are not good at flying, have a strong tendency to yellow, and gradually spread the harm after occurrence of multiple spots in the field. The adult lifespan of *B. tabaci* is generally about 2 weeks, and it can be as long as 1-2 months. *B. tabaci* has obvious photophilia, and its activity peak is from 11:00 am to 3:00 pm every day [57].

B. tabaci adults like to lay eggs on the middle and upper leaves of host plants, mostly in young parts. The egg-laying ability of *B. tabaci* is closely related to environmental temperature, host plant types, and different geographic population densities [63]. According to reports, under the condition of the average temperature of 28.5 ℃ from July to August in China, the average number of eggs laid by *B. tabaci* on cotton is 252/head, and under the condition of the average temperature of 22.7 ℃ from October to November, the average number of eggs laid on cotton is 187/head. The average number of eggs laid was only 61 per head under the condition of an average temperature of 14.3℃ from December to January of the following year [57].

The nymphal period of *B. tabaci* is about 15 days. The first instar nymphs have relatively long tentacles and legs when they hatch, with the body half-bent until the front legs can grasp the leaves, detaching from the discarded soft shell. It generally crawls a few centimeters on the leaves or crawls to other leaves of the same plant to find a suitable feeding point, and then inserts the needle into the phloem on the back of the leaf to feed on the sap. Usually 2-3 days after starting to feed, it sheds its skin and enters the second age. The morphological characteristics of *B. tabaci* vary greatly in the pseudopupa stage. The main reason is that it is related to the host, and different morphological changes occur on different host species. For example, on the leaves of hairy plants, most of the pupal shells have setae on the back, but there are no steel hairs on the smooth plant leaves [64-66].

1.5.2 Biotype of *B. tabaci*

B. tabaci have been described by researchers as a composite species composed of multiple biotypes [7], according to the differences in host plant adaptation, host range and plant virus transmission ability of *B. tabaci* [35,67,68] . The large outbreak of the *B. tabaci* population first appeared in the United States, and entomologists have confirmed that the *B. tabaci* population in the United States is not an original local population, but an invasive species that has newly invaded the United States from other places [63]. Studies have shown that the pseudopupae of *B. tabaci* may change depending on the host plants they feed on. However, in terms of host range and egg production, the newly invaded *B. tabaci* showed a wide host range and large egg production, and transmitted many kinds of plant geminiviruses, such as squash leaf curl virus and tomato necrotic dwarf virus, etc. Injury can cause abnormal plant physiology, including silver leaf reaction of zucchini and irregular ripening of tomato [6,31]. Using isoelectric focusing electrophoresis to study the polymorphism of *B. tabaci* esterase isoenzymes is the earliest method to distinguish *B. tabaci* biotypes based on the differences in the characteristic bands of different biotypes of *B. tabaci* [6]. The polymorphisms of the esterase alleles of *B. tabaci* biotypes A and B, the researchers call the original American *B. tabaci* population type A, while the newly invaded population is called type B [3].

At present, in the phylogenetic and systematic studies of *B. tabaci*, there is sufficient evidence to show that it is a species complex containing at least 31 cryptic species [4]. 15 Cryptophytes of *B. tabaci*, including 13 native species and 2 global invasive species, have been reported in China [34]. *B. tabaci* biotype B and Q invaded China in the mid-to-late 1990s and around 2003, respectively, and quickly replaced the native species in many areas and became dominant [11]. After several years of transmission and spread of *B. tabaci*, *B. tabaci* broke out in various crops in southern China, seriously endangering the safety of China's planting industry. Chu Dong used this marker to report the Q-type *B. tabaci* for the first time in Kunming, Yunnan Province, China. Since 2005, the Q-type *B. tabaci* has successively replaced the B-type

whitefly in many areas, which is likely to be the same as the Q-type *B. tabaci*. The strong resistance is related to [69]. Temperature is an important environmental factor that affects the growth and development of insects. The change of external environmental temperature can directly affect the individual's physiological metabolism, the activity of various enzymes in the individual, and the synthesis and release of hormones [70,71,72]. Under the condition of transient heat shock, It is beneficial to the occurrence of *B. tabaci* population in the summer high temperature season[73,74].

Sex ratio and sex assignment are important biological characteristics and ecological strategies of arthropods, among which sex ratio is an important factor determining the rise and fall of insect populations. Through field observation, we found that the sex ratio of male to female in *B.tabaci* population was related to season, with more female adults in the early growing season and more male adults in the late summer [41]. *B. tabaci* is a haplodiploid species, that is, under normal circumstances, fertilized eggs (diploids) develop into females and unfertilized eggs (haploids) develop into males [6]. Therefore, the effect of high temperature stress on the sex ratio of *B. tabaci* population may be due to its adverse effect on the gonadal development of males, resulting in the reduction of sperm developmental maturity of *B. tabaci*, thereby reducing the fertilization rate of female adult eggs and increasing the proportion of male offspring. Cui et al. found that heat shock of *B. tabaci* biotype B adults at 39℃ and 41℃ for 1h would significantly increase the proportion of female insects in the offspring, but after exposure to 43℃ or above for 1h, the proportion of female insects in the offspring significantly decreased. The sex ratio of *B. tabaci* fluctuates with seasons. The seasonal fluctuations of whiteflies are also different in different countries or regions, the report said. In Israel, the ratio of females to males is as high as 3:1 in spring, falling to 0.43:1 in late summer, and in India, males dominate from March to July. In the season when female whiteflies are dominant, we can achieve better results in the control of whiteflies [73].

1.5.3 Biotype identification method

Isozyme and protein polymorphisms are the main components of biochemical variation. Isozyme polymorphisms are determined by genes, and their activity and content are regulated by genes. According to the different charges of isozymes, isoelectric focusing electrophoresis can be used to distinguish different enzymes. Polyacrylamide gel electrophoresis uses different molecular weights of isozymes to distinguish different enzymes, and then counts the degree of acquaintance or genetics between different biological groups. distance. There are differences in the characteristic bands of different biotypes of *B. tabaci*, and isoelectric focusing electrophoresis was used to study the polymorphism of *B. tabaci* esterases.

Restriction fragment length polymorphism (RFLP) is a DNA molecular marker technology with molecular hybridization as the core developed in the 1980s. Restriction fragment length polymorphisms obtained by treating DNA with restriction enzymes have relative interspecific stability, and the resulting strips The band is stable and reliable, can reflect the evolutionary relationship between species, and is an effective genetic marker for phylogenetic research. Using the RFLP method combined with PCR technology to analyze the rDNA region of *B. tabaci* and to distinguish *B. tabaci* populations from other host populations is the preferred method to identify *B. tabaci* variation among different host plants [75].

Random amplified polymorphic DNA (RAPD) technology is based on PCR technology and uses random primer amplification for DNA polymorphism research. It has been widely used in the identification and differentiation of biological species, strains, populations and individuals. RAPD technology can be used to analyze the unique loci of different biotypes of *B. tabaci*, and can also be used to analyze the genetic differentiation and relationship of different biotypes of *B. tabaci*. RAPD-PCR technique was used to identify the biotype of *B. tabaci*, and found that the Cv type

distributed in Guangzhou area of China may become a new biotype of *B. tabaci* [76-78].

Amplified fragment length polymorphism (AFLP) is a relatively effective DNA molecular marker method based on PCR technology, which was initially applied to the construction of chromosomal linkage maps. AFLP is to digest genomic DNA by restriction endonucleases, and use the selective amplification of the digested fragments to carry out polymorphism analysis, which is used for the definition of closely related species and interspecies monitoring genetic relationship. Chinese scholars used the AFLP method to analyze the genetic diversity and biotypes (type B and type Q) of 27 geographical populations of *B. tabaci* [79].

The mt DNA COI gene has been widely used in the study of the phylogenetic relationship of *B. tabaci*, and is considered to be the most effective method to reflect the variation of *B. tabaci*. For the first time, the Q-type *B. tabaci* was reported in Kunming, Yunnan Province, China [80]. The mt DNA COI gene fragment marker study was conducted in 5 *B. tabaci* populations affected by the outbreak in China, and *B. tabaci* outbreak was proved [81]. In a subsequent study of *B. tabaci* populations in 35 different geographic populations or host plants in China, three distinct non-B haplotypes were found, while the other 32 populations were all *B. tabaci* [82].

1.6 Ecological characteristics of *B. tabaci*

1.6.1 Effects of Temperature on *B. tabaci* populations

Insects are ectothermic animals, and their ability to regulate their body temperature is very poor. Body temperature basically depends on the surrounding environment temperature. Change is one of the important environmental factors affecting the geographical distribution and population changes of insects [83]. Appropriate temperature is a necessary condition for the normal growth and development of insects. Too high or too low temperature stress will adversely affect

the growth and development of insects [84]. Different insects or different developmental stages of the same insect have a temperature range suitable for their growth and development. High or low temperature has a great impact on the growth and development of insects, especially the survival and reproduction, causing the cessation of growth and development, and even death [85]. The change of the external environment temperature can directly affect the individual's physiological metabolism, the activity of various enzymes in the individual's body, and the synthesis and release of hormones. Therefore, changes in temperature not only affect the growth and development of individual insects, but also affect the behavior of insects [86].

B. tabaci have strong resistance to high temperature and low temperature. In general, they can endure high temperature above 40℃, and they become nymphs and worms continue to survive at 5℃ [60]. High temperature and dryness in summer are favorable conditions for the exponential growth and rampant damage of *B. tabaci* population. The minimum temperature for *B. tabaci* development is 26-28℃. The developmental starting point temperature of each state is slightly different. The minimum temperature for the development of the second instar nymph is 10.5℃, and the temperature for the development of other nymph stages and pseudopupa is generally around 12℃. The starting temperature of generation development is 12.4℃. For each state, the egg stage was the longest, about 89.9 d; the pupal stage (4th instar nymph) was the shortest, about 22.8 d. Temperature has a great influence on the development of *B. tabaci*. The developmental duration of *B. tabaci* is the shortest at 29℃, about 13.9 d. The developmental duration of *B. tabaci* is prolonged below or above this temperature. The period can reach 48.7 d, and it is about 20.7 d at 35℃ [87].

Among the various states of *B. tabaci*, the survival rate of eggs and first-instar nymphs was the lowest, and different temperatures also had a great influence on the survival rate of *B. tabaci*. The survival rate of *B. tabaci* at 26℃was the highest,

reaching 67.3%; The lowest temperature was at 35℃, and the generation survival rate was only 27.6%. The lifespan of *B. tabaci* adults generally shortened with increasing temperature, reaching 39.6 days at 20℃and 12.8 days at 32℃. The number of eggs laid by *B. tabaci* adults also decreased with increasing temperature. The intrinsic growth rate of *B. tabaci* population is greatly affected by temperature, and the intrinsic growth rate at 26℃and 29℃can be twice as high as that at 20℃, and the intrinsic growth rate will decrease with continued warming [87, 88].

According to the research on various biological effects of different temperatures on *B. tabaci* population, 26℃is the optimum temperature for *B. tabaci* growth, and too high or too low will have different degrees of adverse effects on *B. tabaci* development [89, 90,91]. In addition, temperature also affects the eclosion behavior of *B. tabaci*, which is reflected in different eclosion rates at different temperatures. At present, there are not many reports on the temperature selectivity of *B. tabaci*. Skinner (1996) found that the most preferred temperature for egg-laying of *B. tabaci* adults in the range of 20℃ to 40℃, optimum temperature 31℃[92]. The effects of temperature on behaviors other than egg-laying behavior of *B. tabaci* are rarely reported [93].

1.6.2 Effects of humidity on *B. tabaci* populations

In addition to temperature, humidity has become another important ecological factor affecting the growth and development of insects. Different insects or different developmental stages of the same insect have a humidity range suitable for their growth and development. High humidity or drought has a great impact on the growth and development of insects, especially survival or reproduction. Drought and less rainfall are beneficial to the growth and reproduction of *B. tabaci* populations [94,95].

B. tabaci adults have a certain degree of selectivity to humidity, and experiments have shown that: *B. tabaci* adults are mostly active in the humidity condition of 80% RH; humidity has little effect on the growth and development of *B. tabaci*, and its

developmental history under different humidity conditions The effect of humidity on the survival of *B. tabaci*, except for the survival rate in the early stage of adult oviposition, has a significant difference in the impact on other insect states, and the humidity of 60% RH is more conducive to The survival of the insect increases; humidity has a significant impact on the lifespan and egg laying of *B. tabaci* adults. Under different humidity conditions, the adult lifespan is 80%RH>60%RH>40%RH>100%RH, and the number of eggs laid is 80%RH>60%RH>40%RH>100%RH [57]. It can be seen that under the condition of humidity of 60% is conducive to the egg laying of adults, and under the condition of humidity of 80% is more conducive to its survival. In addition, the effect of humidity on *B. tabaci* population growth was also significant, and its population growth index was 60%RH>80%RH>40%RH>100%RH. Based on the summary of the humidity requirements of *B. tabaci* in different periods, it can be seen that low temperature drying is conducive to the occurrence of *B. tabaci* population [96]. *B. tabaci* eggs and nymphs of different instars had the highest survival rate at 75% relative humidity, and the total survival rate reached 77.8%. Under the conditions of 27℃ and relative humidity of 55%, 75% and 95%, the average lifespan of *B. tabaci* adults was 27.5, 31.2 and 15.7 days, respectively, and the number of eggs laid by a single female reached 264.3 eggs, respectively 220.0 and 110.4 [97].

Under different humidity conditions, the individual development and population expansion rates of *B. tabaci* are different. Specifically, under low humidity conditions, *B. tabaci* develops over time and lays more eggs, which contributes to the rapid growth of *B. tabaci* population. However, under high humidity conditions, *B. tabaci* population growth was slower [60]. Different instars have different requirements for temperature. The developmental starting point temperature of 2nd instar nymphs is the lowest, while the effective accumulated temperature required for egg stage is the highest (110.22 days). The intrinsic growth rates (r) at 80%RH were 0.0729, 0.0673, 0.0895 and 0.0486 with 30, 25, 20 and 15℃respectively. Humidity has a significant effect on the lifespan and egg-laying ability of *B. tabaci* adults. Both high and low humidity environments are

not conducive to the survival of *B. tabaci* adults. The average lifespan of *B. tabaci* adults was 24.6 days at 50% RH, but was shortened by 5.5, 5.0 and 10.0 days at 90%, 70% and 30% RH, respectively. The environment with high relative humidity is favorable for *B. tabaci* to lay eggs, and the average number of eggs laid per female is 90%>70%>50%>30%RH. The intrinsic growth rates of *B. tabaci* population at 25℃ with 90%, 70%, 50% and 30% RH treatments were 0.0499, 0.0596, 0.0640 and 0.0856, respectively. The generation net proliferation rate of *B. tabaci* at 50% RH was 14.79, which was twice that at 90% RH, but the weekly growth rate was not much different. The average generation duration of *B. tabaci* was as long as 43.6 days at 50% RH, but only 26.4 days at 30% RH. By comparing the intrinsic growth rate and the net proliferation rate of generations, it can be seen that 30% RH is the most suitable environmental humidity for *B. tabaci* population proliferation [98].

1.7 *B. tabaci* forecasting technology

1.7.1 Field investigation of *B. tabaci*

Because there are significant differences in drug resistance among the cryptic species of *B. tabaci*, monitoring the composition of the cryptic species of *B. tabaci* in the field is very important for controlling *B. tabaci* [99,100]. The field survey of *B. tabaci* generally uses the visual method to calculate the number of its insect population [101]. According to the occurrence of *B. tabaci*, We select 1-2 representative fields or greenhouses and use diagonal 5-point sampling. We record 2-4 strains at each point by the size of the insect population and investigate the number of insect strains and adults in each of the upper, middle, and lower leaves of each plant. During the investigation, we gently turned the leaves, calculated and counted the population density of *B. tabaci*, and comprehensively analyzed to determine the best time for *B. tabaci* control [102-105].

1.7.2 Yellow sticky board

Because adults of *B. tabaci* have a strong tropism for yellow [106,107], the standard method to control *B. tabaci* is yellow board trapping, which is also suitable for investigating the occurrence dynamics of *B. tabaci* [108,109]. In each field or shed room, we use the 5-point placement method and insert a yellow board of 20cm×30cm at each point. The height of the yellow board is that the lower end of the yellow board is slightly higher than the top of the host plant; we replace the yellow plate every ten days. The number of male and female adults on each yellow board is recorded in detail to monitor the occurrence of *B. tabaci* adults in the field. There are three plots for each crop, and five yellow boards are placed on each plot, totaling 15 plots [110]. In order to improve the forecasting and continuous pest control level of *B. tabaci*, The researchers systematically monitored *B. tabaci* on vegetable crops in greenhouses for four consecutive years (2009-2012) by using the yellow board trapping method. The occurrence of *B. tabaci* was different on vegetable crops in different facilities, the largest on cucumber, the second on eggplant and tomato, and the least on pepper [111,112].

1.7.3 Prediction and forecast of *B. tabaci*

In the process of field prediction and forecasting of *B. tabaci*, it is necessary to comprehensively analyze and predict the occurrence trend to inhibit the spread of *B. tabaci* according to the development progress of each insect state the number of insects [113,114] and the number of natural enemies in the field [114-116], combined with the growth of field crops and the recent weather forecast [117,118]. It has become a significant problem to be solved urgently in current agricultural production [119,120]. *B. tabaci* can damage facility vegetables every year, and there is obvious overlapping of generations. When the temperature is suitable and the host is abundant, *B. tabaci* is prone to occur, especially in the hot and dry season [8].

1.7.4 Prediction of occurrence period

According to the field investigation of *B. tabaci*, determine the beginning, peak, and end of the nymphs or adults. On this basis, using the existing calendar research data, referring to the climatic conditions at that time, plus the predicted duration of the insect state, the occurrence time of a certain period of *B. tabaci* is predicted to carry out prevention and cure in time [121-124]. Prediction criteria for occurrence period of *B. tabaci*: the initial peak period is when the cumulative development progress reaches 16%, the peak period is when it reaches 50%, and the final peak period is when it reaches 84%[49]. In the process of growth and development, each insect needs to absorb a certain amount of heat from the outside to complete a particular stage of development, and the total heat required by insects at each developmental stage is a constant (Effective Accumulated Temperature Law) [125-129]. When the developmental starting temperature and effective accumulated temperature of a certain instar or insect stage of *B. tabaci* are measured, the generation number of *B. tabaci* can be predicted by the accumulated temperature formula based on the developmental state of *B. tabaci* and the recent temperature forecast [89,130-132]. The formula for calculating the developmental duration is as follows:

$$N = \frac{k}{t - t_0}$$

In the formula, N is the developmental period; k is the effective accumulated temperature; t is the average temperature; t_0 is the temperature at the development point.

1.7.5 Prediction of occurrence quantity

The population growth and decline of *B. tabaci* are affected by various environmental factors. We need to identify dominant factors from complex environmental factors and use the dynamics of these factors as predictors of *B. tabaci* population density [133-137]. Then we combined the occurrence over the years to conduct a comprehensive analysis to predict the occurrence of *B. tabaci* on a specific host plant [91,138].

1.7.6 Control index of *B. tabaci*

Based on the prediction of the occurrence period and amount of *B. tabaci*, and according to the growth status of crops, further research to predict whether the sensitive period of a particular crop to be harmed by *B. tabaci* is consistent with the most significant number of insects and the damage period. So we can infer the degree of occurrence of pests or the size of losses to determine the prevention period and method [139-141].

For the control index and occurrence degree of *B. tabaci*, we usually refer to"Prediction, Forecast, and Control of Major Crop Diseases and Insect Pests"[102]. The classification standard of *B. tabaci* is determined as follows: the number of first-level single leaf insects is less than 10; the number of second-level single leaf insects is 10-30; the number of third-level single leaf insects is 30-50; the number of fourth-level single leaf insects is more than 50 (Unit Quantity: head) [142,143].

1.8 Integrated control of *B. tabaci*

When *B. tabaci* outbreaks are damaged, it cannot be effectively controlled by using a single control technology. We need to pay attention to the daily phytosanitary work. In agricultural production, we should take agricultural prevention and control as the basis, and comprehensively use physical, biological and chemical measures to reduce the damage and reduce the loss to a lower level [144].

1.8.1 Plant quarantine

Plant quarantine is the protection of the life or health of plants in Member States from risks arising from the introduction, establishment or spread of pests carried by plants or plant products, and the prevention or limitation of the introduction, establishment or spread of pests. All official activities of dissemination of other damages. plant quarantine is one of the most cutting-edge measures in plant protection.

The content of plant quarantine involves all aspects of prevention, elimination and eradication in plant protection.

Plant quarantine has a history of more than 100 years in the world, and it has also experienced more than 80 years of development in China. Especially with the development of modern transportation and the increasing circulation of plants and plant products, phytosanitary work has been paid more and more attention by governments of various countries, and countries have generally established legislative systems. Plant quarantine has become an important part of a country's exercise of sovereignty and an important part of plant protection cooperation in the world today [145].

Under natural circumstances, once the pests have the opportunity to enter a new environment, without the control of natural enemies, and without the various factors that restrict their growth, development and reproduction, they often cause serious harm and are difficult to control. Before the 1980s, *B. tabaci* was a major pest of cotton in countries such as the United States, the Soviet Union, Egypt, India, Brazil and Turkey. However, along with human migration and transfer of flowers, *B. tabaci* were also found on crops such as tomatoes in Mexico and flowers in Japan [146,147].

B. *tabaci* was not a major pest in China for a long time. In recent years, *B. tabaci* broke out in some areas in eastern China one after another. At present, *B. tabaci* has occurred on a large scale in China, but for those areas where the occurrence of *B. tabaci* is small or other more harmful *B. tabaci* biotypes, such as biotype Q have not invaded, it is necessary to introduce and adjust *B. tabaci*. The exported vegetables, flowers and plants and their packaging materials are subject to strict quarantine. At the same time, for cassava and sweet potato cuttings from tropical regions, Africa and South Asia, as well as vegetables, flower plants and their packaging materials from other whitefly recurrence areas, we need to carry out phytosanitary, pest control and isolation tests to control Q. The further spread and spread of dangerous biological types such as biotype Q and B [148].

1.8.2 Agricultural control

Agricultural control is to create a farmland ecological environment that is conducive to the growth and development of crops and beneficial organisms but is not conducive to the occurrence of pests by combining the entire agricultural operation process, making full use of the relationship between pests, crops and environmental factors, so as to avoid or A pest control method for inhibiting the development and reproduction of pests and ensuring the safe production of crops. Agricultural control is from the overall point of view of the agro-ecosystem, which can reduce the base of insect sources, thereby reducing the cost of pest control [144].

Type B whitefly likes to feed on crops with hypertrophic, broad leaves and more hairy on the back of the leaves, and likes to feed on crops such as eggplant, soybean, cotton and cucumber, but does not like to feed on plants with smooth and hairless leaves, such as celery , lettuce and spinach. The growth and population growth of *B. tabaci* on host plants that do not like to feed is very slow. It is recommended to rotate the host crops that *B. tabaci* like to eat and crops that *B. tabaci* do not like to eat, such as celery and garlic, cut off the natural life history of *B. tabaci* from the overwintering link and the spreading link, and reduce the number of *B. tabaci* in the field. Alleviate damage to cultivated crops [149].

Appropriate adjustment of the sowing period of crops can effectively prevent the occurrence of *B. tabaci* and the viruses it carries in a continuous large area between crops. For 1-year crops, especially those with short growing seasons, the harm can be mitigated by adjusting the sowing date of the crops. For example, the use of plastic film mulching to increase soil temperature to sow cotton in early spring can significantly reduce the harm of *B. tabaci*. Adjusting the transplanting period of vegetables can not only effectively control the direct damage of *B. tabaci*, but also successfully reduce the infestation of virus diseases[149].

There are often harmful organisms lurking in the dead branches, fallen leaves and fallen fruits of crops. These residues provide a good overwintering place for *B. tabaci* and increase the population base of the following year. After the crops are harvested, deal with the remaining branches and leaves of the previous crops in time, bury them deeply or burn them, and do a good job in cleaning the pastoral fields. The host range of *B. tabaci* is very wide, and some weeds are also good host plants. Removing weeds in the field can help reduce the choice of host by *B. tabaci* and reduce the potential rate of virus infection [150]. The Q-type whitefly can replace the B-type whitefly in some areas because the Q-type whitefly has a stronger survivability on weeds in these areas, plays a role in maintaining virulence, and is an important source of the initial epidemic of the virus .

Strict disinfection should be carried out when raising seedlings, eggs and adults should be completely eliminated, management of seedlings should be strengthened, and insect nets should be strictly covered during the whole seedling raising process. Strictly bring the insect seedlings into the greenhouse for colonization to reduce the number of overwintering insect populations, which is conducive to the control of *B. tabaci* in the following year. The selection and breeding of crop varieties resistant to *B. tabaci* or indirect damage by *B. tabaci* is a measure to prevent crops from being infected by *B. tabaci* and to induce abnormal growth of plants. At present, the identification and evaluation of germplasm technology for isolation of *B. tabaci* resistance has been widely used to screen existing varieties. Criteria for evaluation and screening include: the degree of plant resistance to *B. tabaci*, the choice of feeding and laying eggs, the presence or absence of adverse effects on *B. tabaci*, and the degree of response to viruses and plant irregularities [151].

When the occurrence of *B. tabaci* is serious, its host can be eliminated through the fallow period of the land, and the base number of the pest population can be reduced, so as to inhibit the occurrence and harm of the pest. In the control of *B. tabaci*, the relationship between crops can be used to grow some crops or provide resting places for

natural enemies, or control the behavior of *B. tabaci* to seek hosts for better control, such as the use of melons. Soybeans and pumpkins are the favorite hosts of *B. tabaci*, and melon vegetables are selected as lure fields to trap and kill *B. tabaci*.

Reasonable fertilization can improve the nutritional conditions of plants, promote plant growth and development, accelerate the healing of insect-injured tissues, improve their ability to resist insects, and avoid the heyday of pest damage. The study found that poinsettia with high nitrogen content in the greenhouse has a high number of *B. tabaci*, laying more eggs, and more nymphs develop to adults. Some studies have shown that the higher the amount of nitrogen fertilizer in cotton fields, the higher the population density of *B. tabaci* adults and pupae. Drought and less rainfall, high temperature and less water on the surface of plant leaves, correspondingly increased nutrient concentration, which is conducive to the occurrence of *B. tabaci*. Experiments showed that by adjusting the amount of irrigation or watering of cotton, the population density of *B. tabaci* was significantly affected. It is very important to maintain the appropriate water content of cotton leaves in cotton fields, not too much or too little. Watering once a week is more appropriate, and the number of *B. tabaci* on leaves is significantly less than watering twice a week.

1.8.3 Physical control

Physical control is the application of various physical factors, such as light, heat, electrical temperature, humidity and sound waves, as well as mechanical equipment to eliminate pests or change their physical environment, thereby preventing pests from occurring or invading. It is an ideal pollution-free control method.

B. tabaci adults have a strong tendency to yellow. Hanging yellow sticky insect boards to trap and kill *B. tabaci* adults in field production can effectively reduce the population of *B. tabaci* in greenhouses and fields, and play a better role in the next year's insect source. inhibitory effect. The hanging height of the yellow board is as follows: the lower end of the yellow board is slightly higher than the top of the crop for

non-framed crops, and the middle of the framed crops is appropriate. When using the yellow board to trap *B. tabaci* in cotton fields, under the same height and density, inserting the yellow board into the row of cotton plants has a better trapping effect [152]. The yellow boards were placed at different heights to attract *B. tabaci* on tomato in the sunshade. The experiment showed that the yellow boards hung at the same height as the top of the plants at the same time had the best trapping effect on *B. tabaci* [112].

Greenhouses can control *B. tabaci* by artificially regulating the temperature in the greenhouses. Studies have shown that 26℃ is the optimum temperature for *B. tabaci* population growth [57]. Both low temperature and high temperature have inhibitory effects on the development and survival of *B. tabaci*. In production, the temperature and humidity that are not conducive to the growth, development and reproduction of the pest can be used to reduce the population density [153]. High temperature and stuffy sheds are generally selected on sunny days during the fallow period in summer. The temperature inside the sheds is controlled at 45-48℃, and the relative air humidity is over 90%. A stuffy shed for 24 hours can achieve better results in controlling *B. tabaci* [154]. At the same time, we can also choose to open the greenhouse in winter, and use the natural low temperature to kill the overwintering insect state of *B. tabaci* and reduce the number of overwintering insect populations.

Place insect-proof nets to cover and cultivate to prevent the invasion of *B. tabaci*. When cultivating insect-free seedlings, 60-mesh insect-proof nets can be used to isolate and raise seedlings between the nursery bed and field production to prevent the entry of *B. tabaci*. For crops such as vegetables grown in greenhouses in winter and spring, 60-mesh insect-proof nets can be arranged on the inside of the film around the greenhouse and at the door, and insect-proof nets can be used to cover the entire greenhouse in summer. The harmless treatment of stubble should also be done after harvest to destroy the parasitic sites of pests [57].

1.8.4 Biological control

Biological control is a method of using organisms or their metabolites to control or reduce the degree of harm to harmful animal and plant populations. China began to study the biological control of agricultural pests in the 1930s. Because biological control is safe for humans and animals, it can inhibit pests for a long time, and protect the ecological environment without causing 3R problems, and residues, resistance and pests are rampant again, providing a new way for the control of *B. tabaci* [155]. The biological control of *B. tabaci* is mainly controlled by natural enemies. The natural enemies of *B. tabaci* are rich in resources, mainly including predatory natural enemies and parasitic natural enemies [156].

There are many kinds of predatory natural enemies of *B. tabaci*, mainly Coleoptera, Neuroptera, Hemiptera and predatory mites. Lady beetles are important natural enemies of *B. tabaci*, mainly including lady beetles and lady beetles [157]. The little black lady beetle is native to North America. It is the exclusive predator of whitefly and is a new dominant species of natural enemy to control whitefly pests. According to the selective feeding experiment of the little black lady beetle, it was found that the little black lady beetle likes to feed on the ornamental plants poinsettia, hibiscus and cabbage, and does not like to feed on the nightshade crops tomato and eggplant [158]. The lady beetle is an important predatory natural enemy resource of *B. tabaci* in China, and it is distributed in Zhejiang, Fujian, Sichuan and Guangdong. Both adults and larvae of *L. serrata* can prey on various states of *B. tabaci*, and the predation amount of *B. tabaci* by adults decreases with the increase of state and age of *B. tabaci* [159].

Orius sauteri Poppius are distributed in Liaoning, Beijing, Hebei and Sichuan in China. They can prey on pests such as whiteflies, aphids, thrips and spider mites. They are polyphagous predators in gardens, greenhouses and farmland. *E. asiatica* has a strong foraging activity. The first to second-instar nymphs have very little prey, and the third-instar nymphs start to prey more significantly. They have been successfully bred and used in China [160].

Predatory mites are potential predators of *B. tabaci*. As a common species of natural enemies of *B. tabaci*, amblyseii Cucumis is the main product of various natural enemy companies in the world. It is mainly released in the seedling stage of cucumber and pepper in greenhouse to prevent and control the massive occurrence and outbreak of *B. tabaci* and thrips [161].

Chinese lacewing is distributed in most parts of China. It is a kind of predatory insect that has been successfully studied and utilized in China. It has a wide range of temperature adaptation, strong stress resistance, short generation period, easy feeding and reproduction, and can prey on whiteflies. , aphids, spider mites, and some lepidopteran pests. Generally, when raised indoors, the adult lifespan of Lacewings sinensis is 30-50 days, and one female produces 800-1000 eggs. According to the research results of the Institute of Plant Protection, Beijing Academy of Agriculture and Forestry and other units, one Chinese lacewing can prey on 172 *B. tabaci* during the entire larval stage [57].

Aphid wasps belong to the order Hymenoptera, Aphididae. It reproduces parthenogenetically with females, and can lay eggs and parasites on the larvae of any instar B. tabaci, and like to lay eggs in the third to fourth instar larvae. Under suitable conditions, a female adult can lay a maximum of about 10 eggs per day, with a maximum of 196 eggs produced, and can survive for more than a month. Studies have shown that the control of *B. tabaci* parasitic rate by the release of Aphididae is up to 83%. The Institute of Plant Protection, Beijing Academy of Agriculture and Forestry, used *B. tabaci* to control *B. tabaci* on tomato and cucumber in the greenhouse, and achieved ideal control effects [57].

With the increasing demand for healthy agricultural products, using green control methods and screening effective natural enemies in different regions is an important method to effectively control *B. tabaci*. China reported a total of 109 species of predatory natural enemies in 26 families, and a total of 59 species of parasitic natural

enemies in 2 families. Among them, *B. tabaci* has considerable inhibitory ability to *B. tabaci*, and it is also one of the natural enemies that is widely used to control *B. tabaci* [6].

1.8.5 Chemical control

The key to chemical control lies in rational and timely drug use, and the use of some selective pesticides with high efficiency, low toxicity and low residues. Commonly used chemical control methods mainly include dusting method, particle spreading method, spray method, seedling treatment method, fumigation method and smoke method. The insecticidal effects of chemical agents can be divided into stomach poisoning, contact killing, systemic suction, fumigation, antifeeding and repelling effects.

Because the body surface of *B. tabaci* is waxy, reproduces rapidly, and the generations overlap seriously, it is easy to develop drug resistance. In the early stage of *B. tabaci,* the scientific and rational use of pesticides is a very important control method. In production, it is recommended to use 17% fluoropyranone solution to control *B. tabaci*, and alternately use 25% thiamethoxam water dispersible granules and 10% cyantraniliprole suspension to avoid drug resistance [162].

When using imidacloprid and avermectin insecticides in production, we should pay attention to reasonable rotation and alternate use, and avoid continuous use, so as to delay the emergence and development of *B. tabaci* resistance [163]. At present, there are three main types of conventional chemical insecticides used to control *B. tabaci*: nicotinic insecticides (imidacloprid), synthetic insecticides (pyrethroids), and insect growth regulators (propoxolin), Due to the long-term use of these chemicals to control *B. tabaci*, the *B. tabaci* has developed certain resistance to these types of chemicals, making it difficult to control *B. tabaci*. At this stage, we often use the method of chemical rotation to slow down the resistance of *B. tabaci*. Commonly used chemical

agents such as 10% imidacloprid, abamectin, 25% Aktai water dispersible granules, etc., are used for control in the early stage of *B. tabaci* [164,165].

1.9 Heat shock proteins

Heat shock proteins (HSPs) are anti-stress proteins when organisms are under the pressure of adverse environmental conditions for a certain period [166,167]. HSPs can be used as molecular chaperones to transfer intracellular nascent peptide chains and recognize denatured proteins, and it is an essential mechanism for organisms to cope with adverse environments [168-171].

In 1962, Ritossa [172] first discovered that a brief heat shock could induce the formation of new bulges in the salivary gland chromosomes of Drosophila melanogaster larvae, which is called heat shock response (HSR). After that, many studies have proved that heat shock proteins have the function of conferring heat resistance to organisms [173-176]. Until 1974, Tissieres et al. [177] used SDS-PAGE and autoradiography to confirm that the substance predicted by Ritossa at that time was a group of particular proteins and named these proteins as HSP. Furthermore, whiteflies can utilize heat shock proteins (HSPs) (encoded by Hsp genes) and other stress-related genes to overcome thermal stress [178]. When *B. tabaci* is exposed to harsh environments to a sub-lethal level, heat shock proteins will increase or decrease protein expression to supplement cellular toughness. This paper reviews the different types, characteristics and gene expression of HSP in *B. tabaci*, in order to illustrate the progress of HSP in *B. tabaci* research and provide reference information for further research of *B. tabaci* [179].

1.9.1 Classification of heat shock proteins

In recent years, with the rapid development of biological science and technology and the improvement of sequencing efficiency and accuracy, the research on HSPs has made significant progress. At present, we divide heat shock proteins into five families: Hsp90, Hsp70, Hsp60, small-molecule heat shock proteins, and ubiquitin according to

their molecular weight and homology similarity [175,180-182]. Within the HSPs, Hsp70s are the most studied group[183]. There are many reports on Hsp90 and Hsp70 of *B. tabaci* [184]. Salvucci et al. [178] found that Hsp70 and Hsp90 were the major polypeptides synthesized by *whiteflies* in response to heat stress. Wang et al.[185] observations highlighted the molecular evolutionary properties and the response mechanism to temperature assaults of Hsp genes in *whitefly*.

1.9.1.1 Hsp90

Hsp90 exists in various types of cytoplasm under normal or stress conditions. Its primary function is to bind to denatured proteins as a molecular chaperone and participate in the regulation and maintenance of the conformation and role of various proteins in cells so that cells can usually survive under a stress environment [186-188]. Hsp90 can also interact with signal transduction proteins, promote the binding of steroid hormone receptors and protein kinases to form complexes, and regulate kinase phosphorylation activity [187,189-191]. The interaction between environmental stress and Hsp90 of *B. tabaci* and the analysis of the molecular mechanism has practical significance for further understanding the resistance mechanism of *B. tabaci* to achieve the control effect [186,192]. Kinene [193]investigated the variability of the HSP90 gene in the *B. tabaci* species complex and found evidence of recombination in the coding region of the HSP90 gene in the *B. tabaci* species complex.

1.9.1.2 Hsp70

The Hsp70 family is a class of highly conserved heat shock proteins. Its main functions are: involved in protein folding and unfolding, protein translocation, and multimeric complex translocation. It has weak ATPase activity when combined with ATP [194,195]. When *B. tabaci* is under high-temperature stress, a large amount of Hsp70 is synthesized in the body to protect it from or reduce high-temperature damage [196,197]. Differences in heat shock proteins (HSPs), especially Hsp70, which plays a

vital role in heat tolerance, might cause the observed differences between females and males of *B. tabaci* [192,198].

1.9.1.3 Hsp60

Hsp60 usually exists in the cytoplasm and mitochondria. Hsp60 is not only involved in the folding and assembly of proteins encoded by nuclear genes after entering mitochondria, but also in the folding, assembly and transport of proteins encoded by mitochondria themselves [199]. Under stress conditions, Hsp60 binds to ATP first, causing its own conformational change, so that it can bind proteins for maintenance and repair [200]. Wang et al. [184] employed comprehensive genomics approaches to identify one Hsp60 in the Middle East Asia Minor 1 whitefly genome.

1.9.1.4 Small heat shock proteins

Small heat shock proteins exist in highly ordered oligomers in organisms. Because they have different biological functions in different environments, they are usually in two states of dissociation and aggregation. Their main parts are: participating in protein folding, unfolding, and assembling multimeric complexes [180,201,202]. Improving diapause and cold tolerance for most insects is vital for their safe overwintering. Small heat shock proteins have an essential contribution to enhancing diapause and cold tolerance of insects [203-205]. Small heat shock proteins (sHSPs) are probably the most diverse in structure and function among the various superfamilies of stress proteins, and they play essential roles in different biological processes. Bai et al. [206] confirmed that the sHSP genes of *B. tabaci* had shown differential expression changes under thermal stress.

1.9.1.5 Ubiquitin

Ubiquitin is a protein found in eukaryotic cells either free or covalently joined to a variety of cytoplasmic and nuclear proteins[207]. Its physiological function is to

participate in protein degradation[208]. Xia et al. [209] found that ubiquitin-proteasome system might help the whitefly to counteract the negative influence from TYLCV through degrading the virus directly or activating immune response.

1.9.2 Characteristics of heat shock proteins

Heat shock proteins were initially considered unique proteins expressed by organisms in response to increased temperature. Still, studies have found that a class of heat shock genes is also significantly expressed in unstimulated cells or produced in specific cell cycle stages [204,210]. Meanwhile, studies have shown that many heat shock proteins exist in mitochondria and chloroplasts. Therefore, heat shock protein genes are a multigene superfamily in which not all members are regulated by heat shock [211-213]. Subsequent studies have shown that organisms may induce the synthesis of such stress proteins under stressful environmental conditions such as high temperature, salinity, drought, and osmosis, which function as molecular chaperones in cells and participate in folding new peptide chains, protein assembly, and transport [214,215].

The growth and development of insects are very complex, they go through different developmental stages, and insects in different developmental stages also have significant differences in their morphology [216]. Heat shock proteins can improve the tolerance of organisms to adverse environments and protect organisms or cells from minor damage in subsequent lethal stress [217]. Organisms can often acquire heat tolerance under higher temperature stress after treating sub-lethal high temperatures [173]. Jinn et al.[218,219] showed that the expression of HSPs is related to heat resistance, but also the thermal stability of different kinds of HSPs can substitute for each other. Heat shock proteins (HSPs) as molecular chaperones to assist in the refolding, stabilization, intracellular transport, and degradation of proteins to prevent the accumulation of damaged proteins and maintain the stability of the intracellular environment [167,212,220].

1.9.3 Heat shock protein gene

Studies have found that the heat tolerance of organisms is closely related to the structure and expression of their Hsp genes [192,221,222]. The regulation of heat shock gene expression includes selective transcription and alternative translation; the former is the main one [218]. Studies have shown that heat shock proteins are not directly involved in protecting their intracellular environment in these organisms, but bind to the heat shock element (HSE) through heat shock transcriptional factor (HSF), to form transcription complexes and promote the expression of heat shock protein genes [223,224].

In organisms, the structure and function of HSF have less variation in evolution and have extensive homology. It is a protein that is ubiquitous in eukaryotic cells. We divided them into four types according to their different functions, including Hsf1, Hsf2, Hsf3, and Hsf4 [225]. Hsf1 is considered a major regulator of cellular heat shock protein expression. It is highly conserved in yeast, drosophila, and vertebrates, and the other three HSFs cannot replace Hsf1 [226-229]. Hsf2 is resistant to heat-stimulating signals and is generally more sensitive to signals representing growth, development, and differentiation [230]. Hsf3 is a bird-specific heat-shock regulator [224,231]. Hsf4 only exists in the human body, does not activate the transcription process, and plays an important role in cataract occurrence. Hsf4 can inhibit the expression of heat shock genes under certain conditions [232]. The molecular mechanism of heat tolerance in females of *B. tabaci* MEAM1 cryptic species compared with males shows that the differential expression of multiple genes regulates the heat tolerance of females [233-235].

1.10 Fluorescence real-time quantitative PCR

American scientist Kary Mullis first proposed polymerase chain reaction (PCR) technology in 1983, which is used to amplify specific DNA fragments. It can be regarded as a special DNA replication in vitro [236]. This technology has brought the

research of molecular biology to a new level. It has the advantages of high efficiency, economy and time saving. However, this technology can only detect the end products of amplification, and can not carry out specific quantitative observation. In the experiment, we may prefer to study and understand the initial amount of template (i.e. the specific abundance of a gene expression), Knowing the initial amount of template is very important for studying the function of gene [237]. Therefore, with the gradual in-depth understanding of the principle of PCR technology, Applied biosystems introduced a new technology - real-time fluorescence quantitative PCR in 1996 (real time fluorescent quantitative polymerase chain reaction), real time PCR is a highly sensitive nucleic acid quantitative technology developed on the basis of qualitative analysis of ordinary PCR. Compared with ordinary PCR, it is more sensitive, fast, highly specific, easy to automate, and does not need subsequent processing such as EB staining and electrophoresis detection, so as to avoid cross contamination in the experimental process [238]. At present, as an extremely effective experimental technology, it has been widely used in various fields of molecular biology research, such as medical monitoring, gene expression level analysis, evaluation, transgenic research and food detection, showing a very broad application prospect [239].

1.10.1 Fundamental

Real-time fluorescence quantitative PCR technology refers to adding fluorescent probes or fluorescent dyes to ordinary PCR reactions, using fluorescent signal accumulation to monitor the entire PCR process in real time, and finally quantitatively analyzing unknown templates through standard curves. In the process of Real-time PCR monitoring, it generally goes through the following periods: baseline period, exponential growth period, linear growth period and plateau period. In the baseline period, the amplified fluorescent signal is masked by the fluorescent background signal, and the change in the amount of product cannot be judged; while in the plateau period, the amount of amplification and the amount of template no longer correspond, so real-time monitoring mainly occurs in exponential growth. period and linear growth

period, the amount of template amplification in this period presents an exponentially rising curve. In this way, we can obtain the starting information of the template by monitoring it [239].

The following concepts are often involved in specific quantitative analysis: Baseline: refers to the fact that the fluorescence signal does not change much in the first few cycles of PCR amplification reaction, close to a straight line; The signal is used as the fluorescence background signal, and the fluorescence threshold is 10 times the standard deviation of the fluorescence signal in the 3-15 cycles of PCR, and the fluorescence threshold is set in the exponential phase of PCR amplification; Ct value (C: Cycle, T: threshold): indicates that each. The number of cycles experienced when the fluorescent signal in the PCR reaction tube reaches the set threshold. Research shows that there is a linear relationship between the Ct value of each template and the logarithm of the initial copy number of the template. The more the initial copy number, the higher the Ct value. small and vice versa. Therefore, as long as the Ct value of a sample at a position is obtained, the starting copy number for that sample can be calculated [239].

1.10.2 Main types

At present, fluorescence quantitative PCR technology is mainly divided into two categories: probe method and dye method according to chemical principles. Probe method uses probes that specifically hybridize to target sequences to indicate the increase of amplification products. The main probes include Taqman, Molecular beacons, fluorescently labeled primers and hybridization probes, etc., belong to specific fluorescent materials; the dye method uses fluorescent dyes or specially designed primers to indicate the increase of products, mainly represented by SYBR Green, which is a non-specific fluorescent chemical material [240].

1.10.3 Fluorescent probe TaqMan method

Taq Man technology is a fluorescent quantitative PCR detection technology developed by PE company. It adds a specific fluorescent double-labeled probe (Taq Man probe) to the original pair of primers in ordinary PCR. 3'-5' exonuclease activity, cleaves the probe, producing a fluorescent signal [241]. Since the probe is specifically bound to the template, the intensity of the fluorescent signal represents the amount of the template. Its working principle is: the 5' end of the probe is labeled with a reporter group (reporter, R), and the 3' end is labeled with a fluorescent quenching group (quencher, Q). When the probe is intact, the fluorescence energy emitted by the reporter group is absorbed by the quencher group, and the instrument cannot detect the signal. As the PCR progresses, Taq enzyme encounters the probe bound to the template during the chain extension process, and its 3'-5' exonuclease activity will cut off the probe, and the reporter group will stay away from the quenching group, inhibiting the effect of. When it is released, its energy cannot be absorbed, that is, a fluorescent signal is generated. A cycle of PCR is performed, and as many new chains are synthesized, as many probes are hydrolyzed, the corresponding number of fluorescent groups are released, and the intensity of the fluorescent signal corresponds to the amount of PCR reaction products. As the PCR process progresses, Repeating the above process, the PCR product increases exponentially, and the fluorescent signal also increases accordingly. The intensity of the signal represents the copy number of the template DNA [242].

1.10.4 Fluorescent dye SYBR method

The fluorescent dye SYBR method refers to a method in which SYBR fluorescent dye is added in the PCR reaction to bind to the DNA double-strand to emit a fluorescent signal to detect the PCR product. Specific fluorescent dyes can bind to the minor groove of the DNA double-stranded structure, while free fluorescent dyes do not fluoresce. Therefore, no fluorescent signal can be detected at the beginning of the amplification,

and as the amplification progresses, the fluorescent dye binds to the increasing amount of dsDNA, and the fluorescence intensity gradually increases until it reaches a threshold value, which is detected by the fluorescence detection system [243]. The increase in fluorescence signal intensity is related to the amount of initial template. The less initial template, the more cycles it takes to reach the threshold, thereby quantifying the DNA in the sample. The advantage of the fluorescent dye method is that the detection method is simple and the detection cost is low. The disadvantage is that the detection specificity only depends on the primer specificity. When there is non-specific amplification in the PCR reaction, the fluorescent dye can also bind to the non-specific dsDNA product (such as primer dimer), resulting in fluorescence interference, which makes the experiment easier. produces false positive signals. In this case, specific primers can be designed, the reaction conditions of PCR can be optimized, and the production of non-specific products and primer-dimers can be reduced or eliminated to improve specificity. In addition, melting curve analysis can also be used to distinguish non-specific products and primer-dimers to exclude false positives for qualitative diagnosis.

1.10.5 Quantitative form

Absolute quantitative method: This method is used to analyze and determine the absolute value of a certain target accounting sequence in an unknown sample. It is to obtain the specific copy number of a specific target molecule in the unknown sample on the basis of establishing a standard curve. The standard must be used in each experiment. A standard curve was established using the product [244].

Relative quantification method: This method is used to determine the relative change in the expression of a nucleic acid sequence in a test sample compared to the expression of the same sequence in a calibration sample. The calibration sample can be an untreated control or a sample at time zero in a time course study [245].

1.10.6 Gene expression

The expression level of a specific gene can reflect the growth and survival state of cells, and the quantitative analysis of the transcription level of a specific gene has become the core part of gene function research [246,247]. Due to the application of the Taqman system, the detection of mRNA is much more convenient, faster and more accurate than the previously commonly used methods, such as Northern blotting and RT-PCR quantitative methods, which can study the expression level and dynamic changes of mRNA. It is often used in the following aspects: comparing the differences in the performance of samples processed in different ways to find the best therapeutic drugs and treatment methods; detecting the level of cellular mRNA expression to achieve quantitative detection of gene expression; individual differences may be due to the expression of some genes For this reason, qualitative and quantitative analysis of these genes can be applied to disease resistance breeding and so on.

With the rapid development of modern molecular biology technology, genetically modified products are increasingly entering people's lives. The safety of genetically modified products and the impact of genetically modified organisms on human health and the ecological environment have aroused widespread concern around the world [248]. In recent years, qualitative detection methods of genetically modified food have developed rapidly, and the accurate quantitative detection of genetically modified ingredients has become increasingly important in international trade. China has established a real-time quantitative PCR method, and quantitative detection of genetically modified products has been carried out in some ports, and there are reports of composite fluorescent quantitative PCR detection [248]. RT-PCR is also widely used in veterinary medicine, mainly for the detection and typing of pathogens [249,250].Huang et al. describe a Taqman real-time quantitative PCR detection system, which can rapidly identify *F. occidentalis* from thrips larvae to complement the traditional morphological identification. The rapid method can be used for on-site testing of samples at ports-of-entry in the future [251-253].

RT-PCR technology can not only effectively detect gene mutations[254,255], but also accurately detect the expression of oncogenes, which can be used for early diagnosis, classification, staging and prognosis of tumors, and use RT-PCR to detect the expression of various globin genes difference is an effective method for the diagnosis of thalassemia [256-259]. The expression level of specific genes can reflect the growth and survival status of cells. Quantitative analysis of specific gene transcription levels has become a core part of gene function research [260-267]. Finally, China has established a fluorescent quantitative PCR method, and quantitative detection of genetically modified products has been carried out at some ports [264].

Conclusions to Chapter 1.

As a successful invasive pest, the invasion mechanism of B-type tobacco whitefly has become a research hotspot both domestically and internationally. Previous studies have shown that its adaptability to high temperatures is one of the key factors for its successful invasion and expansion. Under the trend of global warming, the B-type tobacco whitefly, with its strong heat resistance, will have an advantage in competing and replacing other species.

The ability of *B. tabaci* to adapt to new environment is closely related to its tolerance to temperature. Heat shock proteins (HSPs) are a group of proteins produced by cells under the induction of stressors, especially high temperature, to help each cell maintain normal physiological activities. Heat shock proteins play an important role in the adaptability of organisms to the environment. In addition to responding to temperature stress, heat shock protein genes may also improve the stress capacity of cells, especially heat tolerance, in the growth and development of insects.

This experiment mainly starts from the heat shock protein HSPs of *B. tabaci*, and studies its regulatory factor (Heat shock factor 1, hsf1), and uses fluorescence quantitative technology to observe the expression of this regulatory factor under different temperature conditions. *B. tabaci* have induced protection against high

temperature stress. The field control effect test of 10 kinds of insecticides on *B. tabaci* on facility vegetables is to understand the resistance level and development status of *B. tabaci*, and to provide a basis for the rational use of pesticides in agricultural production and to delay the development of *B. tabaci* resistance.

CHAPTER 2

SELECTION AREAS OF RESEARCH, MATERIALS AND METHODS OF RESEARCH

2.1 Research materials

The dissertation work is carried out in accordance with the programs of research work of Henan Institute of Science and Technology (HIST).

Objects of the study -*B. tabaci* biotype B used in the experiment were donated by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences. Using cotton and tomato as hosts indoors, breeding populations were established and subcultured in the artificial climate chamber of Henan Institute of Science and Technology. Environmental conditions: The temperature in the greenhouse is 25-28℃, the relative humidity is 60%-70%, the illumination comes from the sodium lamp, and the alternating day and night sunshine is 12h:12h (the illumination time is 7:00-19:00 every day). At the same time, 50 adult worms were randomly selected from various groups on a regular basis, and RAPD-PCR was used to detect the purity of the population to ensure the singleness of the worm species. The specific method refers to the RAPD-PCR identification method of De Barro and Driver (1997) [270].

The host plants of *B. tabaci* biotype B were cotton and tomato respectively. The cotton variety is Baimian No. 1 (Figure 2.1 and Figure 2.2), which is provided by Henan Bainong Seed Industry Co., Ltd. The tomato variety is zaofen 2, which is provided by the Institute of vegetables and flowers, Chinese Academy of Agricultural Sciences.

The above plants were all cultivated in self-prepared substrates, and the substrates used were all-potential organic matter. All single plants were cultivated in plastic pots. When cotton grew to 7 true leaves and tomato grew to 5 true leaves, they were carefully checked for no insects before being used in the test. The egg stage of *B. tabaci* biotype

B, first, second, third, and red eyes, is to take one or two leaves directly, put them in high and low temperature processors for one hour, and then take them under a microscope and pick them up and centrifuge them with a needle. Check the quantity inside the tube.

The experimental design considered two different populations of B and Q, two high temperature, two low temperature and one control for five temperatures. *B. tabaci* used in the experiments were all maintained in insect cages, and were used for observation and research in part of the experiments. The cage is customized by Jiangnan Instrument Factory, the size is 60 cm×60 cm×60 cm, the frame is a hollow plastic tube, and the eight sides are attached with 120-mesh gauze, and the gauze is customized into a detachable bundle form to facilitate maintenance and cage cleaning. There is an opening with a diameter of about 20 cm in the front center of the insect cage, and a sleeve-shaped gauze with a length of about 40 cm is connected to the opening. Risk of *B. tabaci* flying out of the cage or flying into the cage from the environment (Figure 2.3 and Figure 2.4).

Figure 2.1 Host plant: Cotton

Figure 2.2 Insect cage (Potted cotton)

Figure 2.3 Insect cage

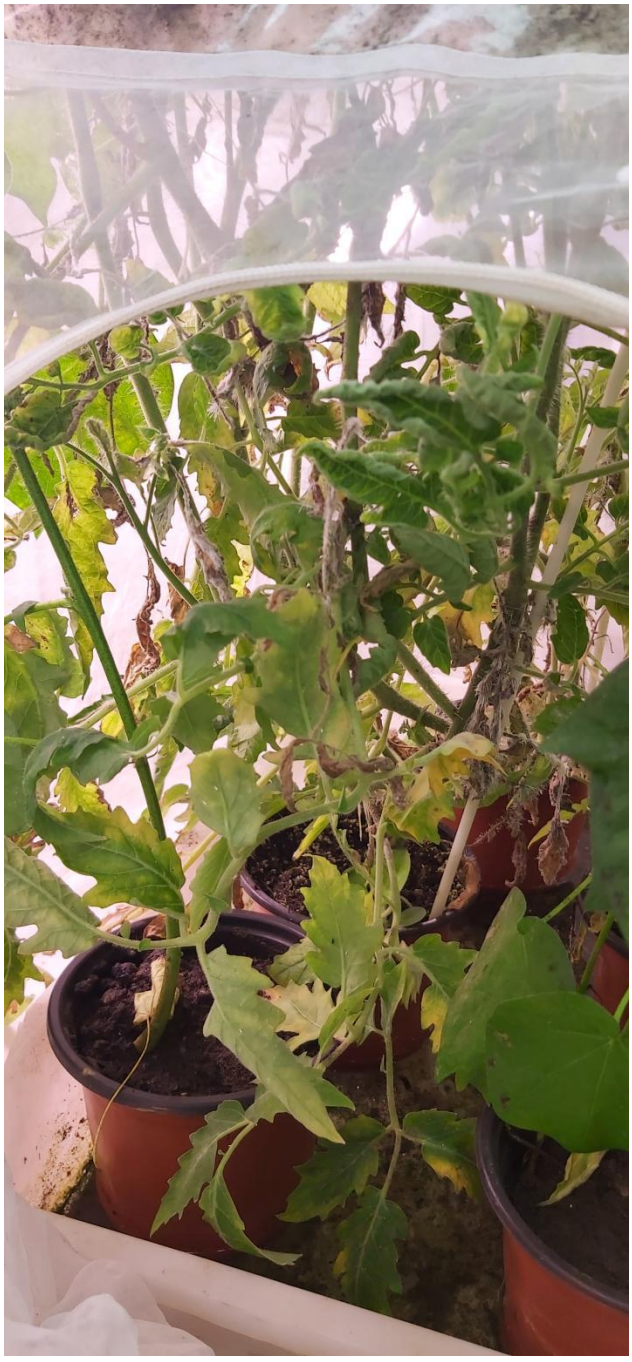


Figure 2.4 Potted tomatoes

Because the adults of *B. tabaci* are very small, artificial flukes are used to facilitate the retrieval of the insects. The artificial fluke consists of a suction nozzle, a rubber tube (about 50 cm in length and 1cm in diameter) and a suction head (Figure 2.5).

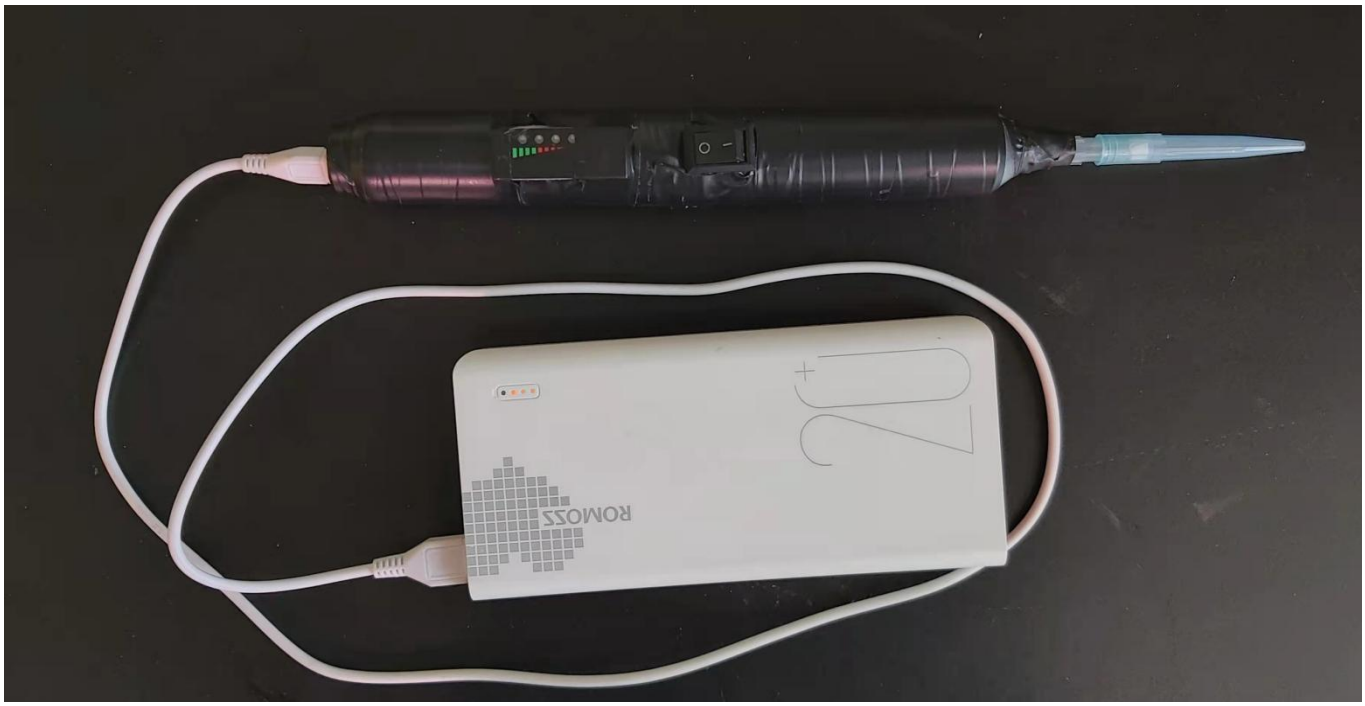


Figure 2.5 The artificial fluke

Stereo microscope can be used to observe the morphology of *B. tabaci* eggs, nymphs, pseudopupae and adults, and can also identify male and female insects. The stereo microscope used in the experiment was produced by Shanghai Wumo Optical Instrument Co., Ltd., model ZOOM-2860.

2.2 Research methods

The total RNA of *B. tabaci* was extracted according to the instructions of the RNA extraction kit (REeasy Mini Kit).

2.2.1 Consumables

1. 3'-Full RACE Kit: The product number is D314, the specification is 20 times, and stored at -20℃.

2. cDNA Synthesis Kit: PrimeScript 1st Strand cDNA Synthesis Kit, Cat. No. D6210A, the specification is 50 times, and stored at -20℃.

3. Fluorescence quantitative kit: SYBR Premix Ex TaqTM, the product number is DRR041A, the specification is 200 times, store at 4°C in the dark, avoid -20°C.

The above reagents were purchased from baoriyi Biotechnology (Beijing) Co., Ltd.

4. RNA extraction kit: purchased from QIAGEN China(Shanghai)Co.,Ltd., RNeasy Mini Kit, the product number is 74104, the specification is 50 times, and stored at room temperature of 15-25℃.

5. Gel extraction kit: purchased from QIAGEN China(Shanghai)Co.,Ltd., Gel Extraction Kit, item number is D2500-01, the specification is 50 times, and stored at room temperature.

6. Marker II: purchased from TIANGEN Company, the product number is MD102, the specification is 300ul, and it is stored at -20℃.

7. DEPC: purchased from Shanghai Soleibo Technology Co., Ltd., the specification is 100ml, and stored at 2-8℃ in the dark.

8. 50×TAE buffer: purchased from Shanghai Double Helix Biotechnology Co., Ltd., the size is 400 ml, and stored at room temperature.

9. Tryptone : purchased from Beijing Shuangxuan Microbial Medium Product Factory, the specification is 250 g, and stored in a cool and dry place.

10. Yeast Extract Powder: purchased from Beijing Aoboxing Biotechnology Co., Ltd., the specification is 205 g.

11. Agarose: purchased from Beijing Bomed Gene Technology Co., Ltd.

12. Eight PCR tubes: purchased from AXYGEN Company, PCR STRIP TUBES, the product number is PCR-0208-C, the specification is 0.2 ml, and it is placed at room temperature.

2.2.2 Experiment equipment

- Intelligent light incubator: Guangdong Shaoguan Keli Experimental Instrument Co., Ltd., PYX-300Q-A type.

- Electric heating constant temperature water bath: Shanghai Precision Experimental Equipment Co., Ltd., model DK-S28.

- High-speed refrigerated desktop centrifuge: Heal Force Development Limited, Neofuge 13R type.

- PH meter: Sartorius Scientific Instruments (Beijing) Co., Ltd., type P8-10.

- Ultra-clean workbench: Wujiang purification equipment factory, CJ-1FD type.

- Electronic analytical balance: Shanghai Precision Scientific Instrument Co., Ltd., type FA1104N.

- Vertical pressure steam sterilizer: Shanghai Shen'an Medical Equipment Factory, type LDZX-40KB.

- Dual-function air bath constant temperature oscillator: Jintan Jieruier Electric Co., Ltd., ZD-85 type.

- Gradient PCR instrument: Biometra, Germany, Gradient type.

- Junyi electrophoresis instrument (JY600C), electrophoresis tank (JY-SPCT): Beijing Junyi Dongfang Electrophoresis Equipment Co., Ltd.

- Tanon gel imaging system: Shanghai Tianneng Technology Co., Ltd., Tanon3500.

- 7300 real-time quantitative PCR instrument (ABI, UK).

- Cryostat (PHY100): Beijing Feike Technology Co., Ltd.

2.2.3 Chemicals and solvents

EB (Shanghai Kaiyin Chemical Co., Ltd.), chloroform and isopropanol (Shanghai Yishi Chemical Co., Ltd.), tryptone and yeast extract powder and agar powder (Beijing Hongrun Baoshun Technology Co., Ltd.).

25% Thiamethoxam water dispersible granules (Zhejiang Qianjiang biochemistry Co., Ltd.), 9% Mineral oil emulsifiable concentrate(Shandong Keda Venture Biology Co., Ltd.), 22.4% Spirotetramat suspension concentrate (Bayer Crop Science), 17% Flurpyrone soluble concentrate(Bayer Crop Science), 70% Acetamiprid water dispersible granules (Shaanxi Thompson Biotechnology Co., Ltd.), 5% Diprofen dispersible concentrate (BASF (China) Co., Ltd.), 10% Cyantraniliprole suspension concentrate (FMC (China) Investment Co., Ltd.), 1.8% Abamectin emulsifiable concentrate (Zhejiang Zhongshan Chemical Group Co., Ltd.), 50% Flonicamid water dispersible granule (Shandong Huimin Zhonglian Biotechnology Co., Ltd.), 20% Mevirpirazone suspension concentrate (Shanghai Shengnong Biochemical Products Co., Ltd.).

2.3 Reagent preparation

1. 10mg/ml Ethidium Bromide (EB): Add 1g of ethidium bromide to 100ml of water, stir with a glass rod for several hours to ensure complete dissolution, then transfer to a brown bottle and store at room temperature (Note: EB is a strong mutagen and is moderately toxic, be sure to use it Wear gloves and a mask when weighing dyes).

2. 1‰ DEPC water: Take 1ml of DEPC water and add it to 1000 ml of distilled water. Shake overnight at 37℃ in the dark.

3. 1×TAE running buffer: Take 2 ml of 50×TAE running buffer, add 98 ml of distilled water, and mix well.

2.4 Save *B. tabaci*

Cut the blue pipette tip with scissors, put the big one on one end of the rubber tube, wrap the other end with gauze, cover the small one, put the small one in the cage, put the small one on the back of the leaf, and use a fluke Collect *B. tabaci*, and change the suction to check the number. After inhaling a certain amount, blow the *B. tabaci* into the prepared polypropylene centrifuge tube (1.5 ml) for use. There should be more than 200 heads in each tube. The corresponding processing temperature is marked on the superscript, and it is taken to the laboratory for use. The temperature treatment is carried out separately. (The high temperature treatment is treated in a high temperature processor for one hour, and then placed in liquid nitrogen for 3 seconds, and then taken to the workbench for operation, and the low temperature treatment is Put all samples in low temperature, process for one hour, take them out, put them in liquid nitrogen for 3 seconds, put them on the workbench), put them in a low temperature processor for low temperature treatment for 1 hour, and then freeze them in liquid nitrogen, save- Store in an ultra-low temperature refrigerator at 80℃ for subsequent RNA extraction, and set 3 replicates for each treatment.

2.5 Instrument Sterilization

Prepare the reagents and utensils used in the experiment. Since RNase is ubiquitous in the air, it is extremely easy to degrade during the extraction process. Therefore, all utensils used in the experiment must be strictly sterilized. Pipette tips The samples and centrifuge tubes are imported sterilized. The glass and metal utensils used in the experiment need to be dry-baked at 180 ℃ for 8 hours or 250 ℃ for more than 3 hours. The operators wear medical masks and disposable gloves during the RNA extraction process. During the test, pay attention to changing gloves frequently. Sterilization steps:

Prepare DEPC water: add 600 μl of DEPC per 600 ml of water to prepare DEPC water, shake on the bed overnight, and sterilize at high temperature and high pressure for 30 min the next day to obtain Treated Water.

1 ml tip, 1.5ml tube and grinding rod are treated with 1/1000 water (DEPC and distilled water are mixed according to the ratio of 1/1000), soak 1 ml tip, 1.5 ml tube and grinding rod overnight , Make sure that the DEPC water fills the entire inner cavity when soaking, and the next day, control the moisture content of the tip and tube, and sterilize at high temperature and high pressure for 30 minutes. Take it out and put it on a clean bench for later use.

2.6 Extraction method of total RNA

Multiple head extraction method, including B-type adult, egg stage, first instar, second instar, third instar, red eye (7℃, 9℃, 25℃, 39℃, 40℃ are all the same experiments step).

Use Trizol method to extract RNA, and operate according to its instructions:

a. Sample treatment at 7℃. When B. tabaci are raised to the egg stage, take two leaves with eggs, put them in an ultra-low temperature processor, set the temperature to

7℃, treat for one hour, and take the eggs with a needle under a microscope , set up three repetitions, 300 centrifuge tubes per centrifuge, and then put it in liquid nitrogen for a few seconds, put it on the ultra-clean workbench, add 1000 ul Trizol solution, fully shake and mix, and let it stand at room temperature for 5min.

b. Add 200 ul of chloroform, shake for 15 s, and let stand for 2 min at room temperature.

c. Centrifuge at 4℃, 12,000 rpm for 15 min, and take the supernatant.

d. Add 500 ul isopropanol, mix the liquid in the tube gently, and let it stand for 10 min at room temperature.

e. Centrifuge at 4℃, 12,000 rpm for 10 minutes, discard the supernatant.

f. Add 1ml of 75% ethanol, gently wash the pellet, centrifuge at 4℃, 7500 rpm for 5 min, discard the supernatant.

g. dry in the air, add an appropriate amount of DEPC water to dissolve (10-20 μl), and promote the dissolution at 65℃ for 10-15 min. After the RNA precipitation is completely dissolved, store it at - 80℃ for standby.

2.7 Synthesize cDNA

The first strand of cDNA was synthesized by primerscript 1st strand cDNA synthesis kit according to its instructions.

a. Use RACE technology to obtain full-length cDNA, prepare the following mixture in a sterilized centrifuge tube , and carry out on an ultra-clean workbench.

reagent	usage amount
Oligo dT Primer(50uM)	1ul
dNTP Mixture(10mM each)	1ul
template RNA	6-8ul
RNase Free dH2O	up to 10ul

b．After incubating at 65℃ for 5 min, cool quickly on ice (Note: this treatment can denature template RNA and improve reverse transcription efficiency).

c. Prepare the following reverse transcription reaction solution in the above Microtube tube, the total amount is 20 ul.

reagent	usage amount
Reaction solution after denaturation	10ul
5×PrimerScript Ⅱ Buffer	4ul
RNase Inhibitor（40U/ul）	0.5ul（20U）
PrimerScript Ⅱ RTase（200U/ul）	1ul（200U）
RNase Free dH2O	up to 20ul

d．Mix slowly.

e. Perform reverse transcription reaction: 42-50℃for 30-60 min, 70℃for enzyme inactivation for 15 min, and then cool on ice for later use or -20℃ for later use. After the reverse transcription was completed, the obtained cDNA template was stored at -20℃for later use.

Among them, there are two pairs of amplification primers, one pair is β-tub-F\R, one pair is P98685-wactR\ P98684-wactF, run the PCR program at 95℃ for 5 minutes and 55℃for 20 minutes. 20s at 72℃, 35 cycles, after the time is up, run electrophoresis and see the electrophoresis chart.

2.8 Agarose gel electrophoresis

a. The purpose of electrophoresis is to detect the integrity of 28S and 18S bands and their ratio. Generally speaking, if the 28S and 18S bands are bright and clear, and the 28S brightness is more than twice that of the 18S band, the quality of RNA is considered to be good.

b. Remove the glue tank and place it on the bench, insert the appropriate glue plate and comb.

c. Weigh 0.2 g of agarose with an electronic balance and put it in a beaker, add 20 ml of TAE buffer to make a 1% concentration, shake it well, heat it in a microwave oven until it becomes transparent, take it out and add it when it cools to 60℃. Micro EB at a concentration of 0.5 ug/ml.

d. Pour it into the glue tank slowly and evenly, the gel is completely solidified in about 20 min, and the comb and glue plate are taken out.

e. Carefully put the prepared gel into the electrophoresis tank, make sure that the loading hole faces the negative electrode of the electrophoresis tank, and add TAE buffer to cover the gel surface.

f. Take a small piece of parafilm, use a pipette to aspirate 4 ul of the sample and 1 ul of the loading buffer evenly, and then add it to the spotting well.

g. The output voltage was 120V, and electrophoresis was performed for 30 minutes. After electrophoresis, the gel was taken out, placed in the Tanon gel image processing system, and photographed and stored.

2.9 Gel recovery and purification of PCR products

Follow the steps of Gel Extraction Kit (OMEGA):

a. Separating DNA fragments by agarose gel electrophoresis, it is recommended to use fresh TAE/TBE Buffer and freshly prepared gel.

b. After the fragments are completely separated, quickly cut the desired band under the UV lamp, and the DNA exposure time under the UV lamp should not exceed 30s.

c. Weigh the gel block, add 1 ml of Binding Buffer to each 1 g of gel, add an appropriate volume of Binding Buffer, and take a water bath at 55-60℃ until the gel is completely dissolved (about 7-10 minutes). Shake every 2-3 min.

d. Note: When the Binding Buffer completely dissolves the gel, please pay attention to the color change of the solution. If the color of the solution has turned purple or red, 5ul of 5M NaAc, pH5.2 must be added to the solution to adjust the pH.

e. Put the HiBind DNA column in a 2 ml collection tube.

f. Transfer the DNA/gel mixture to a HiBind DNA column in a 2 ml collection tube and centrifuge at 10,000xg for 1 min.

g. Pour off the filtrate and put the column back into the collection tube. The HiBind column can hold 700 ul of solution at a time. If the mixture exceeds 700 ul, transfer 700 ul to the column each time, and then repeat steps 5-6.

h. Put the column back into the collection tube, add 300 ul Binding Buffer, centrifuge as above, and discard the filtrate.

i. Put the column back into the collection tube, add 700 ul SPW Wash Buffer, centrifuge as above, and discard the filtrate.

j. Note: SPW Wash Buffer must be diluted with absolute ethanol before use.

k. Discard the filtrate, put the column back into the collection tube, and centrifuge the empty column at 13,000xg for 2 minutes to dry the column matrix.

l. Put the column in a clean 1.5 ml centrifuge tube, add 30-50 ul of Elution Buffer pre-warmed at 65°C to the column matrix, and let stand at room temperature for 2 minutes. cDNA was eluted by centrifugation at ≥13,000×g for 2 min (All cDNA extracted and stored in the refrigerator).

2.10 Real time fluorescence quantitative PCR

Real-time fluorescence quantitative PCR technology is a method of measuring the total amount of products after each polymerase chain reaction (PCR) cycle with fluorescent chemicals in DNA amplification reactions. Real-time detection of PCR progress. Firstly, specific amplification primers Hof339F and 429R were designed according to the full-length cDNA sequence, and the internal reference β-tub-F/R was selected.

Using the cDNA obtained by reverse transcription as the template and β-actin as the internal reference gene, the real-time PCR method was used to detect the expression levels of the two genes in *B. tabaci* at different temperatures. PCR amplification was performed 3 times for each repetition, and the average was taken. value is the Ct value for this treatment.

According to the optimization results, the reaction system used in the experiment is:

reagent	usage amount
SYBR® *Premix Ex Taq*™(2×)	10.0ul
upstream primer(10uM)	0.4ul
downstream primer(10uM)	0.4ul
ROX Reference Dye(50×)	0.4ul
cDNA template	2.0ul
dH_2O	6.8ul
Total	20.0ul

After mixing the above reaction solution evenly, carry out SYBR Green fluorescence quantitative RT-PCR amplification, using three-step PCR amplification, the reaction parameters are as follows:

Stage 1 pre-denaturation: 50℃, 2 min;

Stage 2 PCR reaction: 95℃, 1min-94℃, 15s (Reps 40);

Stage3 extension: 54℃, 30s to 72℃, 30s.

2.11 Statistical analysis

Quantitative fluorescence data were counted, and the gene expression was calculated by the $2^{-\Delta\Delta CT}$ method, where ΔΔCT=(Ct target gene-Ct reference gene) treatment group-(Ct target gene-Ct reference gene) control group [266]. SPSS software was used for data analysis, and one-way ANOVA was used for variance analysis for differences between different temperature treatments, and multiple comparisons were performed. The differences between different sexes and different cryptic species were

analyzed by independent sample T-test. The data were expressed by mean soil standard error, and the significance test level was $p < 0.05$.

Conclusions to Chapter 2

By continuously raising *B. tabaci* under indoor conditions, the egg hatching rate, population dynamics, survival rate, offspring sex ratio, female reproductive capacity, and other indicators of B-type *B. tabaci* were observed to clarify the impact of temperature stress on their offspring sex ratio. The results showed that different feeding temperatures and generations had a significant impact on the above indicators. As the treatment temperature increases, the pre oviposition period of female adults shortens, and their lifespan and egg production both decrease.

The feeding of *B. tabaci* usually induces changes in plant metabolites, which in turn can affect the behavior and fitness of *B. tabaci*, so its feeding behavior is likely to be regulated by changes in plant metabolites. Previous studies have shown that feeding on plants by *B. tabaci* can reduce their resistance and cause them to aggregate on plants infected with the same individual.

A Method for Quantitative RT-PCR Using Fluorescence, Establishing a high-throughput and accurate system for comparing the expression differences of heat shock genes between B-type *B. tabaci* and other whitefly species. It is convenient and fast to compare the differences in Hs ps expression induced by multiple sources of stress.

CHAPTER 3

GENE AMPLIFICATION OF HSF1 REGULATORY FACTOR

3.1 Homology search of HSF1 regulatory factors

Use the NCBI website (http://www.ncbi.nlm.nih.gov/) to query the partial or full-length nucleotide sequence of the hsf1 gene related to *B. tabaci*, and download and organize. Use the Primer (premier5.0 software) to design degenerate primers for the conserved regions of the common gene sequence (Figure 3.1):

SF1：CTG CCG CTG TTG TAG TGC

SF690：GGA AGT AGG GAC GAA TGT

```
1      CTGCCGCTGT TGTAGTGCGT TCGCCTTTTC GCTTCACTCA GATATGAATG CTTGTGACCA
61     TTGTGAGCTT TTTCCCTCAA CATAAGAGGG AAATTTGAAC GCCTTTTCAC ACTGAGATTA
121    CGTGTGGGAT GGACCAGTGT CACAAGGAAT TGGATCAACT TATTGACAAT AGTCTGCTGT
181    TTGGCATGTT TCTGCCTCAG AATTGACAGT TCACGCCACA GAGCTTCATT CTCTTGTTTC
241    ATGGAGGAAA GTTTGGAATC CAAGGATTCT TGTCGTCCTT TCATGGATTT GACATCATTC
301    AGCATCTTGT TCATTATGTC AGGAGCTGCA CGAATTTCAT TGTTTTCCTT GGTGACGGTC
361    ATCTTTCTCT TGATGTGCTC CAAAAGGTAT GGATGACCAC GGATGAAGCA ATGATGGCTG
421    AAAGAGATCT CATCATTGTC AGATAGTCGT AGCCCATTGT TTTCGGCACT GACCACTTTG
481    TGGAAGCCAT ACATATTCAA CTGTCGAATA AAGCTGGCCA TGTTGTTGTG TTTGTAGTAC
541    ATGGGTAACA GCTCTTTTGC AAACTGTGAT TGATTTTTGA TCATGAAAGA TGTGCCATCT
601    GAATTCCAGC AAATGAGATC ATTCGTTTCA GGATCTTCCA CCATCCTCCA TAATTTGGCC
661    AAAAAGGCAG AGACATTCGT CCCTACTTCC GTGATTGGGT GCATTTTGAA TGATGAATAA
721    ATAGCTTACG AACAATATCT GAGCATACGG TGCACAAAAT TAAACAATAT CCGTCACAAA
781    ACTGAAATTA CCACCGACAC TGGTTGTGAA AAACGAAAAA GGAAAAAACG CGAAAAAAGC
841    CAAAAAAAAA ATTTT
```

Figure 3.1 Conservative region gene sequence of heat shock factor1

3.2 RNA extraction

The operation steps are the same as 2.6.

3.3 RT-PCR synthesis of first-strand cDNA

The operation steps are the same as 2.7. (cDNA refers to the formation of complementary DNA under the action of reverse transcriptase using RNA as a template)

3.4 hsf1 gene sequence amplification

3.4.1 Acquisition of the intermediate fragment of hsf1 gene sequence

The first-strand cDNA was used as the template, and the above-mentioned degenerate primers SF1 and SF690 were used to establish the following PCR reaction system.

Table 3.1 PCR reaction system

Reagent	Usage amount
cDNA template	0.5ul
10×PCR Buffer	2.5ul
dNTP Mixture	2.5ul
SF1 Primer	0.5ul
SF690 Primer	0.5ul
ddH_2O	18.2ul
Taq enzyme	0.3ul
Total volume	25ul

The reaction parameters were as follows: pre-denaturation at 94℃ for 3 min; then denaturation at 94℃ for 1 min, annealing at 52℃ for 1 min, and extension at 72℃ for 1 min, a total of 36 cycles; and a final extension at 72℃ for 5 min. The amplified PCR products were detected and separated by 1.5% agarose gel electrophoresis, voltage 120V, 30 min. The gel imaging system was used to observe the products.

The total RNA of *B. tabaci* was extracted and detected by 1% agarose gel electrophoresis. The results show that 18S/28S is bright and clear, with sharp bands, and that 28S is more than twice as bright as the 18S band (Figure 3.2).

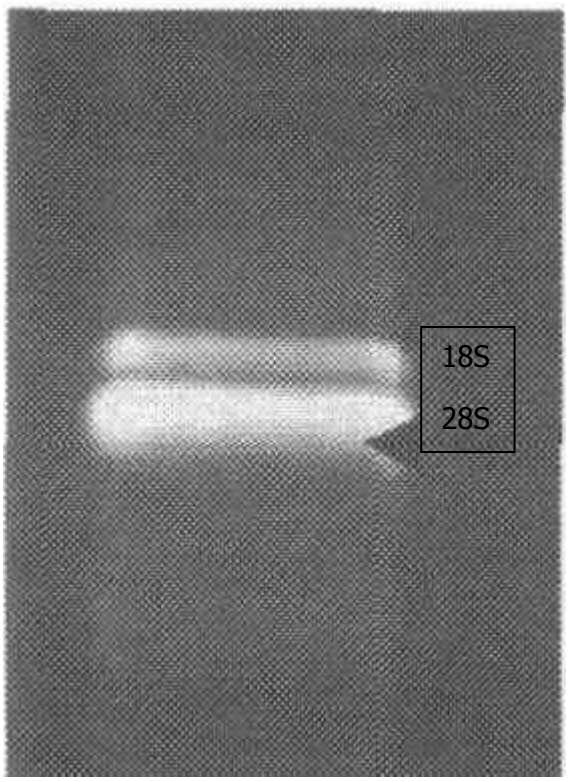


Figure 3.2 Total RNA of *B. tabaci* biotype B

3.4.2 Gel recovery and purification of PCR products

The operation steps are the same as 2.9. Prepare a fluorescence quantitative system, use eight tubes, go to the central laboratory of the school to run the data, and record the Ct value.

3.5 Similarity of gene sequence alignment in *B. tabaci*

The sequenced *B. tabaci* hsf1 and the original conserved gene sequence were compared and verified by DNAMAN, and the similarity of the results was about 60.55%, which satisfies the RT-PCR primer design and can be used for quantitative detection.

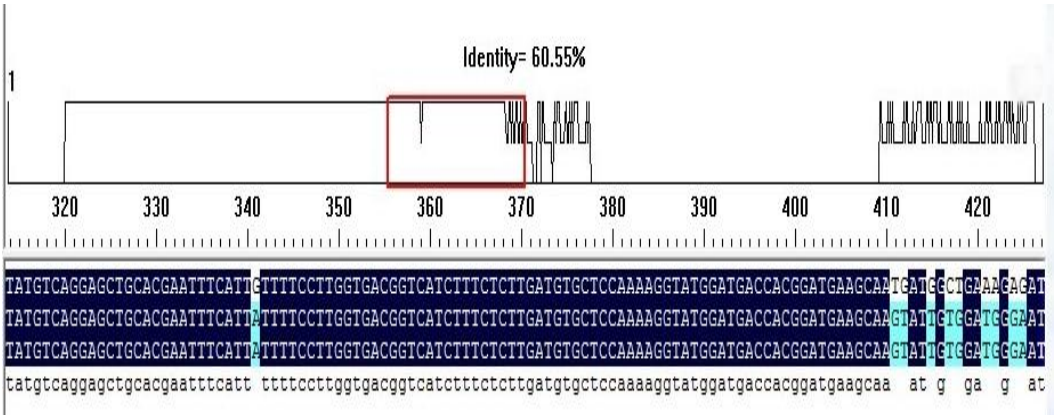


Figure 3.3 Similarity of gene sequence alignment

Conclusions to Chapter 3

1. Through RACE technology, a part of the hsf1 gene sequence (about 900bp) of B. tabaci was amplified. The DNAMAN software analysis and verification showed that the amplified gene sequence conforms to the relevant characteristics of hsf1, and the similarity with the conserved gene sequence of hsf1 reaches 66.55%, which can be used for fluorescence quantitative verification.

2. A Method for RT-PCR using fluorescence, establishing a high-throughput and accurate system for comparing the expression differences of heat shock genes between B-type *Bemisia tabaci* and other whitefly species , It is convenient and fast to compare the differential expression of Hsps induced by various sources of stress.

3. Extracting Total RNA from *Bemisia tabaci* , Detection by agarose gel electrophoresis , The bands of 2 8S and 1 8S are complete and clear, indicating that the extracted RNA has good purity , Good integrity , It can be used to construct cDNA libraries.

CHAPTER 4

CLONING OF THE FULL-LENGTH HSP60 GENE

4.1 Total RNA extraction and RT-PCR

About 300 worms of *B. tabaci* in the greenhouse were taken, ground in liquid nitrogen to extract the total RNA of the worms, and reverse transcribed to synthesize the first strand of cDNA (refer to 2.7 for the method), and then store the synthesized cDNA in the refrigerator at - 20℃for standby.

4.2 PCR amplification and sequencing of hsp60 gene

4.2.1 hsp60 homologous gene sequence search and primer design

Use the homologous gene search function of the NCBI website to search for the hsp60 sequence of related genes, and design degenerate primers using the conserved coding region:

Q60-121：5’-AAGTCGGAGGTTCCAGTG-3’

SP60-892：5’-TCTTGGCTCGGAGTTAGG-3’.

4.2.2 Amplification of hsp60 gene fragment and recovery of gel fragment

PCR amplification was carried out with the first strand cDNA as the template, and the reaction system and reaction conditions were referred to the reaction parameters in Chapter 2.

The electrophoresis detection and gel recovery of amplified fragments are the same as 2.8 and 2.9.

4.2.3 Sequence assembly and verification

The Blast function was performed on the sequenced results. After the alignment, the homologous genes with high similarity were searched and downloaded, and then the full-length sequence was assembled using the Sequence Assembly function of the DNAMAN software, and the full-length hsp60 was electronically cloned to about 2348 bp.

To verify the correctness of the splicing results, design primers at both ends of the sequence:

SP601: 5’-CGTTTACCCTCCGTTCTTCGC-3’,

SP602: 5’-TTATTGTTTCGGTCTTGGCTC-3’.

Using the first strand of *B. tabaci* cDNA as a template, the full-length hsp60 gene of *B. tabaci* was verified.

4.3 Full-length sequence analysis of Hsp60 gene

The full-length cDNA of *B. tabaci* hsp60 was obtained by homologous cloning and electronic splicing clone verification technology. The full-length cDNA and deduced amino acid sequence of Hsp60 gene of *B. tabaci* MED cryptid are shown in Figure 3.3. The full-length cDNA has a 1372 bp open reading frame (ORF), which can encode 608 amino acids, and its predicted isoelectric point is 9.28 , the protein molecular weight is about 49446Da. The 5'UTR of the gene contains 145 bp, and the 3'UTR contains 840 bp. (Figure 4.1).

```
1          TCCGGTCTGTCGGATATCTGTTCCTCGTGTTACTTGCCGCTAGACACTTTTACTGAAACAAATTCTCCTCTATTCTAACTAGAATATTAA
                                                                                          *  N  I  K
91         AAGTTATTCCGTGTCCTCATCTCTTACCTCGAATTGACACCTTGTACCAATTATGTACCGTTTACCCTCCGTTCTTCGCTCAAGTGCAGC
             S  Y  S  V  S  S  S  S  L  T  S  N  W  H  L  V  P  I  M  Y  R  L  P  S  V  L  R  S  S  A  A
181        ACGACAGCTGAACCCTAGCCTTGCTCGATTTTATGCCAAAGATGTGAGATTTGGCCCGGAAGTCAGAGGTTTAATGTTGCAAGGAGTTGA
             R  Q  L  N  P  S  L  A  R  F  Y  A  K  D  V  S  F  G  P  E  V  S  G  L  M  L  Q  G  V  D
271        TATTTTGGCTGATGCTGTAGCTGTCACTATGGGACCCAAGGGCCGAAATGTCATTCTGGAGCAGTCATGGGGATCTCCGAAAATCACCAA
             I  L  A  D  A  V  A  V  T  M  G  P  K  G  R  N  V  I  L  E  Q  S  W  G  S  P  K  I  T  K
361        GGATGGAGTGACGGTCGCTAAAGGAAAAGAACTAAAAGACAAGTTTCAGAACATTGGTGCCAAATTGGTTCAAGATGTAGCCAATAACAC
             D  G  V  T  V  A  K  G  K  E  L  K  D  K  F  Q  N  I  G  A  K  L  V  Q  D  V  A  N  N  T
451        AAATGAAGAGGCTGGCGACGGCACCACTACTGCCACAGTCCTTGCTCGAGCCATTGCCAAGGAAGGCTTTGAGAAAATCAGCAAAGGCGC
             N  E  E  A  G  D  G  T  T  T  A  T  V  L  A  R  A  I  A  K  E  G  F  E  K  I  S  K  G  A
541        AAATCCAATAGAGATAAGGAAAGGTGTAATGTTAGCTGTAGAAACTGTCAAAAACAATTTAAAATCGCTATCAAAGCAAGTAACAACCCC
             N  P  M  E  M  S  K  G  V  M  L  A  V  E  T  V  K  N  N  L  K  S  L  S  K  Q  V  T  T  P
631        AGAAGAGATTGCTCAAGTAGCCACAATCTCTGCCAATGGAGATGGAGCCATTGGAAATCTAATCTCAGATGCTATGAAGAAAGTTGGCAA
             E  E  I  A  Q  V  A  T  I  S  A  N  G  D  G  A  I  G  N  L  I  S  D  A  M  K  K  V  G  K
721        AGAGGGTGTCATCACGGTCAAAGATGGCAAAACTCTGGAAGATGAACTTGAAGTGATTGAAGGGATGAAATTCGACAGAGGTTACATCTC
             E  G  V  I  T  V  K  D  G  K  T  L  E  D  E  L  E  V  I  E  G  M  K  F  D  S  G  Y  I  S
811        CCCCTACTTCATTAACACCACTAAAGGTGCCAAAGTTGAATTTCAAGACGCTCTGTTGCTTCTAAGTGAGAAGAAAATATCAAGCATTCA
             P  Y  F  I  N  T  T  K  G  A  K  V  E  F  Q  D  A  L  L  L  L  S  E  K  K  M  S  S  I  Q
901        GTCGATCATTCCAGCATTGGAACTGGCAAACTCTAAAAGGAGACCCCTCGTCATTATTGCAGAAGACATTGATGGTGAAGCATTGAGCAC
             S  I  I  P  A  L  E  L  A  N  S  K  S  S  P  L  V  I  I  A  E  D  I  D  G  E  A  L  S  T
991        TTTAGTAGTCAATAGGTTGAAAATTGGATTACAAGTTGCCGCTGTGAAAGCGCCAGGTTTTGGTGACAACAGGAAATCTACTCTACAGGA
             L  V  V  N  S  L  K  I  G  L  Q  V  A  A  V  K  A  P  G  F  G  D  N  S  K  S  T  L  Q  D
1081       CCTAGCCATTGCCACCGGTGGACTGGTCTTCGGAGATGAAGGGACCGCCATCAAATTGGAAGATGTTCAACTTCATGATCTCGGTGAAGT
             L  A  I  A  T  G  G  L  V  F  G  D  E  G  T  A  I  K  L  E  D  V  Q  L  H  D  L  G  E  V
1171       AGGAGAGATTGTCATCACGAAAGACGATACCCTCATTCTGAAGGGAAAAGGCAAGAAAGAAGAAATGGATCGAAGAGCAGATCAACTGAG
             G  E  I  V  I  T  K  D  D  T  L  I  L  K  G  K  G  K  K  E  E  M  D  R  S  A  D  Q  L  S
1261       GGATCAAATCGCTGACACAACTTCAGAATACGAAAAAGAAAAGTTGCAGGAACGACTTGCAAGGTTGGCCTCTGGAGTTGCCCTCTTGAA
             D  Q  I  A  D  T  T  S  E  Y  E  K  E  K  L  Q  E  R  L  A  S  L  A  S  G  V  A  L  L  K
1351       AGTCGGAGGTTCCAGTGAAGTTGAGGTGAATGAAAAGAAGGATCGTGTCACAGATGCCTTAAATGCAACCAGAGCAGCAGTGAGAGGAAG
             V  G  G  S  S  E  V  E  V  N  E  K  K  D  R  V  T  D  A  L  N  A  T  S  A  A  V  S  G  S
1441       GCATTGTTCCCGGCGGTGGTCTCGCTCTTCTCCGGTGTATTCCAAAGTTAGATGCCATCTCAAGCAAAAATGAAGATCAGAAAACAGGTA
             H  C  S  R  R  W  S  R  S  S  P  V  Y  S  K  V  S  C  H  L  K  Q  K  W  S  S  E  N  S  Y
1531       TTGAAATTGTCAAGAGAGCATTGAGAATGCCCTGCATGACCATTGCCAGTAACGCTGGAGTCGACGCCTCAATGGTTGTAGCCAAGGTCC
             W  N  C  Q  E  S  I  E  N  A  L  H  D  H  C  Q  *  R  W  S  R  R  L  N  G  C  S  Q  G  P
1621       TAGAATCTCCTCCCGAAATCGGTTACGATGCTTTAAACAATGAATATGTCAACATGATTGAAAAAGGAATCATCGACCCGACCAAAGTTG
             S  I  S  S  R  N  R  L  R  C  F  K  Q  W  M  C  Q  H  D  W  K  S  N  H  R  P  D  Q  S  C
1711       TAAGAACAGCCTTAACAGATGCCGCAGGAGTTGCCTCATTGCTCACAACTGCCGAAGCTGTAGTGACAGAAATCCCGAAAGAAGAGCCGC
             K  N  S  L  N  S  C  R  S  S  C  L  I  A  H  N  C  R  S  C  S  D  S  N  P  E  S  S  A  A
1801       CCATGGGTGGCATGGGTGGAATGGGAGGAATGGGTGGTATGGGAGGAATGGGCGGCATGGGAGGCATGATGTAATCAGCCGCTCGTTTGT
             H  G  W  H  G  W  N  G  S  N  G  W  Y  G  S  N  G  R  H  G  S  H  D  V  I  S  R  S  F  V
1891       AGCTCGATGATCTTAAGTTCCCGAACCAAAAACCTGGTTTTGAATCAAGAAGCATTTAAAAGCGTGAGTATATTAGTAGTTAGAGAGCCA
             A  R  W  S  *
1981       TTGAGACTGAAAACCTGCCCGGTGTAAGAAACTCTGTGAGCCGAATGCTAATTAAACAAACTTTAGGGTGTAGATCAGAGAGCATCTTAC
2071       TCTCAGTGCAATTTTGTAGTGTGCTTGCTCATTTCCTAACTCCGAGCCAAGACCGAAACAATAAATTTAGCTCCAGATGTAGGATAATAA
2161       AGTTCGAATCACACAATTGCAGGGTCACAAGATGATACACTTCCATTCTATCATTTAAATATTGTCCCAAATATTCACTTTAATTTTTTT
2251       CTTTTCTTTTTTATCCCTCTCTCATCAAATTAAAATCTTTGTAAGTTTCTATGGTTCTTTATGAAATTAAAAAAAAAAAAAAAAAAAAA
2341       AAAAAAAAAAAA
```

Figure 4.1 Nucleotide and translation protein sequences of *hsp*60 cDNA from *B. tabaci*

Conclusions to Chapter 4

1. The sequence results from the sequencing were compared by BLAST function. After the comparison, the homologous genes with high similarity were searched and downloaded, and then the full-length sequence was assembled by using the Sequence Assembly function of DNAMAN software, and the full-length Hsp60 was electronically cloned to about 2348 bp. The full-length cDNA of *B. tabaci* Hsp60 was obtained by homologous cloning and electronic splicing cloning verification technology. *B. tabaci*

biotype B Hsp60 gene full-length cDNA and predicted amino acid sequence full-length cDNA has a 1372 bp open reading frame (open reading frame, ORF), which can encode 608 amino acids, the predicted isoelectric point is 9.28, and the protein molecular weight is about 49446Da. The 5'UTR of the gene contains 145 bp, and the 3'UTR contains 840 bp. Heat shock genes can be observed within 1-2 minutes after induction of heat shock, and the maximum transcription rate is reached within 2-5 minutes. After heat shock, RNA polymerase H dissociates from most chromosomal regions and accumulates in new heat shock fluff. The Drosophila uninduced Hsp70 and Hsp26 genes are both pre-bound with an RNA polymerase H complex that synthesizes a short transcript and then stops. No such complex is present in other heat shock genes. There is no such complex in other heat shock genes. Most heat shock genes contain one or several (CT) n repeats in the upstream region of the transcription start site. The (CT) n sequence forms a DNase I hypersensitive (DH) site in the absence of heat shock, and contains the HSE sequence, the GAGA factor constructs and maintains the DH site with high affinity. After heat shock induction, the chromatin structure at the DH site is opened, and the activated heat shock transcription factor (Hsf) recognizes and binds the HSE located at the DH site to initiate transcription [228].

2. Through the amplification verification and quantitative detection of Hsf1 and Hsp60 genes, it was found that the expression of Hsf1 and Hsp60 was positively correlated, which proved that Hsf1 is the main heat-shock regulator of HSPs. When *B. tabaci* is under environmental stress, HSPs are regulated by Hsf1 factor-induced expression to protect themselves. Some studies have put forward the hypothesis of invasion mechanism of *B. tabaci*: the high-temperature tolerance of *B. tabaci* is related to the expression of HSPs in vivo; The difference in HSPs expression may promote the invasive biotype to have strong adaptability to high and low-temperature stress; The low expression of HSPs may be an important reason to limit the high-temperature growth of local biotypes, to limit the expansion of population and distribution. This study starts with this hypothesis and studies the transcription factors of HSPs, trying to reveal the

internal mechanism of *whitefly* invasion and its adaptability to temperature stress from a deeper level. Both genes showed relatively high expression levels at low temperatures, but relatively low expression levels at high temperatures. It was concluded that the expressions of Hsf1 and Hsp60 were positively correlated. It can only be assumed that Hsp60 in *whitefly* is heat shock-inducible through the expression of Hsf1 regulators. It cannot fully explain the specific regulatory function of Hsf1 on HSPs. In the future experimental study, we need to start from the various types of HSPs in *B. tabaci* and conduct overall vertical and horizontal comparisons to more completely explain the relationship between Hsf1 and HSPs.

3. In order to more clearly illustrate the regulatory control relationship between HSF1 and HSPs, we need to consult a lot of previous experimental research results, through quantitative comparison with Hsp20, Hsp70, and Hsp90 in HSPs, etc. RNAi interference was performed to detect the stress expression of different types of HSPs in B. tabaci under the condition that HSF1 was silenced by RNAi technology, to further verify the regulatory function of HSF and the types of HSPs, and to reveal the invasion mechanism of *B. tabaci* from a deeper level. To improve the theoretical hypothesis of the invasion mechanism of *B. tabaci* proposed earlier, and to provide a good theoretical basis and practical guiding significance for the prediction, prevention, control, and management of the invasion process of the "super pest" *B. tabaci.* Both genes showed relatively high expression levels at low temperature, but relatively low expression levels at high temperature, which concluded that the expression of Hsf1 was positively correlated with the expression of Hsp60. This can simply indicate that the Hsp60 gene of *B. tabaci* is heat shock-inducible through the expression of Hsf1 regulators.

4. Studies have shown that the molecular chaperone action of heat shock proteins (HSPs) is an important source of heat resistance. Heat shock protein was originally thought to be a highly conserved stress protein produced by organisms under the action of adverse environmental factors, but studies have found that a type of heat shock gene is activated and expressed in unstimulated cells or the development of organisms. some

stages of expression. The study pointed out that organisms may induce the synthesis of such stress proteins under stressful environmental conditions such as high temperature, salinity, drought, and osmosis, and function as molecular chaperones in cells, synthesizing more heat shock proteins to physiology in cells. The process runs smoothly. Heat shock proteins can be divided into HSP90, HSP70, HSP60, and small-molecule heat shock proteins according to their relative molecular weights. Each family of heat shock proteins is composed of various forms or modified protein molecules.

CHAPTER 5

QUANTITATIVE DETECTION OF HSF1 FACTOR AND HSP60 GENE AT DIFFERENT TEMPERATURES

5.1 Effects of temperature on the development of *B. tabaci*

Temperature has a significant effect on the developmental process of *B. tabaci*. Experiments show that *B. tabaci* treated in the range of 7-39℃ has effects on egg hatching rate and adult reproduction. The developmental duration of *B. tabaci* decreased with increasing temperature (Figure 5.1).

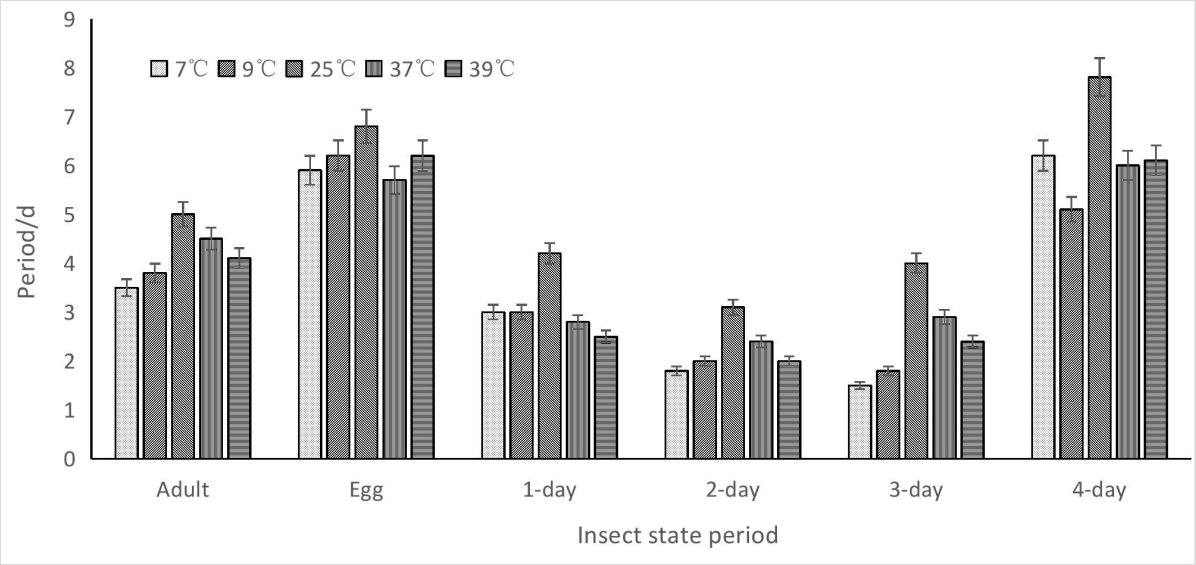


Figure 5.1 Development period of each insect state under different temperatures

5.2 The effect of temperature on the survival of *B. tabaci*

There were significant differences in the mortality rate of *B. tabaci* in different temperature conditions. The test showed that *B. tabaci* had nearly zero death when the optimum temperature was 25℃. There was no significant difference between male and female adults at 37℃ and 39℃(Figure 5.2).

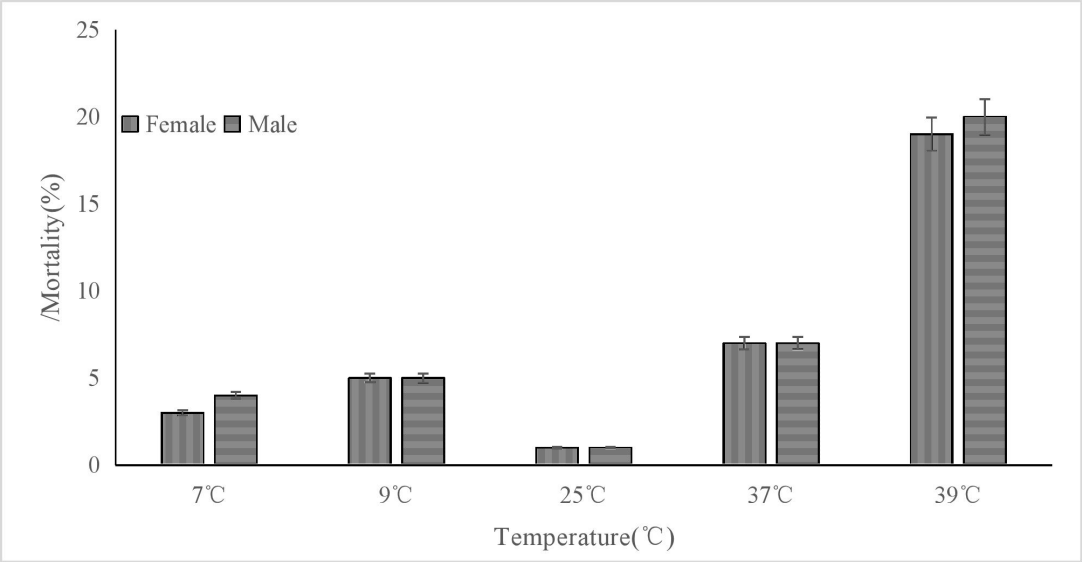


Figure 5.2 Mortality rates of male and female adults at different temperatures

5.3 Effects of Temperature on Reproduction of *B. tabaci* adults

Temperature has a great influence on the reproduction of *B. tabaci* adults, and the sex ratio of male and female adults is different at different temperatures. The research results show that high temperature leads to a decrease in the reproductive fitness of *B. tabaci* (reduced egg production), which will affect the survival of the offspring of the population scale. High temperature may shorten the lifespan of *B. tabaci* female adults and reduce the number of matings, thereby reducing the fertilization rate of female adult eggs and increasing the proportion of male offspring (Figure 5.3).

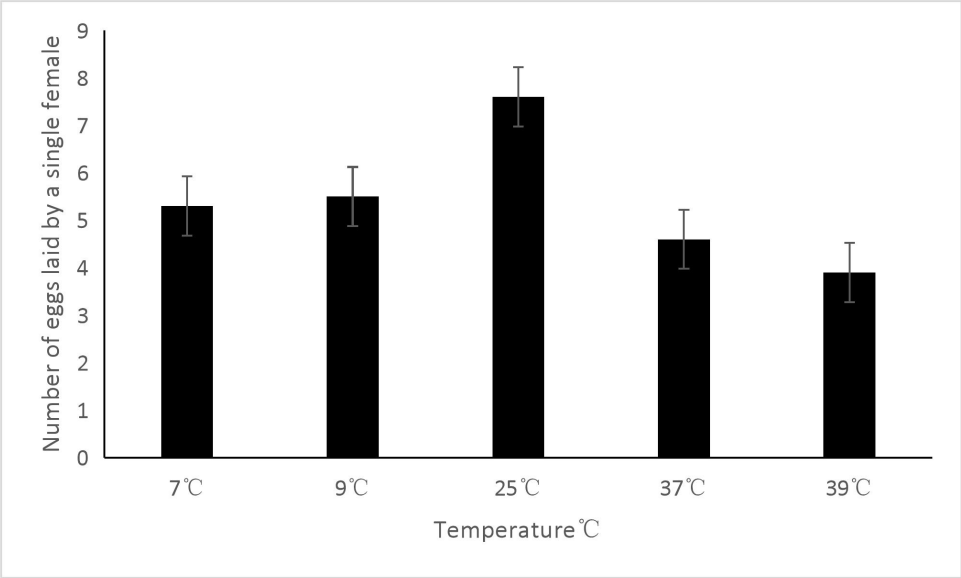


Figure 5.3 The number of eggs laid by *B. tabaci* adults at different temperatures

5.4 Treatment of *B. tabaci* at different temperatures

The long-term *B. tabaci* adults in our laboratory were transferred into polypropylene small tubes (1.5mL) by suction, about 300 per tube. Put it into a preheated artificial climate box for heat treatment for 1h, and the treatment temperatures are: low temperature 7℃, 9℃, high temperature 39℃, 40℃, and at the same time, the normal temperature of 25℃ is used as the control. It was then frozen in liquid nitrogen and stored in an ultra-low temperature freezer at -80℃for subsequent RNA extraction. Each treatment was repeated three times.

5.4.1 RNA extraction and first-strand cDNA synthesis

The method steps are the same as 2.6 and 2.7.

5.4.2 Real-time PCR primer design

Using primer 5.0 primer design software, real-time PCR primers were designed according to the cDNA of hsf1 and hsp60 gene sequences, with a length of about 200 bp. Primers refer to 2.10.

Table 5.1 Primer sequences used in the real-time quantitative PCR

Gene	Primer Name	Primer sequence(5′-3′)
Hsf1	Qsf1	TTA TGT CAG GAG CTG CAC GAA
	Qsf2	AAC AAT GGG CTA CGA CTA TCT
Hsp60	Q60f	TGC CTC ATT GCT CAC AAC TGC
	Q60r	ACA AAC GAG CGG CTG ATT ACA
β-actin	act1	TCA CCA CCA CAG CTG AGA GA
B. tabaci	act2	CTC GTG GAT ACC GCA AGA TT

5.4.3 Real-time PCR quantitative detection

The operation steps are the same as 2.9

5.5 Comparison of differences in the expression levels of hsf1 regulatory factors

Fluorescence quantitative data statistics, according to the relative quantitative analysis method $^{2-\triangle\triangle Ct}$ value analysis method to analyze the relative expression difference, it can be concluded that the highest expression temperature of Hsf1 regulatory factor in *B. tabaci* is about 9℃.It is also concluded that the expression differences of Hsf1 regulatory factors of *B. tabaci* at different temperatures are more obvious.

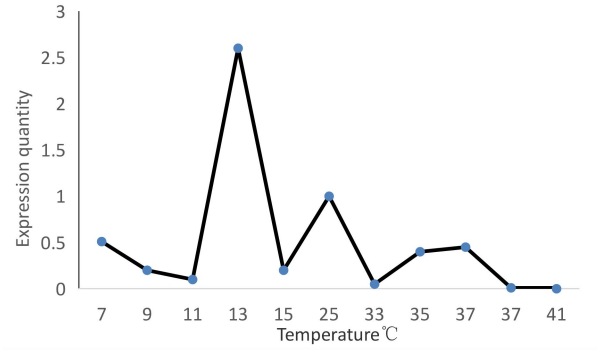


Figure 5.4 The mRNA expression profiles of heat shock factor1 (*hsf*1) genes in *B. tabaci*

Through five different temperature gradient induction treatments, the spss (13.0) software was used to analyze statistics, and the significance was determined by the new multiple range method (Duncan method) test. The difference of the expression level under the treatment was significant ($p<0.05$), and the expression difference under the other different temperatures was not significant. Overall, different high and low temperature treatments had no significant effect on the expression of hsf1.

5.6 Comparison of differences in hsp60 gene expression

The highest expression temperature of Hsp 60 in *B. tabaci* is around 13-25℃.

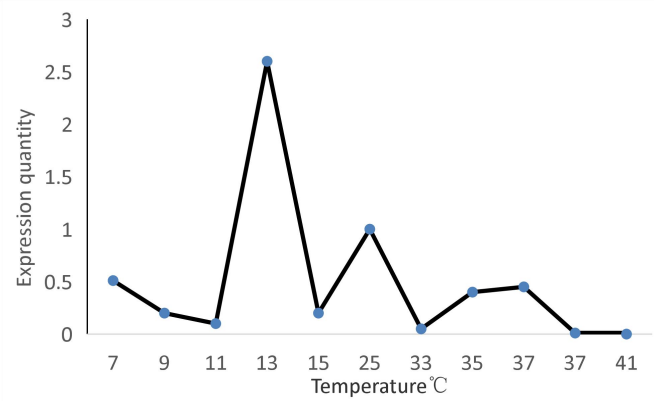


Figure 5.5 The mRNA expression profiles of heat shock protein (hsp60) genes in *B. tabaci*

The significance analysis method is the same as above, and the following conclusions are drawn: the expression of *hsp*60 in *B. tabaci* is not significantly different at different temperatures, and the difference in expression is only significant at 7℃and 41℃($p<0.05$). There were significant differences in the expression of other temperatures ($p<0.05$). Overall, different high and low temperatures had no significant effect on the expression of hsp60.

Conclusions to Chapter 5

1. The effect of different temperatures on the growth and development of *B. tabaci,* the temperature has a significant effect on the developmental process of each stage of *B. tabaci bio*type B. Experiments show that *B. tabaci* are treated in the range of 7-39℃ on egg hatching rate and adult reproduction are affected. The developmental duration of each state of *B. tabaci* biotype B was shortened with the increase in temperature, and the developmental rate was accelerated with the increase in temperature. The developmental starting temperature and effective accumulated temperature obtained by the linear regression method were 12.41℃ and 278.42℃, respectively. With the increase in temperature, the lifespan of B. tabaci adults gradually shortened, and the number of eggs laid gradually decreased. The comprehensive results of various parameters showed that 26-29℃ was the most suitable temperature for the survival and growth of *B. tabaci* biotype B population. The suitable high-temperature limit for the survival of the two types of *whitefly* adults, namely the lethal temperature, is different: the temperature at which adults of the greenhouse *whitefly* die is 39.0-39.5℃, and the temperature of *B. tabaci* biotype B is 41.5-42.0 ℃. In addition, the lethal temperature zones of the two types of *whitefly* overlap very little. It can be seen that *B. tabaci* biotype B adults have higher heat tolerance than greenhouse *whitefly*.

2. The survival rates of *B. tabaci* biotype B were significantly different at different temperatures. From egg development to adult eclosion, the survival rate of *B. tabaci* differs greatly at different temperatures, among which the survival rate is the

highest at 26℃ and the lowest at 32℃, indicating that temperature has a greater impact on the survival of B. tabaci [276]. There is a significant difference with the mortality rate of *B. tabaci* biotype B in this experiment under different temperature conditions. The test shows that at the optimum temperature of *B. tabaci*, which is 26℃, the result is consistent with the near-zero death of tabaci. The average lifespan of females at 20℃ was 38.4 days, but only 12.5 days at 32℃. The number of eggs laid is also more than twice that of 32℃. It shows that temperature has a great influence on the survival and reproduction of *B. tabaci*. As the temperature increased, the number of eggs laid also decreased. The highest egg production of a single female appeared at 20℃. The sex ratio decreased when the temperature increased, but the difference was not significant at different temperatures.

3. Non-lethal high temperature acclimation significantly improved the resistance of *B. tabaci bio*type B adults to extreme lethal heat stress. Acclimation at 33.0℃ significantly improved adult survival by 19% compared to control. The higher the temperature, the more obvious the effect of enhancing heat resistance. The acclimation effect was the best at 37.0 ℃, and the survival rate was significantly increased by 28%. Further increasing the acclimation temperature, the survival rate decreased. 0℃ had little effect on the survival rate of *B. tabaci* eggs, and 78% of the eggs still survived at this temperature for 8 days. However, the survival rate decreased rapidly at temperatures below 0 ℃: 56% of the eggs died after treatment at -2.5℃ for 8 days; almost all eggs died after treatment at -5℃ for 4 days. There are significant differences between temperature treatments, and low temperature exposure time also has a very significant effect. There was no significant difference between male and female adults at 37℃ and 39℃. Heat stress led to a significant decrease in the fecundity of *B. tabaci*. At 25.0℃, the effective number of eggs laid by 20 females reached 114. However, after exposure to 10.0 ℃ for 4 h, the number of eggs laid by adults dropped to 19, which was

significantly lower than that of the control. Continued inoculation at 32.0℃ also resulted in a significant decrease in female egg production. Exposure to 35.0℃ for 1 h reduced the number of eggs laid by females to 4, indicating that the high temperature almost completely inhibited the egg-laying behavior of adult whitefly in the greenhouse. The effect of heat stress on the adult offspring was limited, and the effect on the nymph survival rate, egg hatching rate, and sex ratio (♀:♂) including the offspring was not significant. The next generation of nymphs could develop into adults normally after all treatments, including 32.0℃ treatment of continuous inoculation.

The temperature has a great influence on the reproduction of *B. tabaci* adults, and the sex ratio of male and female adults is different at different temperatures. The research results show that high temperature leads to a decrease in the reproductive fitness of *B. tabaci* (reduced egg production), which will affect the survival of the offspring of the population. scale. High temperature may shorten the lifespan of *B. tabaci* female adults and reduce the number of matings, thereby reducing the fertilization rate of female adult eggs and increasing the proportion of male offspring.

4. The expression levels of Hsf1 regulators in *B. tabaci* were significantly different at 9℃($p<0.05$), while the expression differences at other different temperatures were not significant. Overall, different high and low temperature treatments had no significant effect on the expression of Hsf1. The highest expression temperature of Hsp60 in *B. tabaci* is around 25℃. The expression of Hsp60 in *B. tabaci* was not significantly different at different temperatures, and the difference in expression was only significant at 7℃ and 41℃($p<0.05$), and at 13℃ compared with other temperatures ($p<0.05$), Overall, the different high and low temperature had no obvious effect on the expression of Hsp60. According to the comparison of the expression levels of HSF1 regulatory factor and Hsp60 gene in *B. tabaci* at different temperatures, it can be seen that the two genes have an obvious expression at low temperatures, but there is no significant expression at high temperatures. *B. tabaci* biotype B has a wide

temperature ecological range. At the same time, it can be seen that the expression of HSF1 is positively correlated with the expression of HSP60, and they are induced at low temperatures at the same time, This can clearly show that the Hsp60 gene plays an induced protective role through HSF1 regulatory factor.

5. According to the comparison of Hsf1 regulatory factor and hsp60 gene expression levels in *B. tabaci* at different temperatures, it can be seen that both genes have obvious expression levels at low temperature, but no significant expression at high temperature. *Whiteflies* have a wide temperature ecological range, and it can be seen that the expression of hsf1 is positively correlated with the expression of hsp60, and they are induced at low temperature at the same time, which can clearly show that the hsp60 gene plays a protective role through the hsf1 regulatory factors. It provides a reference for studying the population expansion of other invasive species.

6. The results of this study suggest that hsf1 in *B. tabaci* may be positively correlated with its heat resistance. The invading *B. tabaci* with stronger heat resistance is subject to environmental pressure in the process of adapting to the new environment so that it can be selected in a harsh environment. Under environmental stress, it can induce more heat shock protein gene expression, thereby gaining stronger stress resistance. In the process of long-term adaptation and evolution of insects, the long-term selection of extreme temperatures will affect the temperature adaptability of species. It is difficult for insects to avoid the stress of temperature changes so that their populations can obtain environmental stress that can be maintained and inherited. When *B. tabaci* biotype B is subjected to temperature stress, especially extreme temperature stress, heat shock protein factors are rapidly expressed, which can improve the heat tolerance of *B. tabaci* biotype B under high temperature, and finally survive the competition.

In the process of biological evolution, HSF has less structural and functional variation and is a protein ubiquitously present in eukaryotic cells with extensive

homology. It is divided into 4 types according to their functions, including Hsf1, Hsf2, Hsf3, and Hsf4. Among them, Hsf1 is considered to be the main regulator of cellular heat shock protein expression, it is often highly conserved in Drosophila, yeast, and vertebrates, and its function cannot be replaced by the other three HSF regulators. Hsf1 regulates the expression of stress-induced heat shock genes after acquiring activities such as DNA binding activity, oligomerization, and nuclear localization, enabling organisms to respond to environmental stresses, such as high temperature, heavy metal environments, and protease inhibitors. In rodents, when the organism is exposed to a stressful environment, HSF1 mainly regulates the expression of Hsp70, and the role of Hsf1 is more prominent in the physiological emergency response. In mammals and Drosophila, Hsf1 is constitutively expressed in the cytoplasm and nucleus. In vitro studies have confirmed that purified Drosophila HSF can directly respond to high temperature and oxidative stress, indicating that HSF may act as an intracellular "thermometer" to regulate the stability of the organism's in vivo environment after the organism exceeds the physiological tolerance range.

In conclusion, high temperature stress can reduce the developmental fitness of *B. tabaci*, resulting in reduced survival rate, decreased egg production, reduced proportion of female adults and prolonged developmental duration of *B. tabaci*. However, *B. tabaci* biotype B can have a certain compensatory effect on population development by maintaining the survival rate of offspring and producing enough effective eggs. The adaptability of *B. tabaci* biotype B to high temperature stress is conducive to its further expansion under global warming. Analyzing the adaptability of *B. tabaci* biotype B to high temperature stress is also important for predicting the geographical distribution of *B. tabaci* population and formulating effective control strategies.

CHAPTER 6

FIELD CONTROL EFFECT OF *B. TABACI*

6.1 Test method

The expression of heat shock protein and heat shock factor of *B. tabaci* was the highest at the suitable temperature (25℃), which played a very good role in protecting *B. tabaci*, and played a beneficial role in the reproduction of *B. tabaci*. In the field pesticide test of *B. tabaci*, we ensure that the greenhouse temperature is 25℃ to enhance the viability of *B. tabaci* when applying the pesticide. At the same time, we can better evaluate the pesticide and play a key role in selecting the ideal insecticide of *B. tabaci*.

The test plot is located in the solar greenhouse on the east campus of Henan University of Science and Technology. I started planting tomatoes on August 19, 2020, with 667m^2 planting 2000 plants. The cultivation conditions (cultivation, fertilization, plant, row spacing, etc.) of the test plots are consistent and conform to local cultivation habits. A total of 10 chemical treatment groups and a clear water control were set up in the experiment, treatment A: 25% Thiamethoxam water-dispersible granules (20g for 667m^2), treatment B: 9% Mineral oil emulsifiable concentrate (500g for 667m^2), treatment C: 22.4% Spirotetramat suspension concentrate (30mL for 667m^2), treatment D: 17% Flurpyrone soluble concentrate (40mL for 667m^2), treatment E: 70% Acetamiprid water-dispersible granules (3g for 667m^2), treatment F: 5% Diprofen dispersible concentrate (40mL for 667m^2), treatment G:10% Cyantraniliprole suspension concentrate (40mL for 667m^2), treatment H: 1.8% Abamectin emulsifiable concentrate (40mL for 667m^2), treatment I: 50% Flonicamid water dispersible granule (10g for 667m^2), treatment J: 20% Mevirpirazone suspension concentrate (40mL for 667m^2). Each treatment was repeated 3 times, a total of 33 test plots, each plot area is about 18m^2, arranged in random blocks. The dosage of each test agent is the maximum recommended dosage. The medicament is sprayed with Zhejiang Taizhou Minghui

3WBD-16 electric sprayer, and the water consumption is 30L per 667m^2. The first application will be carried out on October 13, 2020.

6.2 Investigation methods

Investigate the number of insect populations before spraying, and at 1, 3, and 7 days (October 14, 16, and 20) after spraying, the number of insect populations was determined at designated locations. Each plot adopts a 5-point sampling method, and each spot is marked with 2 tomato plants. Investigate the number of adults in the whole plant when the adults are not active in the morning.

The formula for calculating efficacy: Decline rate of insect population = (number of insects before spraying - number of insects after spraying) / number of insects before spraying times 100%.

Corrected control effect = 1- the number of prednisolone in blank control area times the number of insects after chemical treatment / the number of insects after chemical treatment in blank control area times the number of insects before pesticide treatment times 100%.

6.3 Statistical Analysis

DPS software was used to perform statistical analysis on the test data, and Duncan's new multiple range method was used to analyze the variance of different agents against *B. tabaci.*

6.4 Control effect of treatment agent on *B. tabaci*

The effect of different treatments on the control of *B. tabaci* was different 1 day after the medicine. The corrected control effect of treatment F on *B. tabaci* is the best at 41%, which is equivalent to the effects of treatments H, A, B, and J, which is significantly higher than other test reagents, treatment C has the worst control effect on *B. tabaci* on tomato by 28%, None of the treatment reagents showed good quick-acting

properties. 3 days after the treatment, although there are differences in the control effects of different treatments on *B. tabaci*, the overall difference is small. Treatment C has the best control effect on *B. tabaci* at 72%, and treatments I and G have the worst effect at 62%. 7 days after treatment the best control effect of treatment J was 86%, which was equivalent to treatment G and D, and treatment B had the worst effect of 52%, which was significantly higher than other test reagents (Table 6.1, Figure 6.1).

Table 6.1 Control effect of tested insecticides on *B. tabaci*

Treatment	Pre-medicine treatment insect population base/head	1 day after medicine			3 days after the medicine			7 days after the medicine		
		Insect population base/ head	Decline rate of population (%)	Control effect (%)	Insect population base/ head	Decline rate of population (%)	Control effect (%)	Insect population base/ head	Decline rate of population (%)	Control effect (%)
A	139.3	98.3	0.29	0.39	54.2	0.61	0.72	86	0.38	0.58
B	102.1	75.1	0.26	0.37	43	0.58	0.70	72.6	0.29	0.52
C	137.4	115.2	0.16	0.28	53.7	0.61	0.72	92.2	0.33	0.55
D	95	76	0.20	0.31	49.5	0.48	0.63	23.5	0.75	0.83
E	234.7	195	0.17	0.29	105	0.55	0.68	128.1	0.45	0.63
F	187	128.5	0.31	0.41	94.2	0.50	0.64	62	0.67	0.78
G	155.3	117.2	0.25	0.35	82.1	0.47	0.62	34.2	0.78	0.85
H	124.5	86.2	0.31	0.40	57.2	0.54	0.67	82.3	0.34	0.55
I	144	112	0.22	0.33	75.3	0.48	0.62	62.3	0.57	0.71
J	172.6	123.5	0.28	0.38	78	0.55	0.67	34.6	0.80	0.86
CK	113.5	132			157.8			168.5		

Note: The control effect is the average value of each repeat.

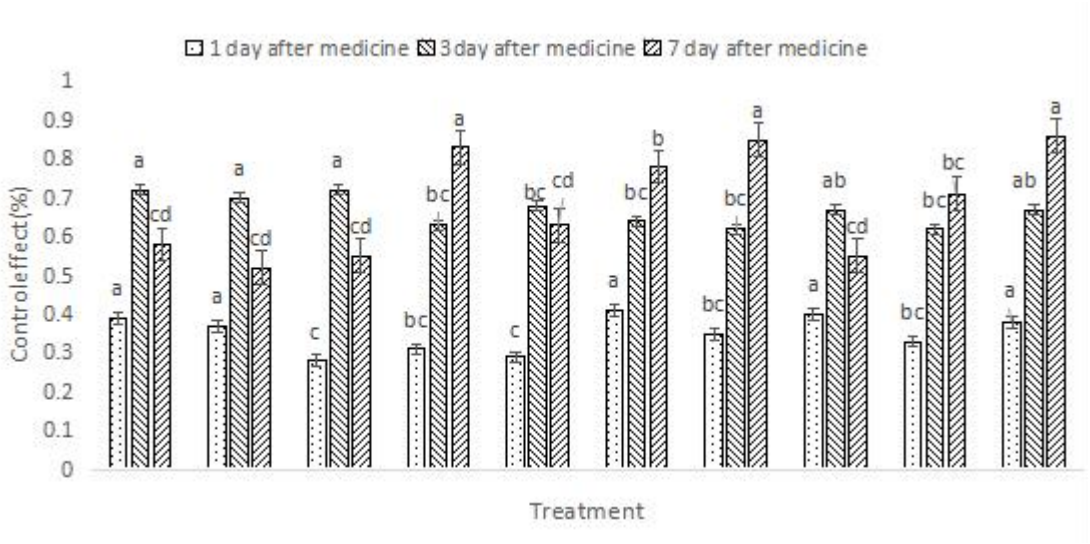


Figure 6.1 10 kinds of pesticides to control *B. tabaci* on different days

Note: The lowercase letters in the table indicate the significance of the difference at the 0.05 level.

6.5 Practical application direction

With the establishment of the green pollution-free food safety production system, the prevention and control of vegetable pests has gradually changed from traditional chemical control to green control, which requires reducing the use of chemical pesticides in green food production [274]. Therefore, biological control measures will become an important means of pest control in this system, especially the utilization of natural enemies. *B. tabaci* is one of the most important pests in the world. With the increasing problem of insecticide resistance and residue on vegetables, biological control has become an important measure to solve this problem. Compared with chemical control, screening effective aerial enemy for biocontrol of *B. tabaci* in different areas is an important method for effective control of *B. tabaci*.

Figure 6.2 Outbreak of *Bemisia tabaci* in Greenhouses in Henan Province, China

In the process of controlling *B. tabaci*, the combination of multiple natural enemies is an important means to increase the biocontrol effect of *B. tabaci* because the

single control effect is not ideal. At present, 168 natural enemies of *B. tabaci* have been reported in China, including 109 predatory natural enemies and 59 parasitic natural enemies [275,276]. Mixed release of different natural enemies can not only improve the control effect of *B. tabaci*, but also indirectly enhance community stability and reduce natural decline of natural enemy population by increasing biodiversity in the same food chain [276].

Ma et al. showed that when Serangium japonicum and Encarsia formosa were jointly released, the functional responses of predation and parasitization were in line with Holling II equation. The predation and parasitization of Serangium japonicum and Encarsia formosa on *B. tabaci* were significantly increased, and the damage control effect was significantly improved, which were better than that of single natural enemy insects. Thus, the control effect of *B. tabaci* is directly improved [277]. Under natural conditions, the release of the little black ladybug Delphastus catalinae (Horn) and the adult Serangium japonicum control cauliflower. *B. tabaci* was released for 3 weeks consecutively, and a total of 150 *B. tabaci* were caught. The results showed that both kinds of ladybugs could lower the population of *B.tabaci*. After the release of adult ladybugs, the number of *B. tabaci* decreased sharply with the increase of time [278].

However, with the increasing demand for healthy agricultural products, the use of green control methods, such as effective control of *B. tabaci* using natural enemy insects, is the future trend. Many predatory and parasitoid natural enemies of *B. tabaci* have been found and studied. From these studies and the actual results of using natural enemies, we can conclude that natural enemies and their role in agroecosystems are very important [279]. To improve the ability of analysis and identification of new natural enemies (especially predatory natural enemies) of *B. tabaci*, and to quantify their effects on *B. tabaci* population, so as to provide more safe and effective measures for the control of *B. tabaci*. So far, although there have been a lot of achievements in biocontrol of *B. tabaci*, most of the experiments have been carried out under indoor conditions, and

there are not many examples of successful control of *B. tabaci* in greenhouse or field, which have not reached the extent of effective control of *B. tabaci* [280].

Therefore, it is necessary to further develop the utilization of natural enemy insects, the cultivation of major crops such as field and greenhouse, and the control method of *B. tabaci* in China and the evaluation of the control effect. China in captivity *whiteflies* predators aspect also has the big gap, due to the high cost of artificial breeding method, process is more tedious, severely restricts the *whiteflies* natural enemy insects of large-scale production, and most farmers are reluctant to use this method to control *whiteflies*, therefore the Chinese government need to spend a lot of financial support to promote biological control.

Conclusions to Chapter 6

1. The damage of *B. tabaci* to crops is mainly manifested in the direct feeding of plant juice, affecting plant nutrient metabolism, resulting in yellow spots on plant leaves, yellowing and falling off in severe cases, and abnormal or irregular fruit structure. Generally, after the outbreak of *B. tabaci*, the virus transmitted by it will occur greatly. These viruses can cause severe damage by causing leaf curling, dwarfing plants and fruit abortion. The best effect of treatment f on *B. tabaci* was 41% one day after treatment, which was significantly higher than other test reagents, and did not show a good quick effect in all treatment reagents; The best control effect of treatment C on *B. tabaci* was 72%, and the worst effect of treatment I and G was 62%. Treatment J 7 days after treatment had the best control effect, which was 86%, significantly higher than other test reagents.

2. Neonicotinoid insecticides are currently the most important type of insecticide to control the harm of *B. tabaci*, but the control effect on Q-type *B. tabaci* has been declining year by year in recent years. Cyaniliprole is a new type of agent developed by DuPont. It belongs to the second generation of fish nitin receptor inhibitors. Sulfoxaflor is a new type of sulfoximine insecticide for the control of piercing-sucking pests. It not

only has a good quick-acting effect on *B. tabaci* but also has a long-lasting effect. During the control of *B. tabaci* in greenhouse cucumber fields, 3.2% abamectin (EC) is the most commonly used pesticide by local farmers, but it is not recommended for use in green food production. The new insecticides 10% bromocyanamide (OD) and 50% fluridonidinitrile (WG) had the highest control effect on *B. tabaci* 10 days after treatment, 94.90% and 94.18% respectively. They could effectively control the harm caused by Bemisia tabaci. The quick effect and lasting effect were as follows: 10% bromocyanamide (OD) > 50% fluridonidinitrile (WG). The second was three new nicotine insecticides, and the control effect was 68.61% -80.69% after 7 days Jia et al. Considered that the Q-type *B. tabaci* hidden species, as the dominant species in Turpan, has shown a high level of resistance to insecticides such as imidacloprid [277]. The control effect of pyridine insecticide 50% pyraphid (WG) was the highest, reaching 93.23% 7 days after treatment, and the effect was good. Pesticide residues are one of the important indicators for screening insecticides to control *B. tabaci*. The average residues of 8 insecticides in cucumber fruit samples are not detected, which is lower than the maximum residue limit standard in China, which can effectively ensure the quality and safety of vegetables [278]. In the three field investigations conducted from October 13 to 20, 2020, there was no drug damage and it was safe for tomatoes. In the process of field control, 20% methotrexate propyl ether suspension can be selected, which can be used together with 22.4% spirochete ethyl ester suspension and 5% dipropyl cyclamate dispersible solution to achieve a better control effect, and can be selected in protected vegetables.

3. The purpose of this experiment is to understand the level and development of *B. tabaci* resistance and to provide a basis for rational use of pesticides and delaying the development of *B. tabaci* resistance. The test results showed that: one day after treatment, treatment F had the best effect on B. tabaci by 41%, which was significantly higher than other test reagents. treatment C had the worst effect on *B. tabaci* control by 28%, and none of the treatment reagents shows good quick-acting. Three days after

treatment, treatment C had the best control effect on *B. tabaci* at 72%, and treatments I and G had the worst effect at 62%. Seven days after the treatment the best control effect of treatment J was 86%, and the worst effect of treatment B was 52%, which was significantly higher than other test reagents. In the three field surveys conducted on October 13-20, 2020, the tomato plants were growing well, and there were no symptoms of wilting, yellowing, and other phytotoxicity, which indicated that the control of *B. tabaci* in accordance with the dosage concentration of this field experiment was safe for tomato crops. In the field control process, you can choose 20% mefenproper suspending agents, which is used together with 22.4% spirotetramat suspending agent and 5% diprofenac dispersible liquid agent.

In nature, insects are very sensitive to temperature changes and it is difficult to avoid the stress of temperature changes. The adaptive process mechanism of insect tolerance to temperature stress can predict the origin, distribution, and dynamics of populations. Summer high temperature and dryness are favorable conditions for the exponential growth of the B. tabaci population and rampant damage.

CONCLUSION

The strong adaptability to temperature stress of *B. tabaci* is an important reason for its successful invasion and colonization. To study the regulation and function of invasive *B. tabaci* hsf1, reveal the mechanism of *B. tabaci* invasion, verify and improve the theoretical hypothesis of *the B. tabaci* invasion mechanism. Further, reveal the internal mechanism of *B. tabaci* invasion and its adaptability to temperature stress, enriching and perfecting the previous researchers' proposals. The hypothesis of *B. tabaci* invasion and adaptive mechanism provides the atheoretical basis for biological invasion control research.

Monitoring the occurrence of *B. tabaci* and timely and accurate trend forecasts can effectively control and reduce hazards. Strengthen the monitoring of *B. tabaci* infestation, based on early prevention and control, to control the base of insect sources and cut off the transmission route as the key measures. The prevention and control of *B. tabaci* should adhere to the concept of "green plant protection," rationally apply relevant green prevention and control technologies and ecological engineering technologies, and reduce the use of chemical pesticides, to ensure the ecological environment and the safe production of vegetables and tobacco. The green and environmental protection control methods of *B. tabaci* include: using modern molecular biology to cultivate new anti-virus varieties. When the crops were planted, yellow sticky boards were hung in the field to trap *B. tabaci* adults and monitor their occurrence dynamics. Planting non-hobby host plants in the greenhouse have a better repelling effect on *B. tabaci*. The release of natural enemies plays a crucial role in controlling the population of *B. tabaci*. The use of a large number of insecticides leads to the drug resistance of *B. tabaci*. We choose high-efficiency and low toxicity chemical pesticides in a reasonable and timely manner. *B. tabaci* young instar nymphs (before the third instar) are thin wax layer, poor drug resistance, and easier to control. When carrying out green prevention and control,

it is necessary to adapt measures to local conditions and take effective prevention and control measures in combination with the local production level at that time.

Predicting the occurrence trend of *B. tabaci* is an essential basis for effectively controlling *B. tabaci* and reducing the degree of damage, and it is a vital prerequisite for ensuring the safety of agricultural production. The statistical establishment of the prediction model for the peak occurrence period and occurrence amount of *B. tabaci* on different crops has guiding significance for scientific and practical control of *B. tabaci* in agricultural production.

The results of this study provide a basis for the research on the resistance and adaptability of *B. tabaci* and further verify that the conserved functional gene heat shock protein can be used as one of the methods to study the development of biological systems. To study the production of *whitefly* heat shock protein, and the law of change can understand the relationship between its growth and development and various influencing factors, and comprehensively provide new ideas for prevention and treatment. In this study, it was found that different temperatures and humidity had different responses to the heat shock of *B. tabaci*.

In practical production, chemical pesticides with strong endoinhalation were usually used to effectively control *B. tabaci* when the heat shock reaction was strong. Based on the change law of greenhouse temperature and humidity, the research is carried out to prevent and control.

The strong adaptability of B. tabaci to temperature stress is an important reason for its successful invasion and colonization. This study is based on the experimental results of the differences in the expression of Hsps under high and low temperature stress between the greenhouse whitefly and the MED cryptic species of *B. tabaci,* and the theoretical hypothesis of the invasion mechanism related to the temperature stress tolerance and Hsps expression between the invading biotypes of *B. tabaci* and native biotypes. To study the regulation and function of HSF1 in the invasive *whitefly*, to

reveal the *whitefly* invasion mechanism from a deeper level, and to verify and improve the theoretical hypothesis of the *whitefly* invasion mechanism proposed earlier. Further reveal the internal mechanism of *whitefly* invasion and adaptability to temperature adversity, enrich and improve the hypothesis of *whitefly* invasion and adaptation mechanism proposed by previous researchers, and provide a theoretical basis for biological invasion control research.

Use 10 kinds of insecticides to control *B. tabaci* on tomatoes in greenhouses. One day after treatment, 70% Acetamiprid water-dispersible granules have the best effect on *B. tabaci* at 41%; 22.4% Spirotetramat suspension concentrate is effective for *B. tabaci* on tomatoes and the worst control effect is 28%. 3 days after treatment, the 22.4% Spirotetramat suspension concentrate has the best control effect on *B. tabaci* at 72%. 7 days after treatment, The best control effect of 20% Mevirpirazone suspension concentrate on *B. tabaci* is 86%. In the reagent production process, we can choose 70% acetamiprid water-dispersible granules,22.4% Spirotetramat suspension concentrate and 20% Mevirpirazone suspension concentrate to promote the prevention and control of *B. tabaci* and achieve better prevention effect.

According to the Intergovernmental Panel on Climate Change (IPCC)(2007), global average temperature has been rising in recent years with the intensification of global Climate Change. By 2010, the global average temperature will rise by 1.4-5.8 ℃ and the frequency of extreme weather events will increase. The average global temperature rose by 0.74 ℃ between 1906 and 2005. In the 50 years from 1956 to 2005, the increase was 0.13℃ per decade, twice that of the past 100 years. Climate change has caused a huge impact on human beings and even the whole ecosystem, and the impact of temperature change on insects has gradually become a research hotspot. The results showed that the reproduction of *B. tabaci* was affected by extreme heat stress in egg and larval stages, but the population was still maintained and expanded rapidly in the

summer heat season through the reproductive adaptation of the offspring, which may be an important reason for the wide distribution and rapid spread of *B.tabaci* worldwide.

Under the trend of global warming, high temperatures will adversely affect other organisms, but B. tabaci has a high heat tolerance. High temperature will affect the physiology and biochemistry of insects, causing the loss of water in insects, changing the concentration of important ions in the cells, collapsing the cytoskeleton, increasing the fluidity of cell membrane, inactivating biological macromolecules, producing heat shock proteins, and enhancing the peroxidation reaction. Sex ratio directly affects insect population and is also an important index for evaluating insect heat tolerance. It is possible that high temperature affects the hormone content or ion concentration related to sex ratio determination in insects, and the occurrence of male spermatozoa, or other factors lead to the change of insect sex ratio. The explanation of the influencing factors of insect sex ratio change will also be the focus of the next work. In addition, *B. tabaci* can undergo long-term and short-term heat stress in natural conditions. The adaptability of *B. tabaci* to heat stress in the above environment needs to be further studied.

REFERENCES

1. Gennadius P. Disease of tobacco plantations in the Trikonia. The aleurodid of tobacco. Ellenike Georgia. 1889. 5. P. 1 - 3.

2. Mound, L., &Halsey, S.H. Whitefly of the world. A systematic catalogue of the Aleyrodidae (Hemiptera) with host plant and natural enemy data. British Museum (Natural History). 1978. P.340.

3. Brown, J.K., Frohlich, D.R., & Rosell, R.C. The Sweetpotato or Silverleaf Whiteflies: Biotypes of Bemisia tabaci or a Species Complex. Annual Review of Entomology. 1995. 40. P. 511 - 534.

4. De Barro, P.J., Liu, S.S., Boykin, L.M., & Dinsdale, A.B. Bemisia tabaci: a statement of species status. Annual Review of Entomology. 2011. 56. P. 1 – 19.

5. Wang, H.L., Lei, T., Xia, W.Q., et al. Insight into the microbial world of Bemisia tabaci cryptic species complex and its relationships with its host. Scientific Reports. 2019. 9(1). P. 6568.

6. Perring, T.M., Cooper, A.D., & Bellows, T.S. Identification of a whitefly species by genomic and behavioral studies.Science. 1993. 259. P. 74 - 77.

7. Perring, T.M. The bemisia tabaci species complex. Crop Protection. 2001. 20(9). P. 725 - 737.

8. Oliveira, MRV., Henneberry, T.J., & Anderson, P. History, current status, and collaborative research projects for Bemisia tabaci. Crop Protection. 2001. 20(9). P. 709 - 723.

9. Dinsdale, A., Cook, L., Riginos, C., Buckley, Y.M., & Barro, P. D. Refined global analysis of genetic boundaries for Bemisia tabaci using mitochondrial CO1: will the real B.tabaci please stand up. Annals of the Entomological Society of America. 2010. 103(2). P. 196 - 208.

10. De Barro, P.J., Liebregts, W., & Carver, M. (1998). Distribution and identity of biotypes of Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) in member countries of the Secretariat of the Pacific Community. Australian Journal of Entomology. 1998. 37(3). P. 214 - 218.

11. Hu, J., De Barro, P., Zhao, H., Wang, J., Nardi, F., & Liu, S.S. An extensive field survey combined with a phylogenetic analysis reveals rapid and widespread invasion of two alien whiteflies in China. PLoS One. 2011. 6. P. F16061.

12. Teng, X., Wan, F.H., & Chu, D. Bemisia tabaci biotype Q dominates other biotypes across China. Florida Entomologist. 2010. 93(3). P. 363 - 368.

13. Rao, Q., Luo, C., Zhang, H., Guo, X., & Devine, G. Distribution and dynamics of Bemisia tabaci invasive biotypes in central China. Bulletin of Entomological Research. 2011. 101(1). P. 81 - 88.

14. Chu, D., Zhang, Y.J., & Wan, F.H. Cryptic invasion of the exotic Bemisia tabaci biotype Q occurred widespread in Shandong Province of China. Florida Entomologist. 2010. 93 (2). P. 203 - 207.

15. Chu, D., Tao, Y.L., & Chi, H. Influence of plant combinations on population characteristics of Bemisia tabaci biotypes B and Q. Journal of Economic Entomology. 2012. 105. P. 930 - 935.

16. Wei, J., Lü, Z.C., Wang, R., & Wan, F.H. Comparative analysis of the contents of the heat shock protein genes hsp70 and hsp90 in the three cryptic species of the greenhouse whitefly and the whitefly. Acta Entomologica Sinica. 2014. 57(6). P. 647- 655.

17. Coppola, V., Coppola, M., Rocco, M., Digilio, M.C., Dambrosio, C., Renzone, G., & Corrado, G. Transcriptomic and proteomic analysis of a compatible tomato-aphid interaction reveals a predominant salicylic acid-dependent plant response. BMC genomics. 2013. 14. P. 515.

18. Dogan,Y.O., Hazir, S., Yildiz, A., Butt, T.M., & Cakmak, I. Evaluation of entomopathogenic fungi for the control of Tetranychus urticae (Acari: Tetranychidae) and the effect of Metarhizium brunneum on the predatory mites (Acari: Phytoseiidae). Biological Control. 2017. 111. P. 6 - 12.

19. Dorta, B., Bosch, A.J., Arcas, A., & Ertola, R.J. High level of sporulation of Metarhizium anisopliae in a medium containing by-products. Applied Microbiology and Biotechnology. 2012. 33. P. 712 - 715.

20. Luo, C., & Zhang, Z.L. Overview of the research on Bemisia tabaci (Gennadius). Beijing Agricultural Sciences, Bemisia tabaci Special. 2000. P. 4 - 15.

21. Chu, D., Zhang, Y.J., & Wan, F.H. Monitoring of Bemisia tabaci biotypes and research on genetic structure. Insect Knowledge. 2008. 45(3). P. 353 - 355.

22. Farooq, M., & Freed, S. Insecticidal activity of toxic crude proteins secreted by entomopathogenic fungi against Musca domestica L. (Diptera: Muscidae). Kuwait Journal of Science. 2018. 45(2). P. 64 -74.

23. Feng, Yuntao., Wu, Qingjun., Wang, Shaoli., Chang, Xiaoli., Xie, Wen., Xu, Baoyun., & Zhang, Youjun. Cross-resistance study and biochemical mechanisms of thiamethoxam resistance in B biotype Bemisia tabaci (Hemiptera: Aleyrodidae). Pest Management Science. 2010. 66. P. 313 - 318.

24. Ferron, P. Fungal control -Comprehensive Insect Physiology, Biochemistry and Pharmacology.Vol.III. (Eds.) G.A. Kerkut and L. Gilbert, ed. Pergamon Press, New York. 2015. P. 313 - 346.

25. Fontes, FVHM., Colombo, C.A., & Lourencao, A.L. Structure of genetic diversity of Bemisia tabaci (Genn.) (Hemiptera: Aleyrodidae) populations in Brazilian crops and locations. Journal of Agricultural Science. 2012. 69. P. 47 - 53.

26. Freed, S., Jin, F.L., & Ren, S.X. Phylogenetics of entomopathogenic fungi isolated from the soils of different ecosystems. Pakistan Journal of Zoology. 2011. 43. P. 417 - 425.

27. Gillespie, A.T., & Crawford, E. Effect of water activity on conidial germination and mycelial growth of Beauveria bassiana, Metarhizium anisopliae, Paecilomyces spp., and Verticiliium lecanii.In Fundamental and Applied Aspects of Invertebrate Pathology, Edited by R.A Samson, J.M. Vlak & D. Peters. Wageningen: Society of Invertebrate Pathology. 2015. P. 254.

28. Guo, C.L., Zhu, Y.Z., Zhang, Y.J., Keller, M.A., & Chu, D. Invasion Biology and Management of Sweetpotato Whitefly (Hemiptera: Aleyrodidae) in China. Journal of Integrated Pest Management. 2021. 12. P. 2.

29. Butter, N.S., & Vir, B.K. Morphological basis of resistance in cotton to the whitefly Bemisia tabaci. Phytoparasitica. 1989. 17(4). P. 251 - 261.

30. Bellows, T.S., Perring, T.M., Gill, R.J., & Headrick, D.H. Description of a Species of Bemisia (Homoptera: Aleyrodidae). Annals of the Entomological Society of America/Oxford Academic. 1994. 87(2). P. 195 - 206.

31. Bedford, I. D., Briddon, R. W., Brown, J. K., Rosell, R. C., & Markham, P.G. Geminivirus transmission and biological characterization of Bemisia tabaci (gennadius) biotypes from different geographic regions. Annals of Applied Biology. 1994. 125(2). P. 311 - 325.

32. Rosell, R. C., Bedford, I. D., Frohlich, D. R., Gill, R. J., Brown, J. K., & Markham, P. G. Analysis of morphological variation in distinct populations of Bemisia tabaci (homoptera: aleyrodidae).Annals of the Entomological Society of America. 1997. 90(5). P. 575 - 589.

33. Qiu, B.L., Chen, Y.P., Liu, L., Peng, W.L., Li, X.X., Ahmed, M.Z., Mathur, V., Du, Y.Z., & Ren, S.X. Identification of three major Bemisia tabaci biotypes

in china based on morphological and DNA polymorphisms. Progress in Natural Science. 2009. 19(6). P. 713 - 718.

34. Liu, S.S., Colvin, J., & De Barro, P.J. Species concept as applied to the whitefly Bemisia tabaci systematics: how many species are there?. Journal of Integrative Agriculture. 2012. 11. P. 176 - 186.

35. Yokomi, R.K., Hoelmer, K.A., & Osborne, L.S. Relationships between the sweetpotato whitefly and the squash silverleaf disorder. Phytopathology. 1990. 80. P. 895 - 900.

36. Liu, B.M., Preisser, E.L., Chu, D., Pan, H.P., Xie, W., Wang, S.L., Wu, Q.J,. Zhou, X.G., & Zhang, Y.J. Multiple forms of vector manipulation by a plant-infecting virus: Bemisia tabaci and tomato yellow leaf curl virus. Journal of Virology. 2013. 87(9). P. 4929 - 4937.

37. Polston, J.E., Barro, P.J.D., & Boykin, L.M. Transmission specificities of plant viruses with the newly identified species of the Bemisia tabaci species complex. Pest Management Science. 2014. 70(10). P. 1547 - 1552.

38. Gilbertson, R.L., Batuman, O., Webster, C.G., & Adkins, S. Role of the insect supervectors Bemisia tabaci and frankliniella occidentalis in the emergence and global spread of plant viruses. Annual Review of Virology. 2015. 2(1). P. 67 - 93.

39. Patil, B. J. Studies on plant extracts as insecticides against whiteflies Bemisia tabaci (gen.) on tomato to protect environmental pollution. Nature Environment and Pollution Technology. 2009. 8(3). P. 515 - 517.

40. Ren, S.X., Wang, Z.Z., Qiu, B.L. & Xiao, Y. The pest status of Bemisia tabaci in china and non-chemical control strategies. Insect Science. 2010. 8(3). P. 279 - 288.

41. Shimron, O., Hefetz, A., & Gerling, D. Arrestment responses of eretmocerus species andencarsia deserti (hymenoptera: aphelinidae) to Bemisia tabaci honeydew. Journal of Insect Behavior. 1992. 5(4). P. 517 - 526.

42. Yee, W.L., Hendrix, D.L., Toscano, N.C., Chi, C.C., & Henneberry, T.J. Diurnal field patterns of honeydew sugar secretion by Bemisia argentifolii (homoptera: aleyrodidae) nymphs on cotton. Environmental Entomology. 1996. 25(4). P. 776 - 782.

43. Muniyappa, V., Venkatesh, H.M., Ramappa, H.K., Kulkarni, R.S., Zeidan, M., Tarba, C.Y., Ghanim, M., & Czosnek, H. Tomato leaf curl virus from Bangalore(ToLCV-Ban4): sequence comparison with Indian ToLCV isolates, detection in plants and insects, and relationships. Archives of Virology. 2000. 145. P. 1583 - 1598.

44. Czosnek, H., Ghanim, M., & Ghanim, M. The circulative pathway of begomoviruses in the whitefly vector Bemisia tabaci—insights from studies with Tomato yellow leaf curl virus. Annals of Applied Biology. 2002. 140. P. 215 - 231.

45. Moreno-Delafuente, A., Garzo, E., Moreno, A., & Fereres, A. A plant virus manipulates the behavior of its whitefly vector to enhance its transmission efficiency and spread. Plos One. 2013. 8(4). P. e61543.

46. Ghanim, M., & Czosnek, H. Tomato yellow leaf curl geminivirus (TYLCV-Is) is transmitted among whiteflies (Bemisia tabaci) in a sex-related manner. Journal of Virology. 2000. 74(10). P. 4738 - 4745.

47. Maruthi, M.N., Hillocks, R.J., Mtunda, K., Raya, M.D., Muhanna, M., Kiozia, H., Coivin, J., & Thresh, J.M. Transmission of cassava brown streak virus by Bemisia tabaci (gennadius). Journal of Phytopathology. 2005. 153(5). P. 307 - 312.

48. Nawaz-ul-Rehman, M.S., & Fauquet, C.M. Evolution of geminiviruses and their satellites. FEBS Letters. 2009. 583(12). P. 1825 - 1832.

49. Azab, A.K. , Megahed, M.M. , & El- Mirasawi, H.D. On the range of host Plants of Bemisia tabaci (Genn.). Bull dela Societe Entomologique Egypte. 1971. 32(54). P. 319 - 326 .

50. Greathead, A.H. Host Plants. In: Cock M J Wed Bemisia tabaci, A Literature Survey on the Cotton Whitefly with an Annotated Bibliography. Food and Agriculture Organization of the UN, CAB. Int. Inst. Biol. Cont. 1986. P. 17 - 25.

51. Sheng, Y. A Study on the Biotypes of Bemisia tabaci in Parts of China. Doctoral dissertation, Yangzhou University. 2007. P. 1 - 2.

52. Drost, Y.C., Van Lenteren, J.C., & Van Roermund, H.J.W. Life-history parameters of different biotypes of Bemisia tabaci (hemiptera: aleyrodidae) in relation to temperature and host plant: a selective review. Bulletin of Entomological Research. 1998. 88(3). P. 219 - 230.

53. Berlinger, M.J. Host plant resistance to Bemisia tabaci. Agriculture Ecosystems & Environment. 1986. 17(1). P. 69 - 82.

54. Jing, Y., Huang, J., Ma, R.Y., & Han, J.C. Host plant preferences of bemisia tabaci gennadius. Entomologia Sinica. 2003. 10(2). P.109 - 114.

55. Zhang, Y.J., Liang, G.M., Ni, Y.X., Wu, K.M. & Guo, Y.Y. Selection of the adult of Bemisia tabaci (gennadius) to different host plants. Plant Protection. 2003. 29(1). P. 20 - 22.

56. Yang, Z.X., Ma, C.S., Wang, X.Q., Long. H.R., Liu, X.Y, & Yang, X. Preference of Bemisia tabaci (gennadius) (Homoptera: Aleyrodidae) to four vegetable hosts. Acta Entomologica Sinica. 2004. 47(5). P. 612 - 617.

57. Zhang, H.Y. Bemisia tabaci and its control, Beijing: Golden Shield Press. 2010. P. 5 - 15.

58. Lin, K., Wu, K., Zhang, Y., & Guo, Y. Overwintering and population dynamics of Bemisia tabaci biotype b in greenhouse during the spring in northern China. Crop Protection. 2007. 26(12). P. 1831 - 1838.

59. Gerling, D., Horowitz, A.R., & Baumgaertner, J. (1986). Autecology of Bemisia tabaci. Agriculture Ecosystems & Environment, 17(17), 5 - 19.

60. Li, L., & Ren, S.X. Development and reproduction of b biotype Bemisia tabaci on variegated leafcrotones. Journal of South China Agricultural University. 2005. 26(2). P. 39 - 42.

61. Xu, J., Lin, K.K., & Liu, S.S. Performance on different host plants of an alien and an indigenous Bemisia tabaci from china. Journal of Applied Entomology. 2010. 135(10). P. 771 - 779.

62. Costa, H.S., Brown, J.K., & Byrne, D.N. Host plant selection by the whitefly, Bemisia tabaci (gennadius), (Hom. Aleyrodidae) under greenhouse conditions. Journal of Applied Entomology. 1991. 112(1). P. 146 - 152.

63. Wan, F.H., Zhang, G.F., Liu, S.S., Luo, C., Chu, D., Zhang, Y.J., Zang, L.S., Min, J., LV, Z.C., & Cui, X.H. Invasive mechanism and management strategy of Bemisia tabaci(gennadius) biotype b: progress report of 973 program on invasive alien species in China. Science in China Series C: Life Sciences. 2009. 52. P. 88 - 95.

64. Zang, L.S., Chen, W.Q., & Liu, S.S. Comparison of performance on different host plants between the b biotype and a non-b biotype of Bemisia tabaci from Zhejiang, China. Entomologia Experimentalis et Applicata. 2010. 121(3). P. 221 - 227.

65. Jiao, X.G., Xie, W., Wang, S.L., Wu, Q.J., Zhou, L., Pan, H.P., Liu, B.M., & Zhang, Y.J. Host preference and nymph performance of b and q putative species of Bemisia tabaci on three host plants. Journal of Pest Science. 2012. 85(4). P. 423 - 430.

66. Rahman, S., Liu, T.X., & Nicolas, D. Feeding experience of bemisia tabaci (Hemiptera: Aleyrodidae) affects their performance on different host plants. Plos One. 2013. 8(10). P. e77368.

67. Costa, A.S., & Russell, L.M. Failure of Bemisia tabaci to breed on cassava plants in Brazil (Homoptera:Aleyroideae). Ciencia E Cultura. 1975. 27. P. 388 - 390.

68. Bird, J. A whitefly transmitted mosaic of Jatropha gossypifolia .Agricultural Experiment Station University of Puerto Rico. 1957. 22. P. 1 - 35.

69. Liu Y.Q., & Liu, S.S. Species status of Bemisia tabaci complex and their distributions in China . Journal of Biosafety. 2012. 21(4). P. 247 - 255.

70. Abdel-Raheem, M., Reyad, N.F., & Al-Shuraym, L. Evaluation of some isolates of Entomopathogenic fungi on some insect pests infesting potato crop in Egypt. International journal of chemtech research. 2016. 9. P. 479 - 485.

71. Alavo, T.B. The insect pathogenic fungus Verticillium lecanii (Zimm.) Viegas and its use for pests control: A review. Journal of experimental biology and agricultural sciences. 2015. 3. P. 337 - 345.

72. Foolad, M.R., & Panthee, D.R. Marker assisted selection in tomato breeding. Critical Reviews in Plant Sciences. 2012. 31. P. 93 - 123.

73. Cui, X.H., Chen, Y.H., Xie, M., & Wan, F.H. Survival characteristics of Bemisia tabaci B-biotype and Trialeurodes vaporariorum (Homoptera: Aleyrodidae) after exposure to adverse temperature conditions. Acta Entomologic Sinica. 2017a. 50(12). P. 1232 - 1238.

74. Cui, X.H., Xie, M., & Wan, F.H. Changes in expression level of heat shock protein 70 gene in Bemisia tabaci B-biotype(Homoptera: Aleyrodidae) under high temperature stress. Acta Entomologic Sinica. 2017b. 50(11). P. 1087 - 1091.

75. Abdullahi, I., Atiri, G.I., Thottappilly, G., & Winter, S. Discrimination of cassava-associated Bemisia tabaci in Africa from polyphagous populations, by PCR-RFLP of the internal transcribed spacer regions of ribosomal DNA. Journal of Applied Entomology. 2010. 128(2). P. 81 - 87.

76. Qiu, B.L., Ren, S.X., Wen, S.Y., & Mandour, N.S. Biotype identification of the populations of Bemisia tabaci (Homoptera: Aleyrodidae) in China using RAPD-PCR. Acta Entomologic Sinica. 2003a. 46. P. 605 - 608.

77. Qiu, B.L., Ren, S.X., Wen, S.Y., & Mandour, N.S. Population differentiation of three biotypes of Bemisia tabaci (Hemiptera: Aleyrodidae) in China by DNA polymorphism. Journal of South China Agricultural University. 2006. 27. P. 29 - 33.

78. Qiu, B.L., Dang, F., Li, S.J. Ahmed, M.Z., Jin, F.L., Ren, S.X. & Cuthbertson, A.G.S. Comparison of biological parameters between the invasive b biotype and a new defined cv biotype of Bemisia tabaci (Hemiptera: aleyradidae) in China. Journal of Pest Science. 2011. 84(4). P. 419 - 427.

79. Zhang, L.P., Zhang, Y.J., Zhang, W.J., Wu, Q.J., Xu, B.Y., & Chu, D. Analysis of genetic diversity among different geographical populations and determination of biotypes of Bemisia tabaci in China. Journal of Applied Entomology. 2005. 129(3). P. 121 - 128.

80. Chu, D., Zhang, Y.J., Cong, B., Xu, B.Y. Wu, Q.J. & Zhu, G.R. Sequence analysis of mtdna coi gene and molecular phylogeny of different geographical populations of Bemisia tabaci (gennadius). Agricultural Sciences in China. 2005. 4(7). P. 533 - 541.

81. Luo, C., Yao, Y., Wang, R.J., Yan, F.M., & Zhang, Z.L. The use of mitochondrial cytochrome oxidase I (mt co i) gene sequences for the identification of

biotypes of Bemisia tabaci (gennadius) in china. Acta Entomologica Sinica. 2002. 45(6). P. 759 - 763.

82. Luo, C. Seed Variation of Bemisia tabaci and its Distribution Status in my country. International Symposium on High-tech Science and Technology for Conservation and Utilization of Biodiversity and International Symposium on Insect Conservation, Utilization, and Industrialization. Beijing China. 2005. P. 52 - 60.

83. Kang, L., Chen, B., Wei, J.N., & Liu, T.X. Roles of thermal adaptation and chemical ecology in liriomyza distribution and control. Annual Review of Entomology. 2009. 54(1). P. 127 - 145.

84. Chen, B., & Kang, L. Wiation in cold hardiness of Liriomyza huidobrenisis (Diptera;Agromyzidae) along latitudinal gradients. Physiological Ecology. 2004. 33. P. 155 -164.

85. Ma, R.Y., Hao, S.G., & Kong, W.N. Cold hardiness as a factor for assessing the potential distribution of the Japanese pine sawyer Monochamus altematus (Coleoptera: Cerambycidae) in China. Annals of Forest Science. 2006. 63. P. 449 - 456.

86. Kostal, V., Dole, M.P. & Rozsypal, J. Physiological and biochemical analysis of overwintering and cold tolerance in two Central European populations of the spruce bark beetle, Ips typographus. Journal of Insect Physiology. 2011. 57. P. 1136 -1146.

87. Qiu ,B.L., Ren ,S.X., Lin, L.,& Musa, PD. Effects of different host plants on the development and reproduction of Bemisia tabaci. Acta Ecologica Sinica. 2003. 6. P. 1206 -1211.

88. Butler, G.D.J., Henneberry, T.J., & Clayton, T. E. Bemisia tabaci (Homoptera: Aleyrodidae): development, oviposition, and longevity in relation to

temperature. Annals of the Entomological Society of America. 1983. 76(2). P. 310 - 313.

89. Bonato, O., Lurette, A., Vidal, C., & Fargues, J. Modeling temperature-dependent bionomics of Bemisia tabaci (Q-biotype). Physiological Entomology. 2007. 32. P. 50 - 55.

90. Tsueda, H., & Tsuchida, K. Reproductive difference between Q and B whiteflies, Bemisia tabaci, on three host plants and negative interactions in mixed cohorts. Entomologia Experimentalis et Applicata. 2011. 141. P. 197 - 207.

91. Han, E.J., Choi, B.R., & Lee, J.H. Temperature-dependent development models of Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) Q biotype on three host plants. Journal of Asia-Pacific Entomology. 2013. 16. P. 5 - 10.

92. Skinner, R.H. Leaf temperature effects on bemisia argentifolii (homoptera: aleyrodidae) oviposition. Environmental Entomology. 1996. 25(6). P. 1371 - 1375.

93. Zhang, Y. Effects of temperature on the behavior of B. tabaci and its adult thermotaxis. Doctoral dissertation, Fujian Agriculture and Forestry University. 2011.

94. Berlinger, M.J., Lehmann-Sigura, N., & Taylor, R.A.J. Survival of Bemisia tabaci adults under different climatic conditions. Entomologia Experimentalis Et Applicata. 2015. 80(3). P. 511 - 519.

95. Chu, D., Tao, Y.L., Zhang, Y.J., Wan, F.H. & Brown, J.K. Effects of host, temperature and relative humidity on competitive displacement of two invasive Bemisia tabaci biotypes [Q and B]. Insect Science. 2012. 19(5). P. 595 - 603.

96. Chen, Y.J., Luo, H.Y., Huang, J., &Yang A.L. Effect of humidity on the experimental population of sweetpotato whitefly, Bemisia tabaci (Gennadius). Entomological Journal of East China. 2001. 10(2). P. 76 - 80.

97. Xiang, Y.Y., Guo, X.J., Zhang, F., Li, Z.Z., & Luo, C. Effects of Temperature and Humidity on the Development and Reproduction of Bemisia tabaci (Homoptera: Aleyrodidae) Biotype B. Acta Agriculturae Boreali-Sinica. 2007. 22(5). P. 152 - 156.

98. Lin, K.J., Wu, K.M., Wei, H.Y., & Guo, Y.Y. Effects of temperature and humidity on the development, survival, and reproduction of B biotype of Bemisia tabaci(Homoptera: Aleyrodidae) from Beijing. Journal of Plant Protection. 2004. 31(2). P. 166 - 172.

99. Tsueda, H., Tsuduki, T. & Tsuchida, K. Factors that Affect the Selection of Tomato Leaflets by Two Whiteflies, Trialeurodes Vaporariorum and Bemisia tabaci (Homoptera: Aleyrodidae). Applied Entomology and Zoology. 2014. 49. P. 561 - 570.

100. Luo, C., Jones, C.M., Devine, G., Zhang, F., Denholm, I. & Gorman, K. Insecticide Resistance in Bemisia tabaci Biotype Q (Hemiptera:Aleyrodidae) from China. Crop Protection. 2010. 29. P. 429 - 434.

101. Ren, S.X., Liu, T.X., Du, Y.Z., Peng, Z.Q., Qiu, B.L., & Ge, F. Systematic Investigation and Monitoring of Whitefly Pests in a Vegetable Ecosystem. Chinese Journal of Applied Entomology. 2014. 51. P. 859 - 862.

102. Yang, Y.F., Yan, F.F., Zhang, R.P., Huang, Y., Shen, X.X., & Li, M.Y. Progresses in the Biology, Forecast, and Management Technology of Bemisia tabaci. Anhui Agricultural Science Bulletin. 2020. 26. P. 101 - 103.

103. Ohnesorge, B., & Rapp, G. Monitoring Bemisia tabaci: A review. Agriculture, Ecosystems & Environment. 1986. 17. P. 21 - 27.

104. Horowitz, A.R. Population Dynamics of Bemisia tabaci (Gennadius): with Special Emphasis on Cotton Fields. Agriculture, Ecosystems & Environment. 1986. 17. P. 37 - 47.

105. Sampson, C., Covaci, A.D., Hamilton, J.G.C., Hassan, N., Al-Zaidi, S., & Kirk, W.D.J. Reduced Translucency and the addition of Black Patterns Increase the catch of the Greenhouse Whitefly, Trialeurodes Vaporariorum, on Yellow Sticky Traps. PLoS ONE. 2018. 13. P. e0193064.

106. Emam, A., Abozeid, S., & Saad, A. Evaluation Efficiency Sticky Traps on Attraction Bemisia tabaci (Genn.) on Cucumber Plants under Greenhouses Conditions. Journal of Plant Protection and Pathology. 2018. 9. P. 649 - 653.

107. Böckmann, E., Pfaff, A., Schirrmann, M., & Pflanz, M. Rapid and Low-cost Insect Detection for Analysing Species Trapped on Yellow Sticky Traps. Scientific Reports. 2021. 11. P. 10419.

108. Gillespie, D.R., & Quiring, D. Yellow Sticky Traps for Detecting and Monitoring Greenhouse Whitefly (Homoptera: Aleyrodidae) Adults on Greenhouse Tomato Crops. Journal of Economic Entomology. 1987. 80. P. 675 - 679.

109. Moerkens, R., Brenard, N., Bosmans, L., Reybroeck, E., Janssen, D., Hemming, J., & Sluydts, V. Protocol for Semi-automatic Identification of Whiteflies Bemisia tabaci and Trialeurodes Vaporariorum on Yellow Sticky Traps. Journal of Applied Entomology. 2019. 143. P. 652 - 658.

110. Yao, F.L., Zheng, Y., Huang, X.Y., Ding, X.L., Zhao, J.W., Desneux, N., He, Y.X., & Weng, Q.Y. Dynamics of Bemisia tabaci Biotypes and Insecticide Resistance in Fujian Province in China during 2005-2014. Scientific Reports. 2017. 7. P. 40803.

111. Qiao, M., Lim, J., Ji, C.W., Chung, B.K., Kim, H.Y., Uhm, K.B., Myung, C.S., Cho, J., & Chon, T.S. Density Estimation of Bemisia tabaci (Hemiptera: Aleyrodidae) in a Greenhouse Using Sticky Traps in Conjunction with an Image Processing System, Journal of Asia-Pacific Entomology. 2008. 11. P. 25 - 29.

112. Hong, W.Y, Wu, Y.J., Wang, D.Z., Wang, H., Wang, A.J., & Zhu X.Y. Study on the Trapping Effect with Yellow Sticky Cards and Forecasting Model of Tobacco Whitefly in Protected Vegetables. Journal of Agriculture. 2015. 5. P. 39-46.

113. Gerling, D. Natural Enemies of Bemisia tabaci, Biological Characteristics and Potential as Biological Control Agents: A Review. Agriculture, Ecosystems & Environment. 1986. 17. P. 99 - 110.

114. Li, S.J., Xue, X., Ahmed, M.Z., Ren, S.X., Du, Y.Z., Wu, J.H., Cuthbertson, A.G.S., & Qiu, B.L. Host Plants and Natural Enemies of Bemisia tabaci (Hemiptera: Aleyrodidae) in China. Insect Science. 2011. 18. P. 101 - 120.

115. Tafaghodinia, B., & Iranmanesh, S. Cotton Whitefly, Bemisia tabaci (Genn.) Degree-hour Model for Predicting Phenological Development. Journal of Insect Science. 2008. 8. P. 46-47.

116. Naranjo, S.E., Castle, S.J., De Barro, P.J., & Liu, S.S. Population Dynamics, Demography, Dispersal and Spread of Bemisia tabaci. Bemisia: Bionomics and Management of a Global Pest, Springer. 2009. P. 185 - 226.

117. Gilioli, G., Pasquali, S., Parisi, S., & Winter, S. Modeling the Potential Distribution of Bemisia tabaci in Europe in Light of the Climate Change Scenario. Pest Management Science. 2015. 70. P. 1611 - 1623.

118. Biber-Freudenberger, L., Ziemacki, J., Tonnang, H., & Borgemeister, C. Future Risks of Pest Species under Changing Climatic Conditions. PLoS ONE. 2016. 11. P. e0153237.

119. Soares, R.R., Lalit, K., Farzin, S., Coutinho, P.M., & Yue, B.S. Mapping Global Risk Levels of Bemisia tabaci in areas of Suitability for Open Field Tomato Cultivation under Current and Future Climates. PLoS ONE. 2018. 13. P. e0198925.

120. Bradshaw, C.D., Hemming, D., Baker, R., Everatt, M., Eyre, D., & Korycinska, A. A Novel Approach for Exploring Climatic Factors Limiting Current Pest Distributions: A Case Study of Bemisia tabaci in North-west Europe and Assessment of Potential Future Establishment in the United Kingdom under Climate Change. PLoS ONE. 2019. 14. P. e0221057.

121. Abisgold, J.D., & Fishpool, L.D.C. A Method for Estimating Population Sizes of Whitefly Nymphs (Bemisia tabaci Genn.) on Cassava. Tropical Pest Management. 1990. 36. P. 287 - 292.

122. Berlinger, M.J., Lehmann-Sigura, N., & Taylor, R.A.J. Survival of Bemisia tabaci Adults under Different Climatic Conditions. Entomologia Experimentalis et Applicata. 1996. 80. P. 511 - 519.

123. Gusmão, M.R., Picanço, M.C., Zanuncio, J.C., Silva, D.J.H., & Barrigossi, J.A.F. Standardised Sampling Plan for Bemisia tabaci (Homoptera: Aleyrodidae) in Outdoor Tomatoes. Scientia Horticulturae. 2005. 103. P. 403 - 412.

124. Hernandez, P.A., Graham, C.H., Master, L.L., & Albert, D.L. The Effect of Sample Size and Species Characteristics on Performance of Different Species Distribution Modeling Methods. Ecography. 2006. 29. P. 773-785.

125. Liu, X.D. Insect Ecology and Forecast. Beijing: Higher Education Press. 2016. P. 278 - 306. (In Chinese)

126. Honêk, A. Geographical Variation in Thermal Requirements for Insect Development. European Journal of Entomology. 1996. 93. P. 303 - 312.

127. Albergaria, N.M., & Cividanes, F.J. Thermal Requirements of Bemisia tabaci (Genn.) B-biotype (Hemiptera: Aleyrodidae). Neotropical Entomology. 2002. 31. P. 359 - 363.

128. Mansaray, A., & Sundufu, A.J. Oviposition, Development and Survivorship of the Sweet Potato Whitefly Bemisia tabaci on Soybean, Glycine Max, and the Garden Bean, Phaseolus Vulgaris. Journal of Insect Science. 2009. 9. P. 1 - 6.

129. Awadalla, S., Bayoumy, M., Khattab, M., & Abdel-Wahab, A. Thermal Requirements for Development of Bemisia tabaci (Hemiptera: Aleyrodidae) Biotype 'B' and Their Implication to Field Sample Population Data, Acta Phytopathologica et Entomologica Hungarica. 2014. 49. P. 289 - 304.

130. Prabhulinga, T., Rameash, K., Madhu, T.N., & Shah, V. Maximum Entropy Modelling for Predicting the Potential Distribution of Cotton Whitefly Bemisia tabaci (Gennadius) in North India. Journal of Entomology & Zoology Studies. 2017. 5. P. 1002 - 1006.

131. Ramos, R. S., Kumar, L., Shabani, F., Silva, R., & Picano, M. C. Climate Model for Seasonal Variation in Bemisia tabaci Using CLIMEX in Tomato Crops. International Journal of Biometeorology. 2019. 63. P. 281 - 291.

132. Zhao, J., Li, Z.P., Zhang, H.W., Chen, H., & Weng, Q.Y. Prediction of Potential Distribution of Tobacco Whitefly Bemisia tabaci in China Based on MaxEnt Niche Model and GIS. Journal of Plant Protection. 2019. 46(6). P. 1292 - 1300.

133. Aslam, M. Evaluation of Different Pest Scouting Methods for Monitoring Whitefly Population in Cotton Fields at Multan (Pakistan). Arab Journal Plant Protection. 2002. 20. P. 52 - 54.

134. Naranjo, S.E., & Ellsworth, P.C. Mortality Dynamics and Population Regulation in Bemisia tabaci. Entomologia Experimentalis et Applicata. 2005. 116. P. 93 - 108.

135. Asiimwe, P., Ecaat, J.S., Otim, M., Gerling, D., Kyamanywa, S., & Legg, J.P. Life-table Analysis of Mortality Factors Affecting Populations of Bemisia tabaci on Cassava in Uganda. Entomologia Experimentalis et Applicata. 2007. 122. P. 37 - 44.

136. Zidon, R., Tsueda, H., Morin, E., & Morin, S. Projecting Pest Population Dynamics under Global Warming: the Combined Effect of inter-and intra-annual Variations. Ecological Applications. 2016. 26. P. 1198 - 1210.

137. Xue, Y., Lin, C., Wang, Y., Zhang, Y., & Ji, L. Ecological Niche Complexity of Invasive and Native Cryptic Species of the Bemisia tabaci Species Complex in China. Journal of Pest Science. 2021. P. 1 - 15.

138. Hirano, K., Budiyanto, E., & Winarni, S. Biological Characteristics and Forecasting Outbreaks of the Whitefly, Bemisia tabaci, a Vector of Virus Diseases in Soybean Fields. ASPAC Food & Fertilizer Technology Center. 1993.

139. McKenzie, C.L., Kumar, V., Palmer, C.L., Oetting, R.D., & Osborne, L.S. Chemical Class Rotations for Control of Bemisia tabaci (Hemiptera: Aleyrodidae) on Poinsettia and Their Effect on Cryptic Species Population Composition. Pest Management Science. 2014. 70. P. 1573 - 1587.

140. Horowitz, A.R., Ghanim, M., Roditakis, E., Nauen, R., & Ishaaya, I. Insecticide Resistance and Its Management in Bemisia tabaci Species. Journal of Pest Science. 2020. 93. P. 893 - 910.

141. Glare, T.R., & Inwood, A. Morphological and genetic characterization of Beauveria spp. From New Zealand. Mycological Research. 2014. 102. P. 250 - 256.

142. Jiangsu Plant Protection Station. Forecast and Control of Main Crop Diseases and Insect Pests. Nanjing: Jiangsu Science and Technology Press. 2006. P. 89 - 93. (In Chinese)

143. Li Huiming. Specification for Investigation and Forecast of Vegetable Diseases and Insect Pests.Shanghai: Shanghai Science and Technology Press. 2006. P. 235 - 239. (In Chinese)

144. Horowitz, A. R., Antignus, Y., & Dan, G. Management of Bemisia tabaci Whiteflies. In book: The Whitefly, Bemisia tabaci (Homoptera: Aleyrodidae) Interaction with Geminivirus-Infected Host Plants. 2011. P. 293 - 322.

145. Xu, W.X., & Wang Y.C. Principles and methods of plant quarantine, Science Press. 2018.

146. Castle, S.J. Concentration and management of Bemisia tabaci in cantaloupe as a trap crop for cotton. Crop Protection. 2006. 25(6). P. 574 - 584.

147. Dennehy, T.J., & Wiliams, L. Management of resistance in Bemisiain Arizona Cotton. Pest Management Science. 1997. 51. P. 398 - 406.

148. Stansly, P. A., & Natwick, E. T. Integrated Systems for Managing Bemisia tabaci in Protected and Open Field Agriculture. In: Stansly, P., Naranjo, S. (eds) Bemisia: Bionomics and Management of a Global Pest. Springer, Dordrecht. 2009.

149. Song, B.B. Comprehensive control measures of Bemisia tabaci in open field vegetables. Agricultural Technology Extension. 2019. 04. P. 204 - 206.

150. Zhao, X.N., & Li, W.H. (2020). Biological characteristics and integrated control of Bemisia tabaci.Yunnan Agriculture. 2020. 09. P. 62 - 63.

151. Lu, D.Y., Liu, Y., Ge, D.Q., Zhang, Z.H., Zhang, D.Y., Tan, X.Q., &Shi, X.B. (2020). Damage characteristics and integrated control techniques of Bemisia tabaci.Yangtze River vegetables,18(022),69-71.

152. Zhao, Y.G., Bian, J.S., & Yu, W. Trap and kill effect of yellow board on Bemisia tabaci and non-target insects in cotton field. Plant Protection. 2008. 34(3). P. 144 - 147.

153. Xiang, Y.Y., Guo, X.J., Zhang, F., Li, Z.Z., & Luo, C. Effects of temperature and humidity on development and reproduction of Bemisia tabaci Biotype B. Acta Agriculturae Boreali-sinica. 2007. 22(5). P. 152 - 156.

154. Wang H.J., & Fan, H.J. Control of Bemisia tabaci in greenhouse by high temperature suffocation. Vegetables. 2003. 10. P. 30 - 31.

155. Naranjo, S. E. Conservation and evaluation of natural enemies in IPM systems for Bemisia tabaci. Crop Protection. 2001. 20(9). P. 835 - 852.

156. Hoddle, M., van Driesche, R., & Sanderson, J. Biology and use of the whitefly parasitoid Encarsia formosa. Annual Review of Entomology. 1998. 43. P. 645 - 669.

157. Gerling, D., Alomar, Ò., & Arnò, J. Biological control of Bemisia tabaci using predators and parasitoids. Crop Protection. 2001. 20. P. 779 - 799.

158. Ellsworth, P.C., & Martinez-Carrillo, J.L. IPM for Bemisia tabaci: a case study from north America-ScienceDirect. Crop Protection. 2001. 20(9). P. 853 - 869.

159. Hodek, I., Honek, A., & Van Emden, H. Ecology and behavior of the ladybird beetles(Coccinellidae). Oxford: Wiley-Blackwell. 2012. P. 1 - 561.

160. Huang, P.Y. Investigation and Identification of the Whitefly Parasitoids. Doctoral dissertation, Fuzhou: Fujian Agriculture and Forest University. 2002.

161. Dou, W.J., Yang, S.W., Liu, Q., Chen, G.H., & Zhang X.M. Progress in the control of predatory and parasitic natural enemies of Bemisia tabaci in China. Journal of Environmental Entomology. 2020. 42 (2). P. 342 - 354.

162. Wang, Y., Jia, X.H., Li, J., Zhang, X.Y., Li, Y.L., Hu, B., Sun, H., Zheng, J.Q., Ca, J.J., Wang, J.X. Field control effects of five insecticides on Tomato Bemisia tabaci. Vegetables. 2020. 11. P. 55 - 58.

163. Hong, B., Cao, N., Zhao, X.Y., Ma, B.L., Dong, Y., Gao, K., Jia, Y.X., &Wang, X.Z. Resistance of Bemisia tabaci to five insecticides in Ningxia. Plant Protection. 2021. 47(2). P. 226 - 232.

164. Kranthi, K.R., Jadhav, D.R., Kranthi, S. Wanjari, R.R., Ali, S.S., & Russell, D.A. Insecticide resistance in five major insect pests of cotton in India. Crop Protection. 2002. 21(6). P. 449 - 460.

165. Kady, H.E., & Devine, G.J. Insecticide resistance in egyptian populations of cotton whitefly. Pest Management Science. 2003. 59(7). P. 865 - 871.

166. Lindquist, S.L. The Heat-Shock Response. Annual Review of Biochemistry. 1986. 55. P. 1151 - 1191.

167. Zhao, L., & Jones, W.A. Expression of Heat Shock Protein Genes in Insect Stress Responses. Invertebrate Survival Journal. 2012. 9. P. 93 - 101.

168. Lindguist, S. & Craig, E.A. The Heat Shock Proteins. Annual Review of Genetics. 1988. 22. P. 631 - 677.

169. Yost, H.J., Peterson, R.B. & Lindquist, S. RNA Metabolism: Strategies for Regulation in the Heat Shock Response. Trends in Genetics. 1990. 6. P. 223 - 227.

170. Feder, M.E., & Hofmann, G.E. Heat-Shock Proteins, Molecular Chaperones, and the Stress Response: Evolutionary and Ecological Physiology. Annual Review of Physiology. 1999. 61. P. 243 - 282.

171. Nusayr, T. The Role of Heat-Shock Proteins, in Vector-virus Transmission. Applied Plant Virology. 2020. 19. P. 249 - 254.

172. Ritossa, F.M. A New Puffing Pattern Induced by Temperature Shock and DNP in Drosophila. Experientia. 1962. 18. P. 571 - 573.

173. Moseley, P.L. Heat Shock Proteins and Heat Adaptation of the Whole Organism. Journal of Applied Physiology. 1997. 83. P. 1413 - 1417.

174. Lin, H., Head, M., & Blank, M. (1998). Myc-mediated Transactivation of HSP70 Expression Following Exposure to Magnetic Fields. Journal of Cell Biochemistry. 1998. 69. P. 181 - 188.

175. Ekengren, S., Tryselius, Y., Dushay, M.S., Liu, G., Steiner, H., & Hultmark, D. A Humoral Stress Response in Drosophila. Current Biology. 2001. 11. P. 714 - 718.

176. Sørensen, J.G., Kristensen, T.N., & Loeschcke, V. The Evolutionary and Ecological Role of Heat Shock Proteins. Ecology Letters. 2003. 6. P. 1025 - 1037.

177. Tissiéres, A., Mitchell, H.K., & Tracy, U.M. Protein Synthesis in Salivary Glands of Drosophila Melanogaster: Relation to Chromosome Puffs. Journal of Molecular Biology. 1974. 84. P. 389 - 392.

178. Salvucci, M.E., Stecher, D.S., & Henneberry, T.J. Heat Shock Proteins in Whiteflies, an Insect that Accumulates Sorbitol in Response to Heat Stress. Journal of Thermal Biology. 2000. 25. P. 363 - 371.

179. Mahadav, A., Kontsedalov, S., Czosnek, H., & Ghanim, M. Thermotolerance and Gene Expression Following Heat Stress in the Whitefly Bemisia tabaci B and Q Biotypes. Insect Biochemistry and molecular biology. 2009. 39. P. 668 - 676.

180. Morimoto, R.I. Cell in Stress: Transcriptional Activation of Heat Shock Genes. Science. 1993. 259. P. 1409 - 1410.

181. Kim, K.K., Kim, R., & Kim, S. Crystal Structure of a Small Heat-shock Protein. Nature. 1998. 394. P. 595 - 599.

182. Buchanan, B.B., & Gruissem, W. Biochemistry and Molecular Biology of Plants. USA: American Society of Plant Biologists. 2000. P. 983 - 987.

183. Kregel, K.C. Invited Review: Heat Shock Proteins: Modifying Factors in Physiological Stress Responses and Acquired Thermotolerance. Journal of Applied Physiology. 2002. 92. P. 2177 - 2186.

184. Elbaz, M., Weiser, M., & Morin, S. Asymmetry in Thermal Tolerance Trade-offs between the B and Q Sibling Species of Bemisia tabaci (Hemiptera: Aleyrodidae). Journal of Evolutionary Biology. 2011. 24. P. 1099 - 1109.

185. Wang, X.R., Wang, C., Ban, F.X., Zhu, D.T., Liu, S.S., & Wang X.W. Genome-wide Identification and Characterization of HSP Gene Superfamily in Whitefly (Bemisia tabaci) and Expression Profiling Analysis under Temperature Stress. Insect Science. 2019. 26. P. 44 - 57.

186. Yonehara, M., Minami, Y., Kawata, Y., Nagai, J., & Yahara, I. Heat-induced Chaperone Activity of HSP90. Journal of Biological Chemistry. 1996. 271. P. 2641 - 2645.

187. Picard, D. Heat-shock Protein 90, a Chaperone for Folding and Regulation. Cellular and Molecular Life Sciences. 2002. 59. P. 1640 - 1648.

188. Zuehlke, A., & Johnson, J.L. Hsp90 and Co-chaperones Twist the Functions of Diverse Client Proteins. Biopolymers. 2010. 93. P. 211 - 217.

189. Christine, Q., Todd, A.S., & Susan, L. Hsp90 as a Capacitor of Phenotypic Variation. Nature. 2002. 417. P. 618 - 624.

190. Rutherford, S.L., & Zuker, C.S. Protein Folding and the Regulation of Signaling Pathways. Cell. 1994. 79. P. 1129 - 1132.

191. Prodromou, C., Roe, S.M., O'Brien, R., Ladbury, J.E., Piper, P.W., & Pearl, L.H. Identification and Structural Characterization of the ATP/ADP-binding Site in the Hsp90 Molecular Chaperone. Cell. 1997. 90. P. 65 - 75.

192. Lü, Z.C., & Wan, F.H. Using Double-stranded RNA to Explore the Role of Heat Shock Protein Genes in Heat Tolerance in Bemisia tabaci (Gennadius). Journal of Experimental Biology. 2011. 214. P. 764 - 769.

193. Kinene, T., De Marchi, B.R., Alicai, T., Luboobi, L.S., Omongo, C.A., Savill, A., & Boykin, L.M. Recombination Detected in the Heat Shock Protein 90 (HSP90) of the Bemisia tabaci Species Complex. BioRxiv. 2019.

194. Guy, C.L., & Li, Q.B. The Organization and Evolution of the Spinach Stress 70 Molecular Chaperone Gene Family. The Plant Cell Online. 1998. 10. P. 539 - 556.

195. Wegele, H., Muller, L., & Buchner, J. Hsp70 and Hsp90-a Relay Team for Protein Folding. Reviews of Physiology, Biochemistry, and Pharmacology, 151, Springer, Berlin, Heidelberg.2004.

196. Wolfe, G.R., Hendrix, D.L., & Salvucci, M.E. (1998). A Thermoprotective Role for Sorbitol in the Silverleaf Whitefly, Bemisia Argentifolii. Journal of Insect Physiology. 1998. 44. P. 597 - 603.

197. Bai, J., Wang, Y.C., Liu, Y.C., Chang, Y.W., Liu, X.N., Gong, W.R., & Du, Y.Z. Isolation of Two New Genes Encoding Heat Shock Protein 70 in Bemisia tabaci and Analysis during Thermal Stress. International Journal of Biological Macromolecules: Part A. 2021. 193. P. 933 - 940.

198. Yu, H., & Wan, F.H. Cloning and Expression of Heat Shock Protein Genes in Two Whitefly Species in Response to Thermal Stress. Journal of Applied Entomology. 2009. 133. P. 602 - 614.

199. Horwich A.L. & Willison K.R. Protein Folding in the Cell: Functions of Two Families of Molecular Chaperone, Hsp60 and TF55-TCP1. In: Ellis R.J., Laskey R.A., Lorimer G.H. (eds) Molecular Chaperones.Springer, Dordrecht. 1993. P. 57 - 70.

200. King, A.M., & Macrae, T.H. Insect Heat Shock Proteins during Stress and Diapause. Annual Review of Entomology. 2015. 60. P. 59 - 75.

201. Nakamoto, H., & Vígh, L. The Small Heat Shock Proteins and Their Clients. Cellular and Molecular Life Sciences. 2007. 64. P. 294 - 306.

202. Garrido, C., Paul, C., Seigneuric, R., & Kampinga, H.H. The Small Heat Shock Proteins Family: The Long-forgotten Chaperones. International Journal of Biochemistry & Cell Biology. 2012. 44. P. 1588 - 1592.

203. Evgen'ev, M.B., Zatsepina, O.G., Garbuz, D., Lerman, D.N., Velikodvorskaya, V., Zelentsova, E., & Feder, M.E. Evolution and Arrangement of the Hsp70 Gene Cluster in Two Closely Related Species of the Virilis Group of Drosophila. Chromosoma. 2004. 113. P. 223 - 232.

204. Rinehart, J.P., Li, A., Yocum, G.D., Robich, R.M., Hayward, S.A., & Denlinger, D.L. Up-regulation of Heat Shock Proteins is Essential for Cold Survival during Insect Diapause. Proceedings of the National Academy of Sciences of USA. 2007. 104. P. 11130 - 11137.

205. Clark, M.S., & Worland, M.R. How Insects Survive the Cold: Molecular Mechanisms-a Review. Journal of Comparative Physiology B. 2008. 178. P. 917 - 933.

206. Bai, J., Liu, X.N., Lu, M.X., & Du, Y.Z. Characterization of Genes Encoding Small Heat Shock Proteins from Bemisia tabaci and Expression under Thermal Stress. PeerJ. 2019. 7. P. e6992.

207. Finley, D., & Varshavsky, A. The Ubiquitin System: Functions and Mechanisms. Trends in Biochemical Sciences. 1985. 10(9). P. 343 - 347.

208. Hoeller, D., Hecker, C.M., & Dikic, I. Ubiquitin and Ubiquitin-like Proteins in Cancer Pathogenesis. Nature Reviews Cancer. 2006. 6. P. 776 – 788.

209. Xia, W. Q. , Liang, Y. , Liu, Y. Q. , Liu, S. S., & Wang, X.W. Effects of Ubiquitin-proteasome System on Tomato Yellow Leaf Curl Virus in Whitefly (Hemiptera: Aleyrodidae). Acta Entomologica Sinica. 2017. 60. P. 1411 - 1419.

210. Xiao, N., Pan, L.L., Zhang, C.R., Shan, H.W., & Liu, S.S. Differential Tolerance Capacity to Unfavourable Low and High Temperatures between Two Invasive Whiteflies. Scientific Reports. 2016. 6. P. 24306.

211. Hartman, D.J., Dougan, D., Hoogenraad, N.J., and Høj, P.B. Heat Shock Proteins of Barley Mitochondria and Chloroplasts Identification of Organellar Hsp 10 and 12: Putative Chaperonin 10 Homologues. FEBS Letters. 1992. P. 305.

212. Wang, W.X., Vinocur, B., Shoseyov, O., & Altman, A. Role of Plant Heat-shock Proteins and Molecular Chaperones in the Abiotic Stress Response. Trends in Plant Science. 2004. 9. P. 244 - 252.

213. Al-Whaibi, M.H. Plant Heat-shock Proteins: a Mini-review. Journal of King Saud University-Science. 2011. 23. P. 139 - 150.

214. Mogk, A., Deuerling, E., Vorderwülbecke, S., Vierling, E., & Bukau, B. Small Heat Shock Proteins, ClpB and the DnaK System form a Functional Triade in Reversing Protein Aggregation. Molecular Microbiology. 2003. 50. P. 585 - 595.

215. Sangster, T.A., & Queitsch, C. The HSP90 Chaperone Complex, an Emerging Force in Plant Development and Phenotypic Plasticity. Current Opinion in Plant Biology. 2005. 8. P. 86 - 92.

216. Jiang, R., Qi, L.D., Du, Y.Z., & Li, Y.X. Thermotolerance and Heat-shock Protein Gene Expression Patterns in Bemisia tabaci (Hemiptera: Aleyrodidae) Mediterranean in Relation to Developmental Stage. Journal of Economic Entomology. 2017. 110. P. 2190 - 2198.

217. Jacques, R. Evolution of Heat Shock Protein and Immunity. Developmental and Comparative Immunology, 2003. 27. P. 449 - 464.

218. Jinn, T.L., Yeh, Y.C., Chen, Y.M., & Lin, C.Y. Stabilization of Soluble Proteins in Vitro by Heat Shock Proteins-Enriched Ammonium Sulfate Faction from Soybean Seedlings. Plant Cell Physiology. 1989. 30. P. 463 - 469.

219. Jinn, T.L., Wu, S.H., Yeh, K.W., Hsieh, M.H., Yeh, Y.C., Chen, Y.M., & Lin, C.Y. Immunological kinship of Class I Low Molecular Weight Heat Shock Proteins and Thermostabilization of Soluble Proteins in Vitro among Plants. Plant Cell Physiology. 1993. 34. P. 1055 - 1062.

220. Becker, J., & Craig, E.A. Heat-shock Proteins as Molecular Chaperones. European Journal of Biochemistry. 1994. 219. P. 11 - 23.

221. Coleman, J.S., Heckathorn, S.A., & Hallberg, R.L. Heat-shock Proteins and Thermotolerance: Linking Molecular and Ecological Perspectives. Trends in Ecology and Evolution. 1995. 10. P. 305 - 306.

222. Hoffmann, A.A., Sørensen, J.G., & Loeschcke, V. (2003). Adaptation of Drosophila to Temperature Extremes: Bringing Together Quantitative and Molecular Approaches. Journal of Thermal Biology. 2003. 28. P. 175 - 216.

223. Wu, C. Heat Shock Transcription Factors: Structure and Regulation. Annual Review of Cell and Developmental Biology. 1995. 11. P. 441 - 469.

224. Pirkkala, L., Nykanen, P., & Sistonen, L. Roles of the Heat Shock Transcription Factors in Regulation of the Heat Shock Response and Beyond. The FASEB Journal. 2001. 15. P. 1118 - 1131.

225. Snoeckx, L.H., Cornelussen, R.N., Van Nieuwenhoven, F.A., Reneman, R.S., & Der Vusse, G.J. Heat Shock Proteins and Cardiovascular Pathophysiology. Physiological Reviews. 2001. 81. P. 1461 - 1497.

226. Salda, L.D., & Romanucci, M. The Role of Heat Shock Proteins in Mammary Neoplasms: a Brief Review. Journal of Cancer Therapy. 2012. 3. P. 755 - 767.

227. Mcmillan, D.R., Xiao, X.Z., Shao, L., Graves, K., & Benjamin, I.J. Targeted Disruption of Heat Shock Transcription Factor 1 Abolishes Thermotolerance and Protection against Heat-inducible Apoptosis. Journal of Biological Chemistry. 1998. 273. P. 7523 - 7528.

228. Xiao, X.Z., Zuo, X.X., Davis, A.A. Mcmillan, D.R., Curry, B.B., Richardson, J.A., & Benjamin, I.J. HSF1 is Required for Extra-embryonic Development, Postnatal Growth, and Protection during Inflammatory Response in Mice. The EMBO Journal. 1999. 18. P. 5943 - 5952.

229. Wiederrecht, G., Seto, D., & Parker, C.S. Isolation of the Gene Encoding the S. cerevisiae Heat Shock Transcription Factor. Cell. 1988. 54. P. 841 - 853.

230. Mathew, A., Mathur, S. K., & Morimoto, R. I. Heat Shock Response and Protein Degradation: Regulation of Hsf2 by the Ubiquitin-proteasome Pathway. Molecular and cellular biology. 1998. 18(9). P. 5091 - 5098.

231. Nakai, A., Kawazoe, Y., Tanabe, M., Nagata, K., & Morimoto, R.I. The DNA-binding Properties of Two Heat Shock Factors, HSF1 and HSF3, are Induced in the Avian Erythroblast Cell Line HD6. Molecular and Cellular Biology. 1995. 15. P. 5268 - 5278.

232. Morimoto, R.I. Regulation of the Heat Shock Transcriptional Response: Cross Talk between a Family of Heat Shock Factors, Molecular Chaperones, and Negative Regulators. Genes Development. 1998. 12. P. 3788 - 3796.

233. Lü, Z.C., & Wan, F.H. Differential Gene Expression in Whitefly (Bemisia tabaci) B-biotype Females and Males under Heat-shock Condition. Comparative Biochemistry and Physiology Part D. 2008. 3. P. 257 - 262.

234. Díaz, f., Orobio, R.F., & Chavarriaga, P. Differential Expression Patterns among Heat-shock Protein Genes and Thermal Responses in the Whitefly Bemisia tabaci (MEAM 1). Journal of Thermal Biology. 2015. 52. P. 199 - 207.

235. Cui, X.H., Wan, F.H., Xie, M., & Liu, T.X. Effects of Heat Shock on Survival and Reproduction of Two Whitefly Species, Trialeurodes Vaporariorum and Bemisia tabaci Biotype B. Journal of Insect Science. 2008. 8. P. 24.

236. Mullis, K.B. Recombinant DNA methodology || specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol, 1989. P. 189 - 204.

237. Mullis, K.B. The unusual origin of the polymerase chain reaction. Scientific American. 1990. 262(4). P. 56 - 61.

238. Heid, C.A., Stevens, J., Livak, K.J., & Williams, P.M. Real time quantitative PCR. Genome Research. 1996. 6(10). P. 986 - 994.

239. Klein, D. Quantification using real-time PCR technology: applications and limitations. Trends in Molecular Medicine. 2002. 8(6). P. 257 - 260.

240. Zhu, G.P. Detection of the distribution of CSISV in SPF chicks by RT-PCR and fluorescence quantitative RT-PCR method. Dissertation, Xinxiang, Henan. Henan Institute of science and technology. 2010. P. 6-7.

241. Qu, S., & Liu, G.Z. Experimental technology of molecular biology. Beijing: Chemical Industry Press. 20087. P. 88 - 91.

242. Huang, L.Y. Principle, method, and application of the latest PCR technology. Beijing: Chemical Industry Press. 2004. P. 135 - 137.

243. Schena, M., Shalon, D. Davis, R.W., & Brown, P.O. Quantitative monitoring of gene expression patterns with a complenmentary DNA microarray. Science. 1995. 270. P. 467 - 470.

244. Li, W.T., Wang, J.D., Yang, L.F., Wang, H.W., & Han, S.J. Real-Time Fluorescent Quantitative PCR Technique and its Application. Letters in Biotechnology. 2006. 17(1). P. 112 - 114.

245. Livak, K.J., & Schmitten, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods. 2001. 25(4). P. 402 - 408.

246. Li, S.Q., Ye, L.G., Li, X.Y., & Deng, S.X. (2009). The design and application of the quantitative real-time PCR. Animal Husbandry and Feed Science, 30(1),43-44.

247. Wang, D.P., Li, H.G., Guo, S.C., Yang, J., Qi, D.L., & Zhao, X.Q. Tissue specific expression of HIF-1 gene in Qinghai domestic Yak. Journal of Anhui Agricultural Science. 2007. 35(29). P. 9173 - 9175.

248. Ma, R.Q., Chen, H.Y., Huang, W.S., & Zhu, S.F. Detection of genetically modified products by real-time quantitative PCR. Plant Quarantine. 2002. 16(1). P. 61 - 65.

249. Yi, D.S., Lou, G.M., Han, X.G., & Feng, S.S. Multiplex PCR Detection and Typing of Porcine Circovirus.Journal of Anhui Agricultural Science. 2007. 35(32). P. 10239 - 10241.

250. Chen, Y.D., Zhang, C.Y., Zou, J.X., Chen, L.X., Li, T., & Guo, C.L. Establishment of a real-time quantitative PCR technique for rapid quantitative detection of swine fever attenuated rabbit vaccine.Virologica Sinica. 2003. 18(2). P. 124 - 128 .

251. Huang, K.S., Lee, S.E., Yeh, Y., Shen, G.S., Mei, E., & Chang, C. M. Taqman real-time quantitative PCR for identification of western flower thrip (frankliniella occidentalis) for plant quarantine. Biology Letters. 2010. 6(4). P. 555 - 557.

252. Zhang, G.M. Overview of plant quarantine in Shenzhen, China. Plant Pathology & Quarantine. 2011. P. 103 - 114.

253. Huang, H.Q. Research progress of application of real time fluorescent quantitative PCR technology in plant quarantine. Hubei Agricultural Sciences. 2012.

254. Hanan A., Nazir T., Basit A., Ahmad S., & Qiu, D. Potential of Lecanicillium lecanii (Zimm.) as a Microbial Control Agent for Green Peach Aphid, Myzus persicae (Sulzer)(Hemiptera: Aphididae). Pakistan Journal of Zoology. 2020. 52(1).

255. Hayet M., Sundararaj R., &Vimala d. The genus Encarsia Forester (Hymenoptera: Aphelinidae) parasitoids of whiteflies (Hemiptera: Aleyrodidae) in India. Journal of Insect Systematics. 2018. 2. P. 44 – 90.

256. Yu Guolong. Application of real-time fluorescent quantitative PCR in medical genetics. Foreign Medical Genetics. 2003. 26(3). P. 125 - 130.

257. Glare, T.R., & Inwood, A. Morphological and genetic characterization of Beauveria spp. From New Zealand. Mycological Research. 2014. 102. P. 250 - 256.

258. Goettel, M.S., Eilenberg, J., & Glare, T. Entomopathogenic fungi and their role in regulation of insect populations. In: Comprehensive Molecular Insect Science, (Eds. L.I., Gilbert, K., Iatrou & S.S., Gill), Amsterdam. 2015. P. 361 - 405.

259. Hanan, A., Basit, A., Nazir, T., Majeed, M.Z., & Qiu, D. Anti-insect activity of a partially purified protein derived from the entomopathogenic fungus Lecanicillium lecanii (Zimmermann) and its putative role in a tomato defense mechanism against green peach aphid. Journal of Invertebrate Pathology. 2020. 170. P. 107 - 282.

260. Chen Suhong, Zhang Minli, Mou Hang,Guan Wei,Li Lu, &Wang Shengqi. Detection of Bacillus anthracis by real-time fluorescent quantitative PCR method. People's Liberation Army Medical Journal. 2003. 28(3). P. 268 - 270.

261. Boulant, S., Stanifer, M., & Lozach, P.Y. Dynamics of virus-receptor interactions in virus binding, signaling, and endocytosis. Viruses. 2015. 7. P. 2794 - 2815.

262. Brodsky F.M. Diversity of clathrin function: new tricks for an old protein. Annu. Rev. Cell Dev. Biol. 2012. 28. P. 309 - 336.

263. Brown, J.K., Zerbini, F.M., Navas-Castillo, J., Moriones, E., Ramos-Sobrinho, R., Silva J.C.F., Fiallo-Olivé, E., Briddon, R.W., Hernández-Zepeda, C., & Idris. A. Revision of Begomovirus taxonomy based on pairwise sequence comparisons. Arch. Virology. 2015. 160. P. 1593 - 1619.

264. Yao Chi, Pan LiLong, Bouvaine S., Fan YunYun., Liu YinQuan, Liu ShuSheng, Seal S., &Wang XiaoWei. Differential transmission of Sri Lankan cassava mosaic virus by three cryptic species of the whitefly Bemisia tabaci complex. Virology. 2019. 540. P. 141 - 149.

265. Czosnek, H., Hariton-Shalev, A., Sobol, I., Gorovits, R., & Ghanim, M. The incredible journey of begomoviruses in their whitefly vector. Viruses. 2017. 9. P. 273.

266. Fiallo-Olivé, E., Pan, L.L., Liu, S.S., & Navas-Castillo, J. Transmission of begomoviruses and other whitefly-borne viruses: dependence on the vector species.Phytopathol. 2020. 110. P.10 - 17.

267. Li, S.Q., Ye, L.G., Li X.Y., & Deng, S.X. Design principles and applications of real-time quantitative PCR experiments. Animal Husbandry and Feed Science. 2009. 30(1). P. 43 - 44.

268. Zhang, G. M., Ling, X. Y., Feng, J. J., Cheng, Y. H., Zheng, Y., Li, Y. N., & Kang, L. Overview of plant quarantine in Shenzhen, China. Plant Pathology and Quarantine. 2011. 1(2). P. 103 - 114.

269. De Barro, P.J. & Driver, F. Use of RAPD PCR to distinguish the B biotype from other biotypes of Bemisia tabaci (Gennadius)(Hemiptera: Aleyrodidae). Australian Journal of Entomology. 1997. 36. P. 149 - 152.

270. Livak, K. J., & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods. 2001. 25(4). P. 402 - 408.

271. Qu, P., Xie, M., Yue, M., Xu, W.A. Effect of temperature on experimental population of biotype B Bemisia tabaci. Shandong Agricultural Science. 2005. 4. P. 36 - 38.

272. Jia, Z.Z., Wang, X.W, Fu, K.Y., Ding, X.H., Guo, W.C., Jiang, W.H. Biotype identification of Bemisia tabaci and its resistance to 11 commonly used insecticides in major agricultural areas of Xinjiang Xinjiang Agricultural Science. 2017. 54 (2). P. 304 - 312

273. Jin, G.L., Zhou, C.S., Zhang, Y.H., Chen, A.S., Ma, D.Y. Eight control effect and residue detection of different types of insecticides on hidden species of MED Bemisia tabaci. Plant protection. 2018. 44 (3). P. 207 - 213

274. Dai, P., Liu, L.Z., Zang, L.S., Zhang, J.J., Yuan, C.C.,& Wan, F.H. Host feeding and parasitism preference in Eretmocerus hayati for nymphs between Q sibling species of Bemisia tabaci and Trialeurodes vaporariorum. Chinese Journal of Biological Control. 2014. 30 (1). P. 20 - 25.

275. Ren, S.X., Wang, Z.Z., & Qiu, B.L. The pest status of Bemisia tabaci in China and non -chemical control strategies. Entomologia Sinica. 2001. 3. P. 279 - 288.

276. Li, S.J., Xue, X., Ahmed, M.Z., Ren, S.X., Du, Y.Z., Wu, J.H., Cuthbertson, A.G.S., & Qiu, B.L. Host plants and natural enemies of Bemisia tabaci (Hemiptera: Aleyrodidae) in China. Insect Science. 2011. 18 (1). P. 101 -120.

277. Ma, L.J., Zhang, S.Z., & Liu, T.X. Influences of interspecific competition between ladybeetle Serangium japonicum and parasitoid Encarsia formosa on predation of tobacco whitefly Bemisia tabaci . Journal of Plant Protection. 2018. 45 (6). P. 1289 -1295.

278. Li, S., Lao, S.B., Wang, S., Guo, X.J., & Zhang, F. Control effect of Orius sauteri collaborated with Encarsia formosa on Bemisia tabaci in the greenhouse. Journal of Environmental Entomology. 20145. 36 (6). P. 978 - 982.

279. Zang, L.S., & Liu, T.X. Intraguild interactions between an oligophagous predator, Delphastus catalinae (Coleoptera: Coccinellidae), and a parasitoid, Encarsia sophia (Hymenoptera: Aphelinidae), of Bemisia tabaci (Homoptera: Aleyrodidae). Biological Control. 2007. 41 (1). P.142 -150.

280. Dou, W.J., Yang, S.W., Liu, Q., Chen, G.H., & Zhang X.M. (2020). Progress in the control of predatory and parasitic natural enemies of Bemisia tabaci in China, Journal of Environmental Entomology. 2020. 42(2). P. 342 - 354.

APPENDICES

Appendix A

CERTIFICATE

OF HONOR

AWARD TO

Shunxiao Liu:

The field control test of 10 insecticides against Bemisia tabaci on facility vegetables has been successful from October 13 to 20, 2020. Researchindicates that we can choose 20% mefenproper suspending agent, which is used in conjunction with 22.4% spirote tramat suspending agent and 5% diprofenac dispersible liquid agent to control B. tabaci, which has a good control effect in facility vegetables.It meets the conditions of scientific and technological achievements after review.This certificate is hereby issued.

Sponsor: Henan Tuofeng Agricultural Technology Co., Ltd.

Signature: Tianchuang Wang

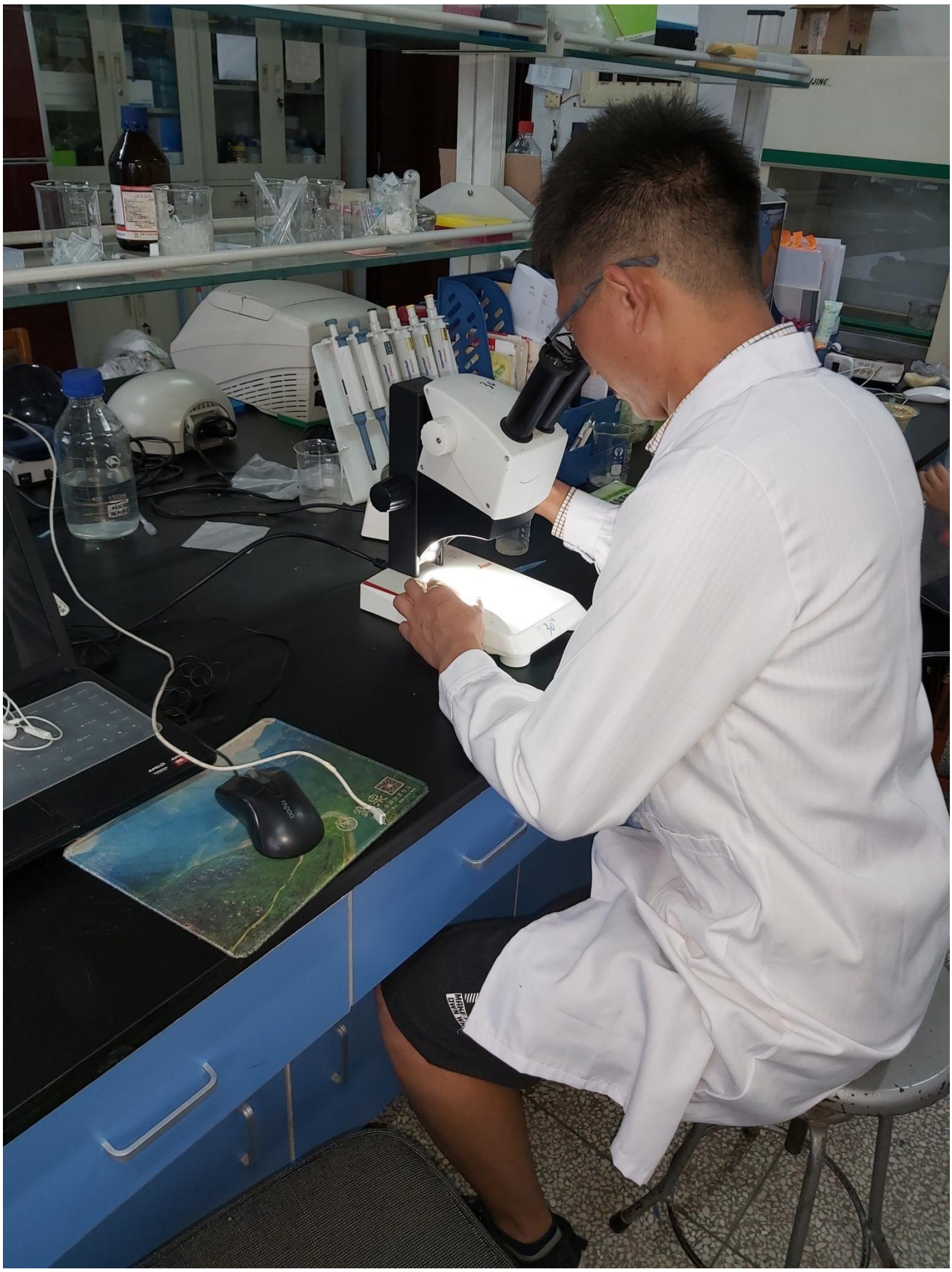
Date: 2020.10.28

Appendix A1

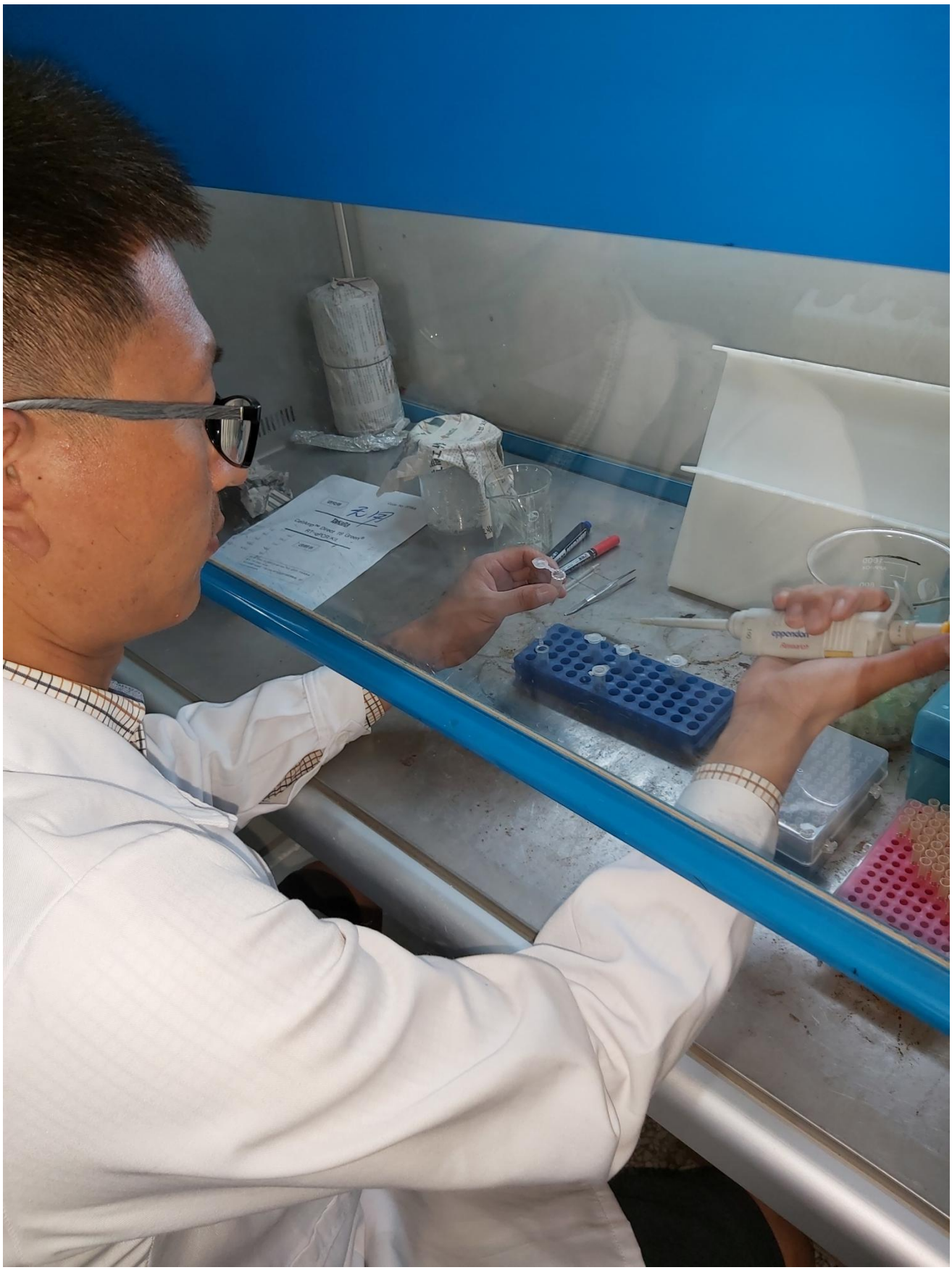

闪拿®
白粉虱/烟粉虱组合套装
规格：（100克+100克）×40套/箱
速度快 随打随落
杀虫杀卵 持效期长达15天以上
低毒 安全 高效
死虫更彻底 死虫率达95%以上
主治白粉虱 烟粉虱
原装正品
白粉虱/烟粉虱组合套装
烯啶虫胺
呋虫胺
拓丰农业
服务电话 0371-55002082
拓丰®农业
服务电话 0371-55002082

Appendix B

Appendix B1



Appendix B2



Appendix C

Appendix C1

Appendix D

ЗАТВЕРДЖУЮ

Проректор з науково-педагогічної роботи

д.біол.н., професор

_____Ігор КОВАЛЕНКО

ДОВІДКА

про впровадження результатів наукових досліджень у навчальному процесі

Видана Шунсяо Лі (Shunxiao Liu) у тому, що матеріали дисертаційної роботи "Molecular mechanism of reproductive isolation of *bemisia tabaci* species complex is for plant protection and quarantine services", які опубліковані в статтях:

- **Лю, Ш.**, Ю, Х., & Власенко, В. (2020). Кількісний аналіз регуляторного гена hsf1 Bemisa tabaci при різних температурах. Вісник Сумського національного аграрного університету. Серія: Агрономія і біологія, 41(3), 49-56.
- **Shunxiao L.**, Hao Y., Vlasenko V. Effect of Different Temperature and Humidity on Bemisia tabaci. Journal of Botanical Research. 2020. Vol. 2, no. 2. P. 21–24.
- **Shunxiao L.**, Hao Y., Vlasenko V. Overview of the Occurrence and Prevention of Bemisia tabaci Invasion in China. NASS Journal of Agricultural Sciences. 2021. Vol. 3, no. 1. P. 18–20.
- **Shunxiao L.**, Zhifang Y., Vlasenko V. Importance of Bemisia tabaci Forecasting Technology: A Review. Advances in Entomology. 2022. Vol. 10, no. 2. P. 149–158.
- **Shunxiao L.**, Kui W., Vlasenko V. A Review of Heat Shock Proteins Research on Bemisia tabaci. Agricultural Sciences. 2022. Vol. 13, no. 3. P. 393–403.
- Characterization and analysis of myosin gene family in the whitefly (Bemisia tabaci) / W. Kui et al. AIMS Molecular Science. 2022. Vol. 9, no. 2. P. 91–106.
- A Hepatocyte Nuclear Factor BtabHNF4 Mediates Desiccation Tolerance and Fecundity in Whitefly (Bemisia tabaci) / W. Kui et al. Environmental Entomology. 2023. Vol. 52, no. 1. P. 138–147.
- Identification and functional analysis of isopentenyl pyrophosphate isomerase genes in the whiteflies Bemisia tabaci (Hemiptera: Aleyrodidae) / Y. Zhifang et al. Journal of Insect Science. 2023. Vol. 23, no. 3. P. 1–9.
- A ferritin protein is involved in the development and reproduction of the whitefly, Bemisia tabaci / Y. Hao et al. Environmental Entomology. 2023. Vol. 52, no. 4. P. 750–758.
- Functional analysis of a NPC1 gene from the whitefly, Bemisia tabaci (Hemiptera: Aleyrodidae) / Y. Hao et al. Archives of Insect Biochemistry and Physiology. 2023.

Включені до навчальних програм (силабусів) дисциплін «Основи біологічного захисту рослин від шкідливих організмів», «Моніторинг шкідників сільськогосподарських культур та організація заходів регулювання їх чисельності», «Управління чисельністю комах-фітофагів», «Технології вирощування і використання організмів у біологічному захисті рослин» та використовуються в навчальному процесі підготовки фахівців спеціальності «Захисті карантин рослин» першого та другого рівнів вищої освіти. Також, практичні результати роботи, впроваджено у виробничу діяльність навчальної лабораторії садівництва та виноградарства.

Довідка видана для надання до спеціалізованої вченої ради.

Завідувач кафедри захисту рослин

к.с.-г.н., доцент

_____*Валентина ТАТАРИНОВА*

Завідувач навчальної лабораторії садівництва та виноградарства

к.с.-г.н., доцент

_____*Сергій ГОРБАСЬ*