

## REVIEW

of the Official Reviewer **Andrii Butenko** Candidate of Agricultural Sciences, Associate Professor of the Department of Agricultural Technology and Soil Science for PhD Thesis **Cao Zhishan** «*Biological Control of Distribution *Grapholita molesta* Through Immunological Features*», Submitted for a Scientific Degree of a Doctor of Philosophy Field of Study: 20 – Agricultural Sciences and Food Specialty 202 – Plant Protection and Quarantine

**1. Relevance of the dissertation topic.** The oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) is a key pest of tree fruits worldwide. The larvae feed on the shoots and fruits of the stone and pome plants, and have been the most headachy insect pest that threatens fruits quality causing great economic losses in many fruit-growing regions.

However, it is difficult to control this pest with traditional chemical control methods because of the larvae has habits of drilling into fruits or twigs. Furthermore, *G. molesta* has developed resistance to some of broad-spectrum insecticides such as carbamates and pyrethroids, and the overuse of insecticides has caused severe harms to the environment. Meanwhile with the increasing demand for food safety and awareness of environmental protection, biological control method to this pest has attracted more and more people's attentions.

*Beauveria bassiana* is one of the most widely studied and used entomopathogenic fungi, which can also infect and kill the *G. molesta* as a biological control agent. The entomopathogenic fungi with a wide range host and they are harmless to the environment, human and animal. Using entomopathogenic

fungi to control pests has many advantages and it has been an important part in biological control of pests.

The dissertation is devoted to addressing the problem of low efficiency of entomopathogenic in biological control by studying the immune response of insects and fungi and immune genes, and put forward a new target gene method to improve the biological control efficiency for *G. molesta*. Through laboratory ecological tests, transcriptome tests, the combination of various molecular methods and field tests, the effective target genes and effective methods for biological control of *G. molesta* were proposed, which laid the foundation for better biological control. This is of great importance for controlling the pest of *G. molesta* worldwide, especially for improving the fruit tree yield of countries around the world.

**2. Connection with scientific programs, topics, plans.** This research was conducted in alignment with the overarching research focus of Sumy National Agrarian University and Henan Institute of Science and Technology within the framework of specific scientific topics: Key Scientific and Technological Research Project of Henan Province (N0. 202102110220); Key Scientific and Technological Research Project of Henan Province (N0. 222102110010), and the Graduate Education Innovation Training Base Project of Henan Province in 2021 (107020221005).

**3. Scientific novelty of the obtained results.** 1) The author of the dissertation for the first time screened the optimal infection concentration under laboratory conditions. It provides a theoretical basis for the better use of *B. bassiana* in field

control of *G. molesta*.

2) The author presents for the first time first time performed the transcriptomics analysis of *G. molesta* larvae after infected by *B. bassiana*. It provides the comprehensive sequence resources of the immune-related genes of *G. molesta* larvae, presenting valuable information for deeply understanding the macular mechanism of innate immunity process in *G. molesta* against *B. bassiana*

3) For the first time, the author provides the complete sequence of immune-related genes: *GmPGRP-SC* gene, *GmBGRP*, *GmSerpin-2* and *GmSerpin-3* identified and characterized. These target genes provide a new way to improve the biological control effect of *G. molesta* and open the door to new methods of study the development of new pesticides

4) The author proposes field investigation and the determination of different biological pesticides provide reliable data and application value for field control of *G. molesta*. The results laid a foundation for the field occurrence rule and biological control for *G. molesta*.

**4. Theoretical significances of the dissertation.** The theoretical significance of the dissertation lies in the following:

1) The study on the concept and mechanism of immune molecular mechanism is an extension of the concept of immune response to insect resistance for external invasion, which has profound scientific value and theoretical significance for pest control, especially for biological control of *G. molesta*. This has laid a theoretical and scientific foundation for the development of biological control of *G. molesta*.

2) In this paper, the sequence, characteristics, evolution and function of the immune gene of *G. molesta* were analyzed and theoretical studies were made, which laid a theoretical foundation for better understanding of the gene and provided a good guarantee for better using the gene as a target gene to develop new pesticides

**5. Practical significance of the results of the dissertation.** The practical significance of the research results in this paper is to provide comprehensive technical suggestions, complete experimental programs and improvement measures for the biological control of *G. molesta*. It also provides good support for the development of new biopesticides using insect immune genes as target genes. The theoretical achievements of target gene sequence and the technological innovation scheme have been certified by Henan Institute of Science and Technology. Most of the results can be applied in the field test station and pesticide development, and will achieve considerable economic benefits and social value. If conditions permit, it is proposed to use the results of this research in Ukraine in order to improve our living standards.

**6. Number of scientific publications.** Based on the results, A total of 10 academic papers were published, including 1 indexed by Scopus or WoS core database, 4 in Ukrainian professional journals, and 5 in conference proceedings.

**7. Complete presentations of the dissertation material in scientific publications.** The main material of the dissertation is fully presented in 5 articles published in scientific publications and 5 papers published in international academic conferences with the applicant as the first or corresponding author.

(1). Cao Zhishan, Cao Jinjun, Vlasenko Volodymyr, Wang Xinfu, Li Weihai. Transcriptome analysis of *Grapholitha molesta* (Busk) (Lepidoptera: Tortricidae) larvae in response to entomopathogenic fungi *Beauveria bassiana*. Journal of Asia-Pacific Entomology, 25 (2022) 101926.

(2). Cao Zhishan, Cao Jinjun, Zhu Hongxia, Vlasenko Volodymyr. Molecular identification of a short-type peptidoglycan recognition protein, *GmPGRP-SC* from *Grapholitha molesta*. Bulletin of the Sumy National Agricultural University of Agricultural Cultures, 3 (45), 2021.

(3). Cao Zhishan, Vlasenko Volodymyr, Li Weihai. Development of biological control of oriental fruit moth and insect immune response induced by entomopathogenic fungi. Bulletin of the Sumy National Agricultural University of Agricultural Cultures, 2 (40), 2020.

(4). Cao Zhishan, Vlasenko Volodymyr. Application and development prospect of RNA interference technology in pest control. Bulletin of the Sumy National Agricultural University of Agricultural Cultures. 2 (48), 2022.

(5). Cao Zhishan, Vlasenko Volodymyr. Laboratory Evaluation of the effect of *Beauveria bassiana* on the vital activity of *Grapholitha molesta* (Lepidoptera: Tortricidae). Bulletin of the Sumy National Agricultural University of Agricultural Cultures. 4 (50), 2022.

(6). Cao Zhishan. Main factors of outbreak damage of oriental fruit moth. International scientific and practical conference dedicated to the 100th anniversary of the birth of Doctor of Biological Sciences, Professor B. M. Lytvynov. October

21-22, 2021.

(7). Cao Zhishan, Vlasenko Volodymyr. The Integrated Pest Management Measures for Fruit Borer in Organic Orchard. The 85th anniversary of the birth of Viacheslav Grhyrovych Mikhailov, a prominent scientist in the field of breeding and seeds for agricultural culture. 5 жовтня 2021 року.

(8). Cao Zhishan, Vlasenko Volodymyr. Research progress on biological control of oriental fruit moth *Grapholitha Molesta*. «Honcharivski Chytannya» dedicated to the 91th anniversary of Doctor of Agricultural Sciences Professor Mykolay Dem'yanovych Goncharovian, 5, 25-26, 2020.

(9). Cao Zhishan, Vlasenko Volodymyr. Application Research Progress of Entomopathogenic Fungus: *Beauveria Bassiana*. International scientific and practical conference "Potter Readings" dedicated to the 92nd birthday of Doctor of Agricultural Sciences, Professor Mykola Dem'yanovych Goncharovian, 5, 25, 2021.

(10). Cao Zhishan, Vlasenko Volodymyr. Research and Application of Nanoparticle-Mediated RNAi Technology in Pest Control. International scientific and practical conference "Potter's Readings" dedicated to the 93rd birthday of Doctor of Agricultural Sciences, Professor Mykola Dem'yanovych Goncharovian, 5, 25, 2022.

**8. The degree of validity of scientific provisions.** The research results of this paper are not only based on professional scientific research methods, systematic theoretical research, but also through extensive experimental research and test verification, and research in practical applications. The validity of scientific

regulations is fully reflected in the published scientific publications and the obtaining of relevant certifications.

**9. The structure and content of the dissertation, its completeness and compliance with the established requirements for design.** The structure and logic of the dissertation are clear, the construction is reasonable, the content is prominent, concise and clear, and the system is complete. It can fully demonstrate the work tasks and main achievements of the research and can reflect the entire research process. The structure, content and completeness of the dissertation fully comply with the established design requirements.

**10. Discussion clauses, comments and wishes regarding the content.** Although entomopathogenic fungi has been used in Ukraine, as well as in China and other countries around the world, this study gives us a profound understanding of the immune mechanism of insects against entomopathogenic fungi. It provides a new way to improve the biological control effect of entomogenous fungi by using the immune mechanism of pear small food worm and develop new pesticide target genes. Although Ukraine has made some achievements in the biological control of *Grapholitha Molesta*, there is relatively little research on the target genes. Therefore, Ukraine should strengthen the research on the immune molecular mechanism and target genes of insects. In addition, the research can provide new ideas for the biological control of insects and the integrated management of agricultural pests.

There are a few points worth discussing regarding the content of the dissertation:

1. Some language errors and formatting errors need to be polished.
2. Gene names in italics, need to be checked in full text.
3. Research methods are formed in a general way. It is desirable to indicate the used scientific research methods and to note exactly which phenomena and parameters were studied by each of the methods; to justify the choice of methods that ensure the reliability of the obtained results and conclusions.
4. Explain all acronyms such as 1, 2, 3, 4, 5, pu such as in Fig. 25.
5. What is the difference in the number of asterisks? Such as in Fig. 27-28
6. The proposals for production are too optimized, it would be desirable to disclose them in more detail.

**General conclusion.** The dissertation work of **Cao Zhishan** intitled “*«Biological Control of Distribution Grapholita Molesta Ththrough Immunological Features»*”, which was submitted for defense to the specialized academic council for obtaining the degree of Doctor of Philosophy in the field of knowledge 20 - “Agricultural sciences and food” in the specialty 202 - “Plant Protection and Quarantine” in terms of its relevance, scientific and theoretical level, main results of validity, main provisions and results published in professional publications, and novelty of the setting and practical significance meets the requirements of the order of the Ministry of Education and Science of Ukraine No.40 dated January 12, 2017 “On approval of requirements for registration dissertation” and the Decree of the Cabinet of Ministers of Ukraine dated January 12, 2022 No.44 “On approval of the Procedure for awarding the degree of Doctor of Philosophy and cancellation of the

decision of the one-time specialized academic council of the higher educational institution, scientific institution on awarding the degree of “Doctor of Philosophy” with amendments made according to the Resolution of the Cabinet of Ministers No. 341 dated 03.21.2022. The PhD candidate **Cao Zhishan** deserves to be awarded the scientific degree of Doctor of Philosophy in specialty 202 - “Plant Protection and Quarantine”.

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