

REVIEW

of the Official Reviewer **Viktor Demenko** Candidate of Agricultural Sciences, Associate Professor of the Department of Plant Protection 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) is considered a major pest in regions, worldwide, and makes a big damage to fruit trees. It is a host-switching pest species, and the larvae always damage bored the inside fruit or stem, so it is difficult to control with traditional chemical methods. Meanwhile with the increasing demand for food safety, biological control method to this pest has attracted more and more people's attentions.

The entomopathogenic fungi with a wide range host. *Beauveria bassiana* is one of the most studied and applied entomopathogenic fungi, can infected and kill the oriental fruit moth as a biological control agent. In order to make good use of it in the future, it is necessary to deeply understand their infection mechanism to insects. When insects are infected by entomopathogenic fungi, their innate immune system will firstly be activated. And the insects will resist the infection by their immune response, which will lead to the reduction of infection efficiency and the control effect. So, it is necessary to study the immune response of insects

introduced by entomopathogenic fungi, and it is a hotspot in pest control.

The purpose of this dissertation is to investigate the occurrence and control technology of oriental fruit moth, the entomopathogenic fungus (*B. bassiana*) and its induced immune mechanism, the development of immune genes of oriental fruit moth, and the effectiveness of five existing biological pesticides on the control of oriental fruit moth in the field. This will greatly deepen the understanding of the mechanism of insects and entomopathogenic fungi. The improvement of the biological control effect of *Beauveria bassiana* on oriental fruit moth was forecasted, which provided theoretical basis for providing better service for plant protection in the future.

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) In this study, the infection mode and the optimal infection concentration of *Beauveria bassiana* infected oriental fruit moth were selected under laboratory conditions. It provides a theoretical basis for better use of

Beauveria bassiana for biological control in field control.

(2) This study is the first time comprehensively analyze the transcriptomic data of oriental fruit moth larvae infected by *B. bassiana* after 24 h, and screened the key immune-related genes. This study laid a foundation for further understanding of the immune molecular mechanism of insect resistance to entomogenic fungi infection, and for further improving the biological control effect of *B. bassiana*.

(3) This study provides the complete sequence of immune-related genes for the first time: GmPGRP-SC gene, GmBGRP, Gmserpin-2 and Gmserpin3. At the same time, RNAi technology is successfully used to silencing the target genes, and RNAi interference efficiency is significantly improved through nanoparticle mediation. These studies provide a new way to improve the biological control effect of pests, and also open up a new research method for the development of new pesticides.

(4) This study provides reliable data and application value for the field control of oriental fruit moth through field investigation and the determination of different biological pesticides. The results laid a foundation for the field occurrence and biological control of this pest.

4. Theoretical significance of the dissertation.

In this study, through the study of the mechanism of resistance to *Beauveria bassiana* infection and the study of transcriptome analysis of immune molecules, the molecular identification of immune genes related to the oriental fruit moth.

The study on the determination of five kinds of biological pesticides in the field laid a solid foundation for the better use of biological control to control the spread of oriental fruit moth, and provided a theoretical basis for the development of new pesticides.

5. Practical significance of the results of the dissertation.

The process of *B. bassiana*'s control of oriental fruit moth is affected by many factors. This study provides a reasonable basis for the better use of *B. bassiana* to control oriental fruit moth by studying the infection mechanism and immune molecular reaction of *B. bassiana*.

Using RNAi interference technology and nanoparticle mediated technology, the immune target genes were successfully silenced, which provided a crucial role for the subsequent development of pesticides. This technological advance provides a reliable data that provides good support for ensuring integrated management of agricultural pests.

6. Number of scientific publications.

Based on these results, a total of 10 academic articles have been published, including 1 Web of Science Core Collection, 4 Ukrainian professional journals, 5 other conference papers.

7. Complete presentation 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 Xinfa, 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 and systematic theoretical research, but also verified by extensive experimental studies and larval mortality tests, and the field effects of five biopesticides commonly used now are tested. The effectiveness of scientific regulations is fully reflected in published scientific publications.

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.

Remarks for the thesis content. Positively evaluating the work, it is necessary to point out the presence of certain discussion points, provisions that require additional arguments and comments of a technical nature:

1. Section 1 does not contain an analysis of the actual state of *Grapholita molesta* biological control indicators in Ukraine and China, nor a comparative analysis.

2. Please check the Latin in the dissertation carefully. Note: species and genus in Latin need to be italicized.

3. All acronyms must be defined upon first usage separately in the abstract and in the body of the manuscript.

4. 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.

5. The dissertation is replete of incorrect or inaccurate statements and contains many other errors that are not material.

6. It would be advisable to make the recommendations for production more detailed, as they reflect the prospect of using the results obtained in the future work of the author and other scholars.

These remarks do not diminish the scientific value of the dissertation and some of them only reflect the reviewer's scientific position, which can also serve as a reason for discussion.

General conclusion. **Cao Zhishan** dissertation on the topic: «*Biological Control of Distribution Grapholita Molesta Through Immunological Features*», which is presented for obtaining a doctor of philosophy, is the independent study containing scientifically based results in the field of 202 – Plant Protection and Quarantine. In terms of content and formal features, the dissertation meets the requirements for the design of dissertations and the Procedure for awarding the degree of Doctor of Philosophy approved by the Resolution of the Cabinet of Ministers of Ukraine dated 12.01.2022 No. 44, which cancels the previous orders of the Ministry of Education and Culture of Ukraine dated January 12, 2017 No. 40 and Ministry of Education and Culture of Ukraine dated May 31, 2019 No.

759 with changes and additions. **Cao Zhishan** deserves to be awarded the scientific degree of Doctor of Philosophy in the specialty of 202 «Plant Protection and Quarantine».

Official Reviewer:

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Associate Professor of the
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ПІДПИС
ЗАСВІДЧУЮ
ПРОВІДНИЙ ФАХІВЕЦЬ



Demenko V