

## REVIEW

of the Official Opponent **Anna Kryvenko** Doctor of Agricultural Sciences, Professor of the Department of Plant Protection, Genetics and Breeding 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

**Relevance of the dissertation topic.** The Oriental fruit moth, *Grapholita molesta* (Busck), is a major pest of fruit trees worldwide. It is difficult to control using traditional chemical methods, as the larvae gnaw through the inside of the fruit or stem. *Beauveria bassiana* is one of the most studied and used entomopathogenic fungi capable of infecting and killing the oriental fruit moth as a biological agent for controlling its population. In this study, through the study of the molecular mechanism of *B. bassiana* and the molecular study of immune-related genes, we were able to lay the foundation for exploring new targets for biological control of *G. molesta* in IPM and improve *B. bassiana* to increase its efficiency in biological defense.

Cao Zhishan's dissertation is devoted to a topical topic – solving an important scientific and practical issue of biological control of the spread of *Grapholita molesta* using immunological features.

**Purpose and objectives of the study.** The aim of the study was to improve the method of controlling *G. molesta* at the biological level and the use of *B. bassiana* to achieve the optimal level of plant protection against the pest.

**Linking the work to research programs, plans, and topics.** 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).

**Scientific novelty of the results.** The scientific novelty of the obtained results is to solve an important scientific problem of developing biological protection at the immune (gene) level of crops against *G. molesta* and increasing the effectiveness of biological control of the pest.

For the first time, the optimal infection concentration was screened in the laboratory. The transcriptomic analysis of *G. molesta* larvae after infection with *B. bassiana* was performed for the first time. The complete sequence of immune-related genes was determined: GmPGRP-SC, GmBGRP, GmSerp-2 and GmSerp-3 – they were identified and characterized for the first time. The effectiveness of various biological pesticides was determined in the field studies, which provide applied value for the field control of *G. molesta*.

The scheme of *G. molesta* control using *B. bassiana* was improved. The immune genes of *G. molesta* were isolated, which contributed to the increase in the efficiency of biological control.

**Research methods.** General scientific methods: analysis, induction, deduction, synthesis; The field method and the best concentration and the best way for *B. bassiana* to *G. molesta* were determined by laboratory virulence assay. Biostatistical analysis methods, corrective mortality analysis methods, transcriptome analysis methods, fluorescence quantitative molecular analysis methods – to summarize and determine certain reliability of experimental results

**The practical significance of the results.** According to the findings, the biological control method of *G. molesta* has been improved. And the exploring of immune genes plays an important role in the subsequent development of other more effective biopesticides and implementation in the educational process of Sumy National Agrarian University. To determine the special role of different immune genes in biological control of *G. molesta*. Meanwhile, the gene silencing effect was effectively improved by nanoparticle mediated method, and the mortality rate of larvae was significantly increased after infection with *B. bassiana*. These studies have potential application value. The application of this gene has obtained the certificate of our university.

**Approbation of dissertation results.** The results of the research were published and discussed at «International scientific and practical conference dedicated to the 100th anniversary of the birth of Doctor of Biological Sciences, Professor B. M. Lytvynov» (2021), at Proceedings of the International Scientific and Practical Conference «Honcharivski Chytannya» international ones (2020, 2021, 2022). The main items, research results and conclusions of the work

during 2020-2023 were presented and discussed at the meetings of the Department of Plant Protection Sumy National Agrarian University.

**The degree of validity of scientific provisions.** This dissertation is a detailed study independently completed by the applicant, aiming to provide a more effective solution for the biological control of the pear small food worm. Full text in English. Taken together, these fully demonstrate the validity of the applicant's compliance with the scientific provisions of the resolutions of the Cabinet of Ministers of Ukraine (Resolution 283 of April 3, 2019, Resolution 502 of May 19, 2023).

**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. The published works effectively reflect and confirm the results of the studies conducted.

**The structure and content of the dissertation, its completeness and compliance with the established requirements for design.** The dissertation consists of 5 sections, including Introduction:

**Section 1** “BIOLOGICAL CONTROL OF DISTRIBUTION GRAPHOLITA MOLESTA (LITERATURE REVIEW)”. The dissertation thoroughly studied the state of the problem on which she performed the work and covered it in a meaningful, scientifically sound manner in the first chapter (this allowed the applicant to justify the direction of experimental research, to foresee their purpose and main tasks.

**Section 2** “MATERIALS AND METHODS”. In the second section provides data on soil and climatic conditions and weather indicators during the years of field research, methods of laboratory research. Data on experimental schemes, the program and research methodology are presented.

**Section 3** “LABORATORY EVALUATION OF THE EFFECT OF *BEAUVERIA BASSIANA* ON THE VITAL ACTIVITY OF *G. MOLESTA*”. *G. molesta* infected by *B. bassiana* through cuticular infection had higher corrected mortality and better weight inhibition than that of digestive tract infection. Meanwhile, *B. bassiana* at concentration of  $1 \times 10^7$  conidia/mL may have potential to be used as control measure against *G. molesta* in fruit orchards. The second-generation high-throughput transcriptome sequencing technology were used, the transcriptomic profile of *G. molesta* larva infected by *B. bassiana* after 24 h were analyzed, the differentially expressed genes (DEGs) were screened and annotated. Totally, 1,755 DEGs were obtained, with 965 up-regulated and 790 down-regulated genes. We focused on the up-regulated genes in *G. molesta* infected by *B. bassiana*, and 14 genes related to immune response of *G. molesta* induced by *B. bassiana* were selected and quantified by qRT-PCR method, and 10 genes were significantly up-regulated. These results indicated the *G. molesta* has changed its physiological and biochemical state as defense responses against *B. bassiana*.

**Section 4** “CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF GENE: *GMPGRP*; *GMBGRP*; *GMSERPIN-2* AND *GMSERPIN-3*”. The complete sequence of GmPGRP-SC gene, GmBGRP, GmSerpIn-2 and GmSerpIn-3 are

obtained and characterized. They have been submitted to NCBI GenBank respectively, and the GenBank accession number is MW773839, ON055286, OQ359960 and OQ35996. The effect of *B. bassiana* on the regulation of immune-related gene expression were analyzed. Finally, we successfully silenced the target gene using RNAi technology, and the effects of target gene silencing on larvae ability to resist fungal infection were analyzed, which laid a good foundation for further improving the control effect of entomopathogenic fungi.

The effects of GmBGRP gene silencing on Toll immune signaling pathway related gene expression and PPO enzyme activity were analysed. And effectively improve the interference efficiency of GmSerpins-2 and GmSerpins-3 genes mediated by nanoparticles M2L and NP5.

**Section 5** “INVESTIGATION ON THE OCCURRENCE AND DYNAMICS OF *G. MOLESTA* IN PEACH ORCHARD AND EVALUATION ON THE CONTROL OF FIVE DIFFERENT BIOLOGICAL PESTICIDES”. The survey results in the Xinxiang area shows that overwintered larva began to pupate in late March, the pupal stage was 10-20 days, and first-generation adult appeared in early April, which reaching a high level around 4, May. Larvae of *G. molesta* had boring into the peach shoots from April 18. The middle May is the key time for controlling of *G. molesta* in Xinxiang area. The damaged peach shoots can be cut off manually and taken out of the orchard for centralized destruction in order to reduce the occurrence of overwintering generation. The damaged shoots rate by *G. molesta* in peach orchard was higher than that in nectarine orchard. And in the same peach

orchard, the damaged shoots are also different due to different planting densities. The shoots damage is higher with planting density of 4×4 meters than that with planting density of 3×3 meters. The five biological agents all had adverse effects on the growth of peach trees. The results of this study indicating that all the five biological agents were safe for the growth of peach trees. And the 5 biological control agents all have certain control effects on *G. molesta*. On day 7 in treatment groups, the control effect of the five agents was 56.3% ~ 86.4%. Its control effect was 63.1% ~ 80.1% on day 14 of treatment. Among them, 60 g/L pinetoram suspension 2000 times liquid and 240 g/L Methoxyfenozide suspension 5 000 times liquid have better control effectiveness with 86.4% and 74.6% control effect on day 7 in the treatment groups. Moreover, the control effect of these two agents was still more than 78% on day 14. The control effects of 0.2% *Celastrus angulatus* MaXim and 10 billion spores *B. bassiana* were 68.25% and 66.8% respectively on day 7 in treatment groups, and the control effects of these two agents were 61.27% and 67.1% on day 14 in treatment groups which indicating that the two agents had relatively good quick and lasting effect on the control of *G. molesta*.

The structure of the dissertation is reasonable and scientific, consistent with standards, clear logical thinking, detailed, systematic and complete. It has a meaningful integrity, consistency and completeness that is entirely consistent with the requirements formulated in the design.

Correspondence of the dissertation to the specialty and the profile of the council. The dissertation totally corresponds to the field of knowledge 10 «Natural

Sciences» specialty 202 «Plant Protection and Quarantine».

Discussion clauses and remarks to the dissertation. Positively evaluating **Cao Zhishan** dissertation work, its important scientific-theoretical and practical significance, novelty and relevance of research, it is appropriate to make the following remarks and wishes:

1. Given that the work was carried out within the framework of international cooperation between Sumy National Agrarian University and Henan University of Science and Technology, the paper should have paid attention to the comparison of weather and climate conditions in Ukraine and China, as well as the relevance of the issue of adapting the biological characteristics of *Grapholita Molesta* development and production processes to the phenomenon under study for these two countries.

2. In section 2, it was necessary to describe the methods of field research in more detail, it would be desirable to indicate the technological methods of growing the crop in the experiments, namely: planning of experiments, phenological observations, accounting and evaluation, harvesting according to generally accepted methods, describing the size of plots, replications, etc.

3. Some of the data presented in Fig. 8 (p. 80), fig. 7 (p. 79), fig. 10 (p. 82), fig. 13 (p. 95), fig. 23 (p. 108) should have been presented in the form of tables.

4. In the conclusions, it was necessary to detail the content of paragraph 5 to a greater extent.

5. Compared with traditional biological control methods, the application of target immune genes has the advantages of specific binding sites and high efficiency.



The research work is of great significance for developing new biological pesticides and better control of *G. molesta*. The author should check the action mechanism of immune genes and screen the optimal target genes

6. The work was carried out with extensive use of mathematical statistics methods, in particular, molecular calculations, analysis of variance. However, the results of these calculations should have been presented in a more detailed form in the appendices.

7. The work contains some grammatical errors and shortcomings related to the formatting of the work (for example, incorrect abbreviations).

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.** Dissertation work of **Cao Zhishan** on the topic «Biological control of distribution *Grapholita molesta* through 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 with a specialty 202 – Plant Protection and Quarantine of agricultural production according to its relevance, scientific and theoretical level, main results of validity, main provisions and the results published in professional publications, the novelty of the formulation and the practical significance meet the requirements of the order of the Ministry of Education and Culture of Ukraine No. 40 of January 12, 2017 «On approval of requirements for the

preparation of a dissertation» and Resolution of the Cabinet of Ministers of Ukraine of January 12, 2022 No. 44 «On approval of the Procedure for awarding a degree doctor of philosophy and cancellation of the decision of the one-time specialized academic council of the institution of higher education, scientific institution on awarding the degree of doctor of philosophy» with changes introduced in accordance with Resolution of the Cabinet of Ministers No. 341 dated 03.21.2022.

Based on the mentioned above, I believe that the author of the dissertation, **Cao Zhishan**, deserves to be awarded the scientific degree of Doctor of Philosophy in the specialty of 202 – Plant Protection and Quarantine of the field of knowledge 20 – «Agricultural Sciences and Food».

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