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DEVELOPMENT OF BIOLOGICAL CONTROL OF ORIENTAL FRUIT MOTH AND INSECT IMMUNE RESPONSE INDUCED BY ENTOMOPATHOGENIC FUNGI

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The oriental fruit moth, Grapholita molesta (Busck) *is a key pest of tree fruit of Europe, Asia, America, Africa, Australia, and New Zealand, which makes a big damage to apple trees, pear tree and the stone fruit of the peach, plum, apricot, nectarine, cherry and so on. It is difficult to control these pests 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. 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. The entomopathogenic fungi with a wide range hosts and they are harmless to the environment, human and animal. Using entomopathogenic fungi to control pests has many advantages and they have been an important part in biological control of pests, although it still has some natural defects, such as long effective time and easy to be affected by environmental conditions. In order to make good use of it in the future, it is necessary to deeply understand their living conditions and infection mechanism to insects. Entomopathogenic fungi can invades the insects from the body wall through contact directly, but also can through the digestive tract, stomata and wounds and other ways into the insect body. But insects have evolved a strong innate immune system to protect themselves from infection by the pathogens and adverse conditions. 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. This article summarized the occurrence and control technologies of oriental fruit moth, and the research status of entomopathogenic fungus (B. bassiana), finally it summarized the insect immune response induced by entomopathogenic fungi. This will provide a significantly deepened the understanding on mechanisms of insect and entomopathogenic fungi. And it prospected the improvement of effective on biological control of oriental fruit moth by B. bassiana, which provide a theoretical basis for supply better services to plant protection in the future.*

Key words: *Grapholita molesta (Busck), Beauveria bassiana (Bals.-Criv.) Vuill., innate immune, biological plant protection.*
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Introduction. The oriental fruit moth (OFM), *Grapholita molesta* (Busk), belonged to *Tortricidae*, *Lepidoptera.* It is a key pest of tree fruits, widely distributed all around the world except Antarctica (Hill, 1987; Rothschild & Vickers, 1991, Natale et al., 2003; Myers et al., 2006; Timm et al., 2008; Bisognin et al., 2012, Ricietto et al., 2016; Kong et al., 2019; Zunic, et al., 2020). In recent years, the infestation by the OFM in pome fruit orchards was on increase in many fruit-growing regions in many countries, and caused big economic losses (Torriani et al., 2010). Currently, the main control methods of OFM are dependent on chemical pesticides (Tian et al., 2019). Due to the characteristics of hidden damage and the transfer of host hazards, it is difficult to get good results with traditional chemical control (Borchert et al., 2004; Pinero & Dorn, 2009; Lu et al., 2012; Zheng et al., 2013). Meanwhile long-term pesticide usage will cause many problems, for example “3R” (Residue, Resistance, Resurgence), and food safety problem related to the human healthy. While using biological method to control OFM could avoid these disadvantages, and this method is attracting more and more attentions (Kanga, 2003; Monteiro et al., 2020; Navarro-Roldan et al., 2020; Zunic et al., 2020).

As a biological control agent, entomopathogenic fungi play important role in the control of pest worldwide and as one of the entomopathogenic fungi, *B. bassiana* can infected and kill the OFM (Feng & Zhang, 1988; Sarker et al., 2020). But the immune response of insects impedes the infection process induced by *B. bassiana*, and always reduces the infection efficiency and the control effect (Wang & Leger, 2006). A better understanding on the immune response induced by entomopathogenic fungi is eagerly needed in order to make good use of entomopathogenic fungi in biological control of this pest. Now adays many researches on the interaction mechanism and immune response of

B. bassiana and insects have been reported including the *Bemisia tabaci*, *Bombyx mori, Ostrinia furnacalis, Diaphorina citri Kuwayama, Plutella xylostella, Drosophila melanogaster, Riptortus pedestris* and so on, but it is blank for the OFM and *B. bassiana* (Xia et al., 2012; Li et al., 2012; Paparazzo et al., 2015; Shen et al., 2014; Lee et al., 2018; Zhang et al., 2020).

1. Oriental fruit moth

1.1. Origin and geographical distribution

The OFM is a native insect of China (Rothschild &Vickers, 1991). And it was mentioned in Japan, in 1901 and 1902, as a special pest of sand pear (Chaudry, 1956). In 1906 it has been found in South Australia as an enemy of peach, under the popular name Peach Tip Moth. This pest also had several more common names in the past, such as Smaller Pear Borer, Oriental Peach Moth, Oriental Peach Worm and Oriental Fruit Moth, and the last one has been adopted as an officially name. It was reported that OFM introduced to the United States around 1913 by cherries, imported from Japan (Rings, 1970). Then the pest had spread to many parts of United States, like New York, New Jersey, California and so on. In Europe, OFM was first recorded in Slovenia in 1920 and in the early 1920-s it was found in southeastern of France and north of Italy. Then it has dispersed throughout eastern, southern and western of Europe (Kirk et al., 2013). Despite the strict quarantine measures, now it is widely distributed in temperate and subtropical regions of the world, including Europe (Italy, France, Greece), Asia and other regions (Japan and South Korea), America (USA), South America (Argentina, Uruguay, Chile), north of Africa, Australia, and New Zealand, becoming the global fruit-boring insect pest (Rothschild and Vickers, 1991; Natale et al., 2003; Myers et al., 2006; Timm et al., 2008; Bisognin et al., 2012; Kirk et al., 2013). It is also an important pest and widely distributed in China, occurred in almost all fruit regions of China except Tibet, especially in the northern and eastern regions (Chen et al., 2009).

1.2. Host and harm

The OFM is an invasive oligophagous insect (Kong et al., 2020), most of its host plants belong to the family *Rosaceae,* including many economic fruit trees, such as peach, pear, apple, plum, apricot, cherry, hawthorn, jujube etc. In addition, the host plants also including many wild and ornamental plants, for example begonia and loquat (Myers, 2006; Varela et al., 2011; Piskorski et al., 2011; Du et al., 2015), and a special plant of *Myrtaceae* which found in South America (Rothschild and Vickers 1991). Among all of the host plants, the stone fruits peaches and nectarines sever as primary hosts, while the pome fruits apples and pears are considered as secondary hosts (Rothschild & Vickers, 1991; Myers, et al., 2007).

The OFM is a host-switching pest species, it always switching from stone-fruit orchards to pome-fruit orchard during the growing season (Yang et al., 2016; Graillot et al., 2017; Li et al., 2019). The larvae of the first generations can damage the tender shoots of peaches, plums, quinces, and apples in the spring. Each larva needs three or more shoots for its development period, that often cause the tree nutritional deficiency. More seriously, the attacked tree would wither and die, so the OFM is also called "folded worms". Sometimes the first-generation of larvae also do harm to small fruit (Rings, 1970). The second and third generations of larvae transferred to fruits and continue to damage the plants, the larvae always bored into the fruits and remain there throughout their feeding stages. The damages made by larvae cause the tree with miscellaneous bacteria, make fruit rotted and lost its edible value, even drop down. This pest always causes considerable economic lose (CABI, 2016). In some locations even over 45 % of fruits has been infected by the larvae (Kanga et al., 2003). When the OFM population are high the incidence of the fruits or shoots damage even reach to 80 % (Zhao, 2004).

Due to the characteristics of hidden damage and the transfer of host hazards, it is difficult to get good results with traditional chemical control that is the main reason for its generally occurrence, spread and seriously harm (Borchert et al., 2004).

1.3. Occurrence regularity

The duration and occurrence of each generation of OFM are different influenced by climate and nutritional condition of the orchard (Zhang, 1980). The number of larval instars is widely reported as 4 or 5 per year (Peterson & Haeussler, 1928; Yokoyama et al., 1987). But different number of larval instars was also report in some areas each year, such as in the southern of United States and parts of Europe, 7~8 generations occurred annually (Reichart and Bodor, 1972). Even in China, OFM occurs for different generations per year in different parts, 3~4 generations in most areas of north-east China, 4~5 generations in Guanzhong area, 5 generations in Xingjian province, and 6~7 generations in south of China (Wang, 2006). The OFM has overlapping generations and wintering phenomenon (Borchert et al., 2004, Magalhaes & Walgenbach, 2011). The OFM survives the winter as pupa in the soil or the rough seams of fruit trees. The overwintering pupae begin to emerge when the average temperature reaches 5 ºC for seven consecutive days. When the average temperature reached 11~12 ˚C for five consecutive days, the emergence of adult worms reaches to the peak (Wang YH, 2012). The occurrence of the OFM is multi-generations, with serious intergenerational overlap and complex life cycle. As a result, it is difficult to predict and control it, which is also the reason that the harm increases and spreads year by year.

1.4. Control technologies of OFM

There are many reports about the control of OFM around the world. They are mainly focusing on the following aspects: phytosanitary control, agricultural control, physical and mechanical control, biological control, chemical control etc.

(1) Phytosanitary control. Russia, Mexico and other countries take OFM as quarantine object and treat imported fruits at low temperature. It was reported that 1~3 instar larvae could not develop to adult after treatment at 0 ºC for 21 days (Yokoyama & Miller, 1989).

(2) Agricultural control. Avoid mixed planting of peach and pear in new orchard or too close with each other. This will reduce the source of this pest transferred by host. Grass was tied to orchard trunks to attract mature larvae and reduce the cardinal number of overwintering insects. Clean the orchard, remove diseased branches and drop fruits, through the larval overwintering period when fruit tree dormancy. Remove and destroy any infested fruit every few days. Clean up the storage place after harvest the fruit (Ma et al., 2016).

(3) Physical and Mechanical control. Taking advantage of the tendency to sweet and sour liquid of OFM. Sweet and sour liquid bowls was hanged on fruit trees in order to trap and kill the adult of this pest in the growing season. It was demonstrated that the liquid decoy works best when the ratio of sugar, acetic acid, alcohol, water was 3:1:3:80 (Li et al., 2006; Zhai et al., 2019). The fruit bagging also has a certain effect on the control of OFM. It is reported that the damage rate of fruits bagged are below 1 %, while the average insect fruit rate of unbagged fruits reaches 8 %, when they are with the same pesticide treatment (Zhi, 2008).

(4) Chemical control. The pesticide should be applied during the peak period of overwintering adults and the first-generation adults. According to the literature, the mixture of 48 % Lorsban missible oil, beta-cypermethrin, 2.5 % cyhalothrin, and 5 % jipronil SC shows a better effect to control the OFM (Chen, 2007).

(5) Biological control. The biological control of OFM has been widely studied, but mainly focus on natural enemies, sex pheromone and plant source volatiles (Stelinski et al., 2006; Rodrigues et al., 2011; Barros-Parada et al., 2018; Robledo et al., 2018; Li et al., 2019; Guo et al., 2019; Chen et al., 2020; Liu & Kainoh, 2020). Additionally, it was reported that *Beauveria bassiana* species can kill this pest and it can parasitic in wintering larvae of OFM. Parasitic rate up to 20–40 %, even as high as 80 % when the conditions are suitable (Feng & Zhang 1988; Sarker et al., 2020). And the mortality rate of OFM caused by *B. bassiana* was 47.2 % (Song et al., 1993). Mix *B. bassiana* with sulfur as a gelatinizing agent is more effective than using this fungus alone on controlling of OFM (Zhao et al., 2010). However, there were a few studies on the biological control with *B. bassiana* and the studies were not in-depth at present to our knowledge.

2. Entomopathogenic fungi: *B. bassiana*

2.1. Overview of B. bassiana

Entomopathogenic fungi can infect their host insects with directly contact and do not need to be consumed by their host, this always lead to infection under normal physiological conditions. It can cause disease or death to the insects by proliferation in insects' body, and play an important role in biological control throughout the world (Ferron, 1978; Mora et al., 2017). *B. bassiana* is one of the most studied and applied entomopathogenic fungi (Clark, 1982). According to the field investigation of overwintering insects, among all fungal diseases, 21 % of deaths are caused by *B. bassiana* (Li et al., 1983). It was reported that *B. bassiana* has been used for control purpose against as many as 149 families and more than 700 species of pest insects in agriculture, forestry and veterinary (Zimmermann, 2007). As a broad host rang insect pathogen, not only for its safety, but also its less dosage, it has been widely used biological insecticide at present. *B. bassiana* has significant broad prospect of development in the future with the biotechnological innovations. *B. bassiana* not only as a pest biological control agent, but also as a model organism can be used to study the mechanism of interaction with insects and entomopathogenic fungal (Lewis et al., 2001; Wanchoo et al., 2009).

2.2. Biological characteristics of B. bassiana

B. bassiana is a globally distributed *Hyphomycete* and its strains infect a range of insects. Colonies of *B. bassiana* grow relatively slowly with colors ranging from white to yellow. The hyphae are hyaline, smooth and septate. The aerial conidia are spherical or nearly spherical single spore, smooth and hyaline (De Hoog, 1972; Huang et al*.,* 2002). Germination of the conidia with an optimum temperature of 22~26 ºC, and when the temperature reaches to 32 ºC, it cannot germinate any more (Kuang et al., 2005). The most suitable pH for its own sporulation is 5.5~6.5. Conidia germinate in an environment with high relative humidity. The germination percentage reaches to the top when the relative humidity is above 95 %. With the humidity decreases, the rate significantly slow down, and it nearly not grow any more when the humidity decreases to 53 % (Guo et al., 2010). Humidity plays a vital role in activation of the conidia independent of the host (Boucias & Pendland, 1988). P. Ferron found that insects are more easily infected by *B. bassiana* at high ambient relative humidity. *B. bassiana* conidia are also easily influenced by light and other factors (Ferron, 1977). The destruction effect of ultraviolet light on the spores will cause unstable characters of strains, which will lead the field control effect become worse.

2.3. The infection process of B. bassiana

B. bassiana can infect the insect host through spiracles and especially through the surface of integument by directly contacting with the insect, which is the main infection pathway (Portilla et al., 2014). Although it can also entry through the alimentary tract as the same with other hyphomycetes (Hu & Fan, 1996). The initial and important steps in the process are attachment of the spores and penetration of the host cuticle. It is also dependent on various enzymatic activities primarily including degradation of proteins, chitin and lipids in the insect integument (Ferron, 1978). These enzymes work with the mechanical pressure of the hyphae to make the fungi penetrates the host cuticle and enters to hemocoel. When the fungal hyphae reach to the hemocoel, it will germinate to produce new hyphae, and thus the fungi spread throughout the host in the body cavity (Johnson & Goettel, 1993). But the proliferation of fungal in the hemocoel, muscles and other tissues of the insects must overcome the host response and immune defense reactions firstly (Srivastava et al., 2009). Immunity system of the host can make a strong immune response to entomopathogenic fungi and may even eliminate the fungi. But meanwhile entomopathogenic fungi could secrete special substances to inhibit or evade immune response of host and establish fungal infection (Wang et al., 2006). In the process of infection *B. bassiana* and the host are inhibited and competed with each other, so the relationship between fungi and the host and the immune response of the host should be deeply studied.

3. Innate immune response of insects after infected by entomopathogenic fungi

Insects can survive infestation and attack by many pathogens, thanks to their innate immune system (Khush et al., 2000; Hoffmann et al., 2002). They have developed an efficient host defense system against the invasion of fungi. When insects are being infected by fungi, the defense mechanism begins to work at the same time. The innate immune system which is usually divided into cellular and humoral defense, in insects plays a decisive role in the process of resistance to the fungi invasion (Vey & Dumas, 2002). Currently, there are a lot of researches on innate immune response of the insects, but mainly focus on the model insects such as *Drosophila melanogaster*, *Anopheles gambiae*, *Bombyx mori* etc. (Hoffmann et al., 2002; Christophides et al., 2002; Hiromitsu et al., 2008). However, there is relatively little research on agricultural and forestry pests.

3.1. Cellular defense

Cellular defense in insects refers to the defense response such as phagocytosis and encapsulation that are mediated by hemocytes (Strand & Clark, 1999; Irving et al., 2005). There are plasma blood cells, granular blood cells, bead blood cells and granulosa cells in insect hemolymph. These cells can recognize and resist entomopathogenic fungi invasion. Lin found that most spores could not germinate after cytophagy of blood cells in the not yet dead larva of *Dendrolimus spectabilis* infected with *B. bassiana* (Lin et al., 1998). The number of blood cells in the insects increased after infected with *B. bassiana*, which was mainly because of the increasing of plasma and granular blood cells that participated in cellular immunity (Ren et al., 2013).

3.2. Humoral immune defense

Humoral defense refers to the humoral immune factors that exists in insects normally or induced by fat bodies and blood cells, including the antimicrobial peptides, phenol oxidase and lysozyme (Gillespie et al., 2000). When entomopathogenic fungi invaded insects, the recognition mechanism of insects can activate various signal transductions and immune pathways. Then these signal transductions and immune pathways can regulate the expression of various immune-related genes, which will produce effector molecules with antibacterial activity, such as reactive oxygen species and the antimicrobial peptides.

Then pathogens will be killed by the antimicrobial peptides (AMPs), and keep insects homeostatic (Lemaitre & Hoffmann, 2007; Govind, 2008). The antimicrobial peptides have been proposed play the role in limiting fungal proliferation and contributing to the host defense. Nowadays, there are more than 200 kinds of insect antibacterial peptides have been reported, involving *Lepidoptera*, *Diptera*, *Coleoptera*, *Hemiptera*, *Isoptera* and *Hymenoptera* (Dimopoulos et al. 1997; Cudic et al. 2002; Bulet & Stöcklin, 2005; Ran et al., 2016). There are two signaling pathways in insects that induce the production of antimicrobial peptides: one of them is the immune deficiency pathway induced by Gram-negative bacteria, and the other is the Toll pathway activated by fungi and most Gram-positive bacteria (Morisato & Anderson 1994; Levashina et al., 1995; Lemaitre et al.,1996; Akira et al. 2006). Fungal infection activates the Toll signaling pathway and regulates the expression of antifungal peptide genes. In addition, the expression of antimicrobial peptide genes is also regulated by IMD and JAK/STAT signal transduction pathways, that is a complex process and involves large number of genes need to be further studied in the future (Ryu & Ha, 2006; Uvell & Engstr, 2007; Ryu et al. 2010). We can reduce the insect immune response introduced by entomopathogenic fungi, by regulating the expression of immune-related genes.

Conclusion. OFM as an important fruit pest, cause damage and economic losses in many fruit tree areas. With people's attention to environmental and food problems, biological control methods are urgent for controlling this pest. *B. bassiana* as a biological control agent have been studied for many years and has good effect on the prevention and control of OFM. But in the process of pest control, due to the slow action of fungi, the low efficiency insecticidal and the great influence of environmental factors on the infection efficiency, these may impede the role of fungi in controlling the pest (Faria & Wraight, 2001). Meanwhile the immune response of insects can also resist the action of *B. bassiana* and reduce the control effective. The research on how to improve the insecticidal effect of *B. bassiana* and improve the pathogenicity of *B. bassiana* have become a research focus in biological control field and need to be deeply studied in the future. These can be achieved by studying the immune system of OFM and identify immune target sites for attack. With the development and application of molecular biology, the interaction between *B. bassiana* and OFM at the molecular level and analysis of the immune-related gene of OFM will be the focuses research for the biological control of OFM in the future.

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РОЗРОБКА БІОЛОГІЧНОГО КОНТРОЛЮ СХІДНОГО ФРУКТОВОГО МЕТЕЛИКА ЧЕРЕЗ ІНДУКОВАНУ ЕНТОМОПАТОГЕННИМИ ГРИБАМИ ІММУННУ ВІДПОВІДЬ КОМАХ

Східна плодова моль, Grapholita molesta (Busck) – ключовий шкідник плодових дерев Європи, Азії, Америки, Африки, Австралії та Нової Зеландії, що завдає великих збитків яблуням, грушам, а також кісточковим – персику, сливі, абрикосу, нектарину, вишні тощо. Боротися з цим шкідником за допомогою традиційних хімічних методів важко. Тим часом зі збільшенням попиту на безпеку харчових продуктів біологічний метод боротьби з цим шкідником привертає все більше уваги людей. Beauveria bassiana є одним з найбільш вивчених та застосовуваних ентомопатогенних грибів, що може заражати та знищувати східну плодову моль як біологічний засіб боротьби. Ентомопатогенні гриби мають широке коло господарів, але вони нешкідливі для навколишнього середовища, людини та тварин. Застосування ентомопатогенних грибів для боротьби зі шкідниками має багато переваг і вони відігравали важливу роль у біологічній боротьбі зі шкідниками. Проте, вони все ще мають деякі природні дефекти, такі як тривалий ефективний час дії, і на них легко впливають умови довкілля. Для ефективного використання методу у майбутньому, необхідно глибоко розуміти умови життя та механізм зараження комах. Ентомопатогенні гриби можуть потрапляти в тіла комах безпосередньо контактуючи з його покривними стінками, але також і через шлунково-кишковий тракт, продихи, рани та іншими шляхами. Однак комахи сформували потужну вроджену імунну систему, щоб захистити себе від зараження патогенами та несприятливих умов. Коли комахи заражаються ентомопатогенними грибами, спочатку активізується їх вроджена імунна система. Тоді комахи протистоятимуть інфекції своєю імунною реакцією, що веде до зменшення ефективності зараження та контролюючого ефекту. Отже, необхідно вивчити імунну відповідь комах, інтродукованих ентомопатогенними грибами і це є актуальним напрямом у боротьбі з шкідниками. У цій статті підсумовано технологію виникнення та боротьби зі східною плодовою моллю, а також досліджений статус ентомопатогенного гриба (B. bassiana), нарешті, узагальнено імунну відповідь комах, індуковану ентомопатогенними грибами. Це забезпечить значне поглиблене розуміння механізмів дії комах та ентомопатогенних грибів. Також це сприятиме удосконаленню ефективного біологічного контролю східної плодової молі за допомогою B. bassiana, що забезпечує теоретичну базу для надання у майбутньому кращих послуг із захисту рослин.

***Ключові слова:** Grapholita molesta (Busck), Beauveria bassiana, природний імунітет, біологічний захист рослин.*

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