



# Biological control of arthropod pests in citrus orchards in China



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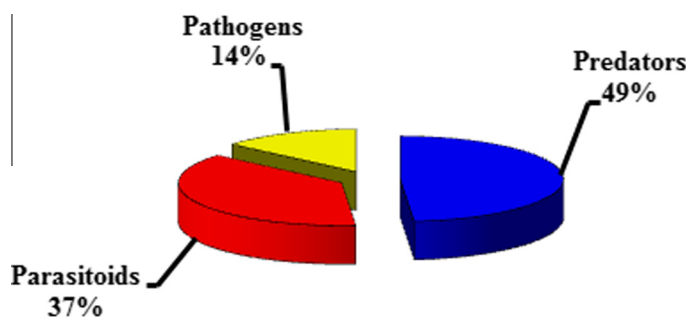
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## HIGHLIGHTS

- A total of 109 natural enemies against citrus insect pests were found in China.
- Field applications of different biological control approaches were evaluated in China.
- Conclusion and perspectives are discussed for the future biological control of citrus pests in China.

## GRAPHICAL ABSTRACT



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## ABSTRACT

China has a very long history of biological control in citrus. Hundreds years ago, predatory ants (*Oecophylla smaragdina* Fabricius) were used to manage pests by Chinese citrus growers. Considerable effort has been placed on the use of biological control in citrus plant landscapes during last three decades. Many scientific studies have now been published, and some additional implementations have already working. In this paper, we review the research, development and application of biological control of citrus insect pests in China. In addition, the importance of biological control for the future control of citrus insect pests is also discussed.

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## 1. Introduction

China is one of the most important citrus origins and has had a significant influence on the development of citrus industry throughout the world. At present, citrus growing acreage in China is an estimated 1.7 million hectares with an annual output of about 19 million tons (Shan, 2008). Citrus has already become a superior agricultural product in China and a supporting industry in the rural areas in southern China. Since 1970s, Chinese scientists have made remarkable scientific and technological advancements to citrus production such as: collection, evaluation and utilization of germ-plasm resources; selection and breeding of variety; cultivation and physiology; pest prevention and control; fruit storage and

processing; quality and safety control of citrus products and standards formulation (Shen, 2009).

In China, citrus orchard insect pests include more than 74 species among 36 families in 9 orders (Yang, 2004). However, only a few are widely distributed and occur consistently to have a significant economic importance. The pests, *Aleurocanthus spiniferus* Quaintance, *Anoplophora chinensis* Forster, *Aphis gossypii* Glover, *Bactrocera dorsalis* Hendel, *B. minax* Enderlein, *Chelidonium arentatum* Dalman, *Dialeurodes citri* Ashmead, *Diaphorina citri* Kuwayama, *Frankliniella occidentalis* Pergande, *Myzus persicae* Sulzer, *Nadzhdiella cantori* Hope, *Panonychus citri* McGregor, *Phyllocnistis citrella* Stainton and *Toxoptera* (*Aphis*) *citricidus* Kirkaldy, have been a major problem to citrus production in the past 40 years. Failure to implement timely pest management has adversely affected citrus harvests quantity and qualities (weight, sugar content, and appearance) (Wang et al., 1999). In recent years, many of these pests, such as the *B. dorsalis* complex, have become

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more abundant and have greatly expanded their geographic distribution from initial points of entry to new areas, increasing the damage to citrus production (Chen and Ye, 2008).

Although multiple control methods (physical, chemical, agricultural and biological control) have been used in citrus orchards, only chemical control has been preferred by citrus growers as it was easy to follow and insecticide applications yielded visible results with the reduction of all arthropods, both pest and beneficial (Xiao et al., 2010). Chemical control strategies were effective control measures in the past; however, their continued use has disrupted natural biological systems and led to dramatic resurgences in insect pest populations (Wu and Nan, 1989). Resurgences are often a result of undesirable effects of insecticides on non-target organisms and the development of insecticide resistance (Zhao, 2000). Increasing resistance levels to the most commonly used insecticides (organochlorines, organophosphates, carbamates and pyrethroids) has led to multiple treatments, including overdoses, thereby raising serious environmental and human health concerns (Chen, 2009). In addition, pesticide resistance has led to the loss of the comparable advantage in modern farming with high input of low cost chemical control. With insecticide resistance and environmental pollution, as well as growing concerns about pesticide residue buildup in soils caused by chemical control, it has become necessary to search for alternative means of pest control in citrus orchards so that the future use of insecticides can be minimized. Therefore, other control strategies, including physical, agricultural and biological control methods, were developed quickly in the last 40 years in China (Li et al., 2010; Luo et al., 2008; Yang and Wang, 2008). Among these control methods, biological control is thought to be a promising replacement by both scientists and citrus growers.

China has a very long history of biological control, especially in citrus. For example, hundreds years ago, nests of predatory ants (*Oecophylla smaragdina* Fabricius) were placed under trees to manage leaf-feeding pests by Chinese citrus growers (Doutt, 1964; Yang, 2002). Considerable effort has been placed on the use of biological control within citrus orchards, especially, during last three decades, many of the scientific achievements about biological control have been published and some additional implementations are already working (Li et al., 2005; Wei et al., 2007; Yang and Wang, 2008).

The aim of this paper is to review the research, development and application of citrus biological control methods in China. Firstly, surveys and classification of natural enemies in citrus orchards, three parts were included: predators, parasitoids and pathogens. Secondly, field applications of different biological control approaches, including classical biological approach, augmentation approach and conservation approach. Finally, conclusion and perspectives were given based on the current biological control research progress in China. In addition, this paper also raised the importance of biological control for the future control of citrus pests.

## 2. Surveys and classification of natural enemies in citrus orchards

Comparatively speaking, biological control research in citrus has developed quickly in China. Between 1981 and 1984, a survey of citrus insect pests and their natural enemies had been conducted resulting in the identification of 53 insect pests species and 100 natural enemies (Lo and Chiu, 1986). For this review, we conducted a thorough search of published literature (main databases: CNKI and CQVIP) about Chinese citrus biological control. This search yielded a list of 109 natural enemies that attack citrus insect pests belonging to 72 genera, 23 families, and 18 orders.

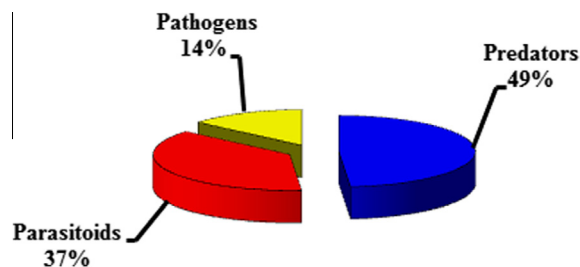


Fig. 1. The percentage of pathogens, predators and parasitoids taken up of the total natural enemies.

Among these natural enemies 53 species (49% of the total) can be classified as predators, while 41 species (37% of the total) are parasitoids and 15 species (14% of the total) are pathogens (Fig. 1).

### 2.1. Predators

The largest groups of natural enemies in citrus orchards that control insect pests are predatory in nature. Of the 53 species recorded, all fall into one of 9 orders with a majority (30 species) belonging to the Coccinellidae family (Table 1). Among these predators, 24 species were found to prey on Asian citrus psyllid (*D. citri* Kuwayama). *D. citri* Kuwayama can not only cause direct feeding damage to young shoots, but is the only known vector in Asia of the bacterium *Candidatus Liberibacter* sp. that causes Huang Long Bing (HLB) citrus greening disease, one of the most serious citrus diseases around the world (Zhang et al., 2009c). However, the utility of these species in China is still scant, therefore, more research efforts should be launched to make better use of these predators to control this serious citrus pest.

The orange spiny whitefly *A. spiniferus* Quaintance is an important pest of economic crops such as tea and citrus (Qi, 2007; Xiao et al., 2009a). *A. spiniferus* Quaintance adults produce honeydew as a result of their feeding and lead directly to the decline of plant vigor and the growth of sooty mold reducing fruit yield (Muniappan et al., 2006). Of the predators recorded in Chinese citrus orchards (mainly *Citrus unshiu* Marcovitch) their control effectiveness was significant if citrus growers protected them. For example, by supplying weed refuges and using fewer chemical pesticides whitefly damage was reduced by 9.2–37% (Guo et al., 2007; Li, 2009).

The aphid species in citrus orchards, such as *A. citricola* von der Goot, *A. gossypii* Glover, and *T. citricidus* Kirkaldy, also cause serious damage to citrus production by direct feeding and the production of honeydew and the growth of sooty mold (Zhang et al., 2009b). In our review, we found 11, 12, and eight predators for *A. gossypii* Glover, *T. citricidus* Kirkaldy, and *A. citricola* von der Goot, respectively. Among these, *Chrysopa septempunctata* Wesmael was found to be a common predator that attacked all aphid species (Ren, 2008; Xiong, 2004). Recently, the chemical iridodial has been found to be a semiochemical attractant to *C. septempunctata* Wesmael (Zhang et al., 2006) and may provide a mean for luring lacewings into aphid-infested orchards. In addition, some species of the genus *Amblyseius*, such as *Amblyseius barkeri* Hughes, *A. cucumeris* Oudemans and *A. orientalis* Ehara, have been used widely to successfully control both *F. occidentalis* Pergande and *Panonychus citri* McGregor in citrus orchards (*C. sinensis* Osbeck, *C. reticulata* Blanco, and *C. grandis* L.), in some citrus plant area (Li et al., 2007; Ling et al., 2008; Ou-Yang et al., 2007; Zhang et al., 2002).

### 2.2. Parasitoids

The 41 parasitoids that have been found to attack citrus insect pests have been classified in only one order, Hymenoptera, and

**Table 1**

Predators of insect pests on citrus in China.

| Insect pest                                    | Pest family (order)            | Pest origin | Predators                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | No. of families | No. of orders | References                                                                                                                                    |
|------------------------------------------------|--------------------------------|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|---------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Aleurocanthus spiniferus</i> Quaintance     | Aleyrodidae (Hemiptera)        | Native      | <i>Adalia bipunctata</i> L., <i>Ankylopteryx octopunctata</i> Fabricius, <i>Anystis baccarum</i> L., <i>Chilocorus gressitti</i> Miyatake, <i>C. kuwanae</i> Silvestri, <i>C. rubidus</i> Hope, <i>Chrysopa septempunctata</i> Wesmael, <i>C. sinica</i> Tjedefa, <i>Erigonidium graminicolum</i> Sundevall, <i>Harmonia axyridis</i> Pallas, <i>Mallada desjardinsi</i> Navas, <i>Propylaea japonica</i> Thunberg, <i>P. quatuordecimpunctata</i> L., <i>Serangium japonicum</i> Chapin, <i>Telsimia emarginata</i> Chapin                                                                                                                                                                                                                      | 4               | 4             | (Guo et al., 2007; Li, 2009; Ren, 2008; Zhang et al., 2004)                                                                                   |
| <i>Aphis citricola</i> Van der Goot            | Aphididae (Hemiptera)          | Native      | <i>C. septempunctata</i> Wesmael, <i>Coelophora saucia</i> Mulsant, <i>Cryptogonus orbiculus</i> Gyllenhal, <i>H. axyridis</i> Pallas, <i>Lemnia biplagiata</i> Swartz, <i>Menochilus sexmaculatus</i> Fabricius, <i>P. japonica</i> Thunberg, <i>Synharmonia octomaculata</i> Fabricius                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 2               | 2             | (Ren, 2008; Xiong, 2004)                                                                                                                      |
| <i>Aphis gossypii</i> Glover                   | Aphididae (Hemiptera)          | Native      | <i>C. septempunctata</i> Wesmael, <i>C. saucia</i> Tjedefa, <i>C. orbiculus</i> Gyllenhal, <i>H. axyridis</i> Pallas, <i>Hylyphantus graminicola</i> Sundevall, <i>L. biplagiata</i> Swartz, <i>Lycosa grahami</i> Fox, <i>M. sexmaculatus</i> Fabricius, <i>P. japonica</i> Thunberg, <i>S. octomaculata</i> Fabricius, <i>Tetragnatha</i> sp.                                                                                                                                                                                                                                                                                                                                                                                                  | 5               | 3             | (Ren, 2008; Xiong, 2004)                                                                                                                      |
| <i>Clitea metallica</i> Chen                   | Chrysomelidae (Coleoptera)     | Native      | <i>Labidura riparia</i> Pallas                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 1               | 1             | (Nin and Qin, 2009)                                                                                                                           |
| <i>Diaphorina citri</i> Kuwayama               | Psyllidae (Hemiptera)          | Introduced  | <i>A. bipunctata</i> L., <i>Agistemus exsertus</i> Gonzalez, <i>Cheilomenes sexmaculata</i> Fabricius, <i>C. quadriplagiata</i> Swartz, <i>C. pallens</i> Rambur, <i>C. septempunctata</i> Wesmael, <i>C. rufilabris</i> Burmeister, <i>Coccinella septempunctata</i> L., <i>Coelophora biplagiata</i> Swartz, <i>C. inaequalis</i> Fabricius, <i>H. axyridis</i> Pallas, <i>H. graminicola</i> Sundevall, <i>L. circumsta</i> Mulsant, <i>Megalocaria dilatata</i> Fabricius, <i>Olla vni-grum</i> Mulsant, <i>Orius minutus</i> L., <i>P. japonica</i> Thunberg, <i>Rodolia cardinalis</i> Mulsant, <i>R. limbata</i> Motschulsky, <i>Scolothrips longicornis</i> Priesner, <i>Sospita chinensis</i> Mulsant, <i>S. octomaculata</i> Fabricius | 6               | 5             | (Chen, 1992; Nin and Qin, 2009; Ren, 2008; Yu, 2001; Zhang et al., 2009c)                                                                     |
| <i>Frankliniella occidentalis</i> Pergande     | Thripidae (Thysanoptera)       | Native      | <i>Amblyseius (Neoseiulus) barkeri</i> Hughes, <i>A. cucumeris</i> Oudemans, <i>O. sauteri</i> Poppius                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | 2               | 2             | (Li et al., 2007; Liu et al., 2007; Ren, 2008; Xiong, 2004; Zhang et al., 2007; Zhi and Ren, 2006)                                            |
| <i>Myzus persicae</i> Sulzer                   | Aphididae (Hemiptera)          | Native      | <i>C. septempunctata</i> Wesmael, <i>C. saucia</i> Mulsant, <i>C. orbiculus</i> Gyllenhal, <i>H. axyridis</i> Pallas, <i>H. graminicola</i> Sundevall, <i>L. biplagiata</i> Swartz, <i>L. grahami</i> Fox, <i>M. sexmaculatus</i> Fabricius, <i>P. japonica</i> Thunberg, <i>S. octomaculata</i> Fabricius, <i>Tetragnatha</i> sp.                                                                                                                                                                                                                                                                                                                                                                                                               | 5               | 3             | (Ren, 2008; Xiong, 2004)                                                                                                                      |
| <i>Panonychus citri</i> McGregor               | Tetranychidae (Trombidiformes) | Native      | <i>A. barkeri</i> Hughes, <i>A. cucumeris</i> Oudemans, <i>A. orientalis</i> Ehara, <i>E. graminicolum</i> Sundevall, <i>Oligota</i> sp., <i>Stethorus siphonulus</i> Kapur                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | 4               | 3             | (Gan et al., 2001; Gao and Pan, 2007; Ling et al., 2008; Ou-Yang et al., 2007; Wei et al., 1997, 2007; Xiao et al., 2005; Zhang et al., 2002) |
| <i>Phyllocnistis citrella</i> Stainton         | Gracillariidae (Lepidoptera)   | Native      | <i>C. boninensis</i> Okamoto                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 1               | 1             | (Zhen and Yang, 2009)                                                                                                                         |
| <i>Toxoptera aurantii</i> Boyer de Fonscolombe | Aphididae (Hemiptera)          | Native      | <i>C. septempunctata</i> Wesmael, <i>C. saucia</i> Mulsant, <i>C. orbiculus</i> Gyllenhal, <i>H. axyridis</i> Pallas, <i>H. graminicola</i> Sundevall, <i>L. biplagiata</i> Swartz, <i>L. grahami</i> Fox, <i>M. sexmaculatus</i> Fabricius, <i>P. japonica</i> Thunberg, <i>S. octomaculata</i> Fabricius, <i>Tetragnatha</i> sp.                                                                                                                                                                                                                                                                                                                                                                                                               | 5               | 3             | (Ren, 2008; Xiong, 2004)                                                                                                                      |
| <i>Toxoptera citricidus</i> Kirkaldy           | Aphididae (Hemiptera)          | Native      | <i>Alesia discolor</i> Fabricius, <i>C. septempunctata</i> Wesmael, <i>C. saucia</i> Mulsant, <i>C. orbiculus</i> Gyllenhal, <i>H. axyridis</i> Pallas, <i>H. yedoensis</i> Takizawa, <i>Ischiodon scutellaris</i> Fabricius, <i>L. biplagiata</i> Swartz, <i>M. sexmaculatus</i> Fabricius, <i>P. japonica</i> Thunberg, <i>S. octomaculata</i> Fabricius                                                                                                                                                                                                                                                                                                                                                                                       | 3               | 3             | (Ren, 2008; Xiong, 2004)                                                                                                                      |

are represented by 23 genera, 5 families (Table 2). Among them, 18 species have been reported to attack orange spiny whitefly (*A. spiniferus* Quaintance) and are confined to five genera: *Amitus*, *Encarsia*, *Prospaltella*, *Aphytis* and *Comperiella*. Biological control success of *A. spiniferus* Quaintance was first reported in Japan using *Encarsia* (= *Prospaltella*) *smithi* Silvestri and *Cryptognatha* beetles

imported from China (Kuwana and Ishii, 1927). More recently, control has been achieved in citrus crops of Swaziland, South Africa using *E. smithi* Silvestri (Van den Berg and Greenland, 1997) and in the ornamental citrus and rose plants of Hawai'i (Nafus, 1988). However, while species in the genus *Encarsia* have the potential to be successful biological control agents, the research on *Encarsia*

**Table 2**  
Parasitoids of insect pests on citrus in China.

| Insect pests                               | Pest family (order)       | Pest origin | Parasitoids                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | No. of families | No. of orders | References                                                                                                                                               |
|--------------------------------------------|---------------------------|-------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|---------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Aleurocanthus spiniferus</i> Quaintance | Aleyrodidae (Hemiptera)   | Native      | <i>Amitus hesperidum</i> Silvestri, <i>A. longicornis</i> Förster, <i>Aphytis chrysomphali</i> Mercet, <i>Comperiella unifasciata</i> Ishii, <i>Encarsia albiscutellum</i> Girault, <i>E. aseta</i> Hayat & Polaszek, <i>E. azimi</i> Hayat, <i>E. collecta</i> Chou & Su, <i>E. formosa</i> Gahan, <i>E. ishii</i> Silvestri, <i>E. japonica</i> Viggiani, <i>E. lahorensis</i> Howard, <i>Psytalia lounsburyi</i> Silvestri, <i>E. nipponica</i> Silvestri, <i>E. obtusiclava</i> Hayat, <i>E. smithi</i> Silvestri, <i>Eretmocerus silvestrii</i> Gerling, <i>Prospaltella ishii</i> Silvestri | 4               | 1             | (Guo et al., 2006a; Huang et al., 2000; Nin and Qin, 2009; Yang and Wang, 2008; Ye et al., 1996; Zhu and Chen, 1994)                                     |
| <i>Anoplophora chinensis</i> Forster       | Cerambycidae (Coleoptera) | Native      | <i>Aprostocetus prolixus</i> LaSalle & Huang, <i>Brulleia shibuensis</i> Matsumura, <i>Scleroderma</i> sp.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 3               | 1             | (Shen, 2010; Zhang, 2001)                                                                                                                                |
| <i>Bactrocera dorsalis</i> Hendel          | Tephritidae (Diptera)     | Introduced  | <i>Aceratoneuromyia indica</i> Silvestri, <i>Aganaspis</i> sp., <i>Diachasmimorpha longicaudata</i> Ashmead, <i>Dirhinus giffardii</i> Silvestri, <i>Fopius arisanus</i> Sonan, <i>F. vandenboschi</i> Fullaway, <i>P. fletcheri</i> Silvestri, <i>P. incise</i> Silvestri, <i>Spalangia longepetiolata</i> Boucek                                                                                                                                                                                                                                                                                | 5               | 1             | (Guo et al., 2006b; Liang et al., 2007; Lin et al., 2006; Lu et al., 2007; Shao et al., 2009; Yao et al., 2008; Zhang et al., 2008a; Zheng et al., 2006) |
| <i>Chelidonium arentatum</i> Dalman        | Cerambycinae (Coleoptera) | Native      | <i>A. prolixus</i> LaSalle & Huang, <i>B. shibuensis</i> Matsumura, <i>Scleroderma</i> sp.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 3               | 1             | (Shen, 2010; Zhang, 2001)                                                                                                                                |
| <i>Dasineura citrigemma</i> Yang & Tang    | Cecidomyiidae (Diptera)   | Native      | <i>Neanastatus</i> sp.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 1               | 1             | (Ren, 2008)                                                                                                                                              |
| <i>Dialeurodes citri</i> Ashmead           | Aleyrodidae (Hemiptera)   | Native      | <i>E. formosa</i> Gahan                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | 1               | 1             | (Nin and Qin, 2009)                                                                                                                                      |
| <i>Diaphorina citri</i> Kuwayama           | Psyllidae (Hemiptera)     | Introduced  | <i>Diaphorencyrtus aligarhensis</i> Shafee, Alam and Argarwal, <i>D. diaphorinae</i> Lin & Tao, <i>Psyllaephagus diaphorinae</i> Lin & Tao, <i>Tamarixia radiata</i> Waterston, <i>Tetrastichus</i> sp.                                                                                                                                                                                                                                                                                                                                                                                           | 2               | 1             | (Mao et al., 2010; Nin and Qin, 2009; Ren, 2008)                                                                                                         |
| <i>Nadezhdiella cantori</i> Hope           | Cerambycidae (Coleoptera) | Native      | <i>A. prolixus</i> LaSalle & Huang, <i>B. shibuensis</i> Matsumura, <i>Scleroderma</i> sp.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 3               | 1             | (Shen, 2010; Zhang, 2001)                                                                                                                                |

has initially focused on species investigation, identification and classification in citrus orchards (Guo et al., 2006a; Huang et al., 2000).

Other parasitoids have also been recorded attacking *A. spiniferus* Quaintance in citrus orchards including the platygastroid wasp *Amitus* sp. (Nin and Qin, 2009; Yang, 2004; Ye et al., 1996; Yu, 2001). *Amitus* wasps locate their hosts by following the chemical signals emanating from honeydew released and exuviae shed by the feeding whitefly larvae (Li et al., 1993) and the wasps were thought to be the dominant parasitoid to the 1st to 2nd instar larvae of *Angoliuma hesperidium* Quaintance (Zhu and Chen, 1994). On the Pacific Island of Guam, use of both *A. hesperidium* Silvestri and *E. smithi* Silvestri achieved 80–95% parasitism (Peterson, 1955). Clearly, collection of these parasitoids indicates a beneficial presence in Chinese citrus orchards and a potential for commercial biological control.

Probably no other citrus insect pest is more famous than the oriental fruit fly (*B. dorsalis* Hendel). The *B. dorsalis* complex includes 52 species, 8 of which are of economic importance in Asia and North America (Drew and Hancock, 1994). In recent years, many aspects of *B. dorsalis* Hendel control have been studied. At least 10 parasitoid species (Table 2) may contribute to a potential control strategy for effective management of this pest. In an inventory of citrus orchards in Guangdong Province, eight species within four families of hymenopterous parasitoids of *B. dorsalis* Hendel were found (Yao et al., 2008). Of those eight species efforts have been made to study the functional response of the chalcidoid *Spalangia longepetiolata* Boucek, and the braconids *Diachasmimorpha longicaudata* Ashmead, *Fopius vandenboschi* Fullaway, and *Fopius arisanus* Sonan on the developmental stages of the *B. dorsalis* Hendel host (Guo et al., 2006b; Lin et al., 2006; Lu et al., 2007; Shao et al., 2009; Zheng et al., 2006). In addition, *F. vandenboschi* Fullaway has been successfully reared in the laboratory, which infers its potential application in the field (Zhang et al., 2008a), but as

of yet no mass rearing efforts have been made for release in citrus orchards.

### 2.3. Pathogenic natural enemies

Microbial or biological pesticides may consist of bacteria, entomopathogenic fungi, or viruses used to control pests, especially insects. Two decades ago microbial pesticides accounted for <1% of the total insecticide market (Starnes et al., 1993), however, awareness of alternative microbial insect pest control is increasing in both the public and agricultural sectors. Although only 15 pathogens of citrus insect pests were recorded in the current literature (Table 3), work with this natural enemy group began in China in the 1960s (Feng, 1986). As of 2008, 327 bio-pesticides, a majority of which are based on *Bacillus thuringiensis* Berliner, were registered in China, accounting for 1.6% of total registered pesticide products (Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA, 2008) indicating that bio-pesticides hold great promise for the biological control of pests. However, to date most of those registered are for general use and not specifically targeted to citrus pests. The current literature lists 14 entomopathogenic fungi from 8 families and the bacterium *B. thuringiensis* Berliner as endemic to citrus orchards (Table 3). The fungal pathogen *Beauveria bassiana* Balsamo has a long history of biological control application in China and has been adopted as a conventional, and oftentimes preferred, choice for forest farms against defoliating caterpillars in southern China; the routine use of *B. bassiana* Balsamo has resulted in a great reduction in chemical insecticide use in forestry (Xu, 2004). *B. bassiana* Balsamo is thought to be the best entomopathogenic candidate to control citrus insect pests, such as *B. dorsalis* Hendel, *D. citri* Kuwayama, *M. persicae* Sulzer and *F. occidentalis* Pergande (Nin and Qin, 2009; Pan et al., 2006, 2008). Another entomopathogenic fungus, *Entomophthora aphidis* Hoffm., has not been developed as a biological control agent, but may play

**Table 3**

Pathogens of insect pests on citrus in China.

| Insect pests                                   | Pest family (order)          | Pest origin | Pathogens                                                                                                                                                                                         | No. of families | No. of orders | References                                                                               |
|------------------------------------------------|------------------------------|-------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|---------------|------------------------------------------------------------------------------------------|
| <i>Aleurocanthus spiniferus</i> Quaintance     | Aleyrodidae (Hemiptera)      | Native      | <i>Aschersonia aleyrodis</i> Webber, <i>Paecilomyces aleurocanthus</i> Petch, <i>Isaria fumosoroseus</i> Wize, <i>Pleurodesmospora coccorum</i> Li & Huang, <i>Verticillium lecanii</i> Zimmerman | 4               | 3             | (Guo et al., 2006a; Han and Cui, 2004; Zhang et al., 2004)                               |
| <i>Aphis gossypii</i> Glover                   | Aphididae (Hemiptera)        | Native      | <i>Cladosporium</i> sp., <i>Entomophthora aphidis</i> Hoffm                                                                                                                                       | 2               | 2             | (Feng, 1986; Li et al., 1997)                                                            |
| <i>Bactrocera dorsalis</i> Hendel              | Tephritidae (Diptera)        | Introduced  | <i>Beauveria bassiana</i> Balsamo                                                                                                                                                                 | 1               | 1             | (Pan et al., 2006, 2008; Yuan et al., 2010; Zhu, 2010)                                   |
| <i>Diaphorina citri</i> Kuwayama               | Psyllidae (Hemiptera)        | Introduced  | <i>B. bassiana</i> Balsamo                                                                                                                                                                        | 1               | 1             | (Nin and Qin, 2009)                                                                      |
| <i>Dialeurodes citri</i> Ashmead               | Aleyrodidae (Hemiptera)      | Native      | <i>A. placenta</i> Berk, <i>A. aleyrodis</i> Webber                                                                                                                                               | 1               | 1             | (Fu, 1998; Tong, 1992)                                                                   |
| <i>Diaphorina citri</i> Kuwayama               | Thripidae (Thysanoptera)     | Native      | <i>B. bassiana</i> Balsamo, <i>Metarhizium anisopliae</i> Metsch, <i>V. lecanii</i> Zimmerman                                                                                                     | 2               | 1             | (Ding et al., 2009; He, 2010; Yang and Yu, 2005)                                         |
| <i>Frankliniella occidentalis</i> Pergande     | Aphididae (Hemiptera)        | Native      | <i>B. bassiana</i> Balsamo, <i>Pandora neoaphidis</i> Remaudière & Hennebert, <i>P. nouri</i> Humber, <i>Zoophthora anhuiensis</i> Li                                                             | 2               | 2             | (Bao and Feng, 2006; Feng, 1986; Gui et al., 2005; Li et al., 2003; Zhang et al., 2008b) |
| <i>Phyllocnistis citrella</i> Stainton         | Gracillariidae (Lepidoptera) | Native      | <i>Bacillus thuringiensis</i> Berliner                                                                                                                                                            | 1               | 1             | (Zhang, 2001)                                                                            |
| <i>Toxoptera aurantii</i> Boyer de Fonscolombe | Aphididae (Hemiptera)        | Native      | <i>E. aphidis</i> Hoffm                                                                                                                                                                           | 1               | 1             | (Feng, 1986)                                                                             |
| <i>Toxoptera citricidus</i> Kirkaldy           | Aphididae (Hemiptera)        | Native      | <i>E. aphidis</i> Hoffm, <i>Fusarium lateritium</i> Nees                                                                                                                                          | 2               | 2             | (Feng, 1986; Song, 2001)                                                                 |
| <i>Toxoptera citricolavan</i> der Goot         | Aphididae (Hemiptera)        | Native      | <i>E. aphidis</i> Hoffm                                                                                                                                                                           | 1               | 1             | (Feng, 1986)                                                                             |

a very important role in citrus orchards in controlling aphid pests, such as *A. gossypii* Glover, *A. citricola* von der Goot, *Toxoptera aurantii* Boyer de Fonscolombe and *T. citricidus* Kirkaldy (Feng, 1986). In 1996, the fungus *Fusarium lateritium* Nees was isolated from dead brown citrus aphid bodies in Nanping city, Fujian Province. Interestingly, bioassay results indicated that this fungus was extremely virulent to brown citrus aphid nymphs. In addition, in a controlled field test when a spore suspension of *F. lateritium* Nees was applied, aphid mortality was 91.80%; an excellent example of the possible utility of microbial pesticides (Song, 2001).

#### 2.4. Field applications of different biological control approaches

Exploration and classification of natural enemies in Chinese citrus orchards supplied basic foundation for field application, especially, research on pest control effectiveness of certain natural enemies and massive reproduction of natural enemies, would help scientists to find out the appropriate agents and put into practice by using different approaches. Generally, there are three approaches for biological control, including: classical biological control (importation), augmentation and conservation of natural enemies. In China, these three approaches were applied in citrus orchards to control the dominant pests during different times. In some cases, the combination of different approaches was also used.

#### 2.5. Classical biological control

*Rodolia cardinalis* Mulsant was introduced in China around 1950s for controlling citrus pests. With high reproduction rate and sufficient ability of adaptation, it had successfully settled and spread in some citrus growing area of China, which inhibited the frequently and massively occurrence of *Icerya purchasi* Maskell. *A. cucumeris* Oudemans were imported for control mites. In China, scientists also used this approach for long distance nature enemies importation, especially, the ladybeetles of other species, such as, *R. rufopilosa* Mulsant, and *R. pumila* Weise, were

transported across provinces in China to control *I. purchasi* Maskell (Yang and Wang, 2008). Besides, application of nature enemies from other pests to citrus pests was found to be promising to control *Homona coffearia* Meyrick and *Adoxophyes cyrtosema* Meyrick by *Trichogramma* sp., which was originally nature enemy for *Pectinophora gossypiella* Saunders in China. Some citrus pests, such as *B. dorsalis* Hendel and *D. citri* Kuwayama were introduced from abroad or other location in China, so the survey of nature enemies from their origin could help to find the useful candidate agents to control them in the introduced area. In the future research, the strategies, such as, introduction nature enemies from international level, national level, and pest level, should be considered together and choose the appropriate strategy to control citrus pests.

#### 2.6. Augmentations

The release of additional numbers of natural enemy in citrus orchards critically relies on the ability to mass-produce large numbers of the natural enemy. In 1974, massive release of *Trichogramma dendrolimi* matsumura in large scale of citrus orchards (*C. reticulata* Blanco; *C. sinensis* Osbeck; and *C. poonensis* Hort) significantly caused the reduction of *Homona coffearia* Neitnar. Field experiments revealed that *E. citrina* Craw was more effective to control *Aonidiella aurantii* Maskell than *Aphytis melinus* DeBach, *A. lingnanensis* Compere, separately release, and even better than both *A. melinus* DeBach and *A. lingnanensis* Compere released together (Zhang and Gu, 1994). Other massive agents release also can significantly reduce the population of pests in field, such as *Gampylomma chinensis* Schuh for control of *D. citri* Kuwayama; *F. arisanus* Sonan for control of *B. dorsalis* Hendel; and *C. sinica* Tjeder for control of *P. citri* McGregor (Peng, 1985). However, there are still limited commercial nature enemies for citrus pests control in the market, which largely influence citrus growers to use these agents in field. The main species of citrus pests in China was changed in different times corresponding to several factors, such as pesticides, pests invasion and spread, natural enemies homeostasis

and so on (Woo et al., 1975), which have great influence on sustainable biological control. Currently, the citrus spider mite, *P. citri* McGregor, and citrus rust mite, *Phyllocoptura oleivora* Ashmead, are considered some of the most important pest arthropods in citrus production areas of China. Chemical control of mites constitutes more than half of all pest control in one year (Ho, 2000; Lu et al., 2009). However, biodiversity of both pest and beneficial arthropods were rapidly decreased due to the intensive application of acaricides (Zhao, 2000). Since 1974, the goal of many plant protection agencies and research facilities has been to find an effective biological control agent for these mites by using both classical biological control and augmentation approaches. One of the first large scale projects was conducted by the consortium of Guangdong Entomological Institute, Department of Plant Protection at Southeast University, and Citrus Institute of the Chinese Academy of Agricultural Sciences. Up until the end of 1987, integrated control of citrus pest mites included the rearing and mass release of predatory mites over 8650 hectares, approximate 26% of the total citrus planting area, in the south eastern provinces (Zhang et al., 2002). Herein we review the mass rearing and release of the predatory mites *A. cucumeris* Oudemans and *A. barkeri* Hughes for the control of citrus mite pests. As early as 1964, the predatory mite *A. cucumeris* Oudemans had been investigated as a biological control agent for orchard management (Burrell and McCormick, 1997). By the early 1990s, *A. cucumeris* Oudemans was being used not only for the control of mites, but also thrips (McMurtry and Croft, 1997).

In 1996, the Fujian Academy of Agricultural Sciences introduced *A. cucumeris* Oudemans into China from England. Successful rearing protocols and mass production logistics were developed at the first predatory mite laboratory built in Fujian, with production capacity reaching at 800 billion individuals per year. After release, *A. cucumeris* Oudemans effectively suppressed both the citrus spider mite and the rust mite on citrus in China (Ou-Yang et al., 2007; Wei et al., 2007). By 2005, the Fujian Yanxuan Biocontrol Company Limited factory for predatory mite production had been built in Fuzhou, China. Mass-produced predators can be transported by air conditioned truck or airplane within 1–4 days to destinations in China or around the world (Yu et al., 2008; Zhang et al., 2002). Currently, *A. cucumeris* Oudemans are applied on not only in citrus trees, but also in cotton, moso bamboo, tea, apples, peaches and many greenhouse crops (Zhang et al., 2002). Use of *A. cucumeris* Oudemans now accounts for 193,000 ha on 20 species of plants in 358 regions in 20 provinces in China, reducing chemical use by 3234 tons, saving control costs by US\$ 500,000, and reducing the use of water by 940,000 m<sup>3</sup> annually (Yu et al., 2008; Zhang et al., 2002). Although *A. cucumeris* Oudemans as sufficient in the field to control citrus red mites, the control effectiveness showed slight different among different citrus species. After 120 days releasing of *A. cucumeris* Oudemans in orchards of *C. sinensis* Osbeck, *C. reticulata* Blanco, and *C. grandis* L., the control effects of *P. citri* McGregor were 100%, 86.8%, and 98.1%, respectively.

*Amblyseius barkeri* Hughes has been commercially available as a biological control agent in Europe since 1981 (Van Lenteren, 2003). *A. barkeri* Hughes was found attacking the citrus red mite, *P. citri* McGregor, in Ganzhou, Jiangxi province. They are now known to be distributed throughout the Chinese citrus growing area. Since 2005, efforts to understand and use the biological and ecological characteristics that enhance artificial production and application technology for the control of *P. citri* McGregor have been under way in China (He et al., 2008; Ling et al., 2008; Xu et al., 2010; Zhang and Fan, 2005). Once protocols were in place for successful factory production of *A. barkeri* Hughes and field application trials using *A. barkeri* Hughes to control *P. citri* in pomelo orchards (*C. grandis* var. *shatinyu* Hort) were conducted in Jiangxi province in 2007 and 2008 (Xu et al., 2010). *A. barkeri* Hughes were able to successfully establish a large, reproducing population in pomelo

orchards and could effectively control *P. citri* McGregor (Xu et al., 2010). The density of *P. citri* McGregor was significantly reduced to below economic damage thresholds after releasing 1800–2400 individuals on each pomelo tree (Xu et al., 2010). Currently, use of *A. barkeri* Hughes is increasing to control mite pests around Chinese citrus production areas. This has benefited the Chinese citrus growers by decreasing the amount of pesticides and consequently decreasing cost of controlling the pests while improving the environment and the quality of fruits.

## 2.7. Conservation

Every organism has natural enemies. Conservation and use of natural enemies is one of the most important and potentially cost-effective biological control practices available to growers. From the private garden to the open field, pests and their enemies will adapt to favorable habitats that provide resources for growth and reproduction. Inappropriately using pesticides could probably kill beneficial insects, such as dominate natural enemies, so it is important to select the pesticides which are safe to natural enemies in orchards before using chemical control (Zhang et al., 2009a). Normally, the chemical control and biological control were used together, therefore, future analysis could be made to combine these two control methods well, for example, control effect of mineral oil combining with predatory mites on *P. citri* McGregor was optimal (Fang et al., 2012). Recent studies have shown that cultural practices can influence the diversity and abundance of natural enemies. Trap crops (Corbett and Plant, 1993), crop rotation (Xia, 1995), creation of barriers such as hedgerows (Coombes and Sotherton, 1986; Dennis et al., 2000; Wratten and Thomas, 1990), and manipulation of non-crop habitats, such as refuges, can increase natural enemy diversity and abundance (Andow, 1991; Banks, 1955; Landis et al., 2000; Perrin, 1975; Schellhorn and Sork, 1997). In China, the common weeds *Ageratum conyzoides* L., *Perilla frutescens* L., *Agastache rugosa* Fisch. Et Mey, *Lolium perenne* L., *Medicago sativa* L., *Ageratum houstonianum* Miller, *Trifolium repens* L., and *Paspalum notatum* Flügge are widely used as “companion weeds” to enhance biological control in orchards (Chen, 2007; Li et al., 2005; Qiu, 2007). In 2004, a comparison survey of orchards with and without companion weeds found that the biodiversity of natural enemies was 65% greater in orchards with companion weeds than those without (Wu, 2005). In addition, refuges help maintain predator populations and reduce the need for reintroduction from year to year (Fu and Huang, 2007). The planting of companion weeds in citrus orchards may not only attract and maintain natural enemy populations by supplying alternate food resources (Xiao et al., 2009b), but may also improve the environment and lead to an increase in the density and diversity within the orchard ecosystem (Wu, 2005). Companion weeds have been found to help regulate the microclimate within the orchard by reducing water evaporation and aerating the soil (Li et al., 2005).

## 3. Conclusion and perspectives

In China, research and development of biological control of citrus pests in some academies and agricultural research institutions has led to a significant reduction in the need for classical pesticides, an expanding technology base and a growing recognition of China's commitment to science. As one of China's highest quality exports, biological control in citrus has been placed as a key tactic for the integrated pest management (IPM) program. Although considerable effort and even appreciable progress, has been made in biological control in citrus, there are opportunities to improve this field. For instance, chemical control remains the main control method in Chinese citrus growing areas (Ho, 2000; Lu et al.,

2009); local or rural citrus growers remain uninformed about biological control methods. In addition, only few natural enemies, such as *A. cucumeris* Oudemans and *A. barkeri* Hughes, are commercialized which highly restricts the development and application of massive biological control efforts. In conclusion, the demand for biological control methods are increasing as governments and growers world-wide become aware of the benefits, both economic and environmental, to using biological control methods. Thus the use of pesticides will no doubt decline over the next few decades, reducing the biochemical hazards they pose to our health. However, our collective responses to these challenges will determine how effectively biological control is maintained as a viable discipline. With our continuous studies and research perspectives, the application of biological control will continue to add to new frontiers for citrus insect pests control in China.

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