THE CHINESE CITRUS FLY, *Bactrocera minax* (DIPTERA: TEPHRITIDAE): A REVIEW OF ITS BIOLOGY, BEHAVIOUR AND AREA-WIDE MANAGEMENT

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SUMMARY

The Chinese citrus fly *Bactrocera minax* (Enderlein) is a major pest of citrus in some Asian countries. It is a univoltine, oligophagous pest, which strictly infests *Citrus* species and varieties, and has an exceptionally long pupal diapause. *B. minax* has great socio-economic importance in China and its neighbouring countries because citrus production is a key fruit industry in these countries. We review the biology and management of this pest with a focus on its distribution, life cycle, diapause, behavioural ecology, and host preferences. We further review potential area-wide integrated pest management (AW-IPM) strategies, including chemical control, but also various eco-friendly, locally developed and adopted techniques applied mainly in China and Bhutan. After years of continuous efforts in AW-IPM of *B. minax*, significant progress has been achieved in suppressing *B. minax* populations to a level of less than 5% infested fruit and a 60-80% reduction in the use of synthetic insecticides against this pest in China.

Key Words: temperate fruit fly, life cycle, behavioural ecology, diapause, *Tetradacus*, Dacinae, oranges, *Citrus*, AW-IPM, China, Bhutan, IPM

1. INTRODUCTION

Citrus fruits rank first across the world in the international fruit trade in terms of value (Liu et al. 2012; Srivastava 2012). The Chinese citrus fly *Bactrocera minax* (Enderlein) (Diptera: Tephritidae) is a major pest of *Citrus* spp. in China, Bhutan, India, Viet Nam and other neighbouring countries (White and Wang 1992; Dong et al. 2014a). It is a univoltine insect with a long pupal diapause that feeds solely on different *Citrus* species and varieties (Chen et al. 2016). The Chinese citrus fly is thought to be endemic to China as its presence could have been recorded in a poem written about 1000 years ago during the Song dynasty:

“The yellow oranges drop to the ground of the garden due to the wind in autumn. When the oranges opened, there were maggots inside the oranges instead of dragon” (Yang et al. 2013).

In the 1940s *B. minax* was only recorded in Guizhou and Sichuan, China (Chen and Wong 1943). Currently, it is reported to occur in the major citrus growing provinces of China (Chongqing, Guangxi, Guizhou, Hubei, Hunan, Shanxi, and Sichuan, see Fig. 1), climatically ranging from temperate to subtropical (Wang and Zhang 2009; Gao et al. 2013).

Figure 1. The occurrence and distribution of *Bactrocera minax* in China (indicated with dark marks showing location within different provinces).

Following a population outbreak in 2008 in south-western China, and the resulting heavy losses incurred by farmers, *B. minax* was removed from the national quarantine plant pest list in 2009 (Announcement No. 1216 of the Ministry of Agriculture of the People's Republic of China). Thus, the current management against *B. minax* in China has shifted from eradication to suppression.

1.1. Nature of Damage and Economic Importance

The adult female fly oviposits its eggs under the peel of green, immature citrus fruits with the aid of its elongated ovipositor (Fig. 2). The eggs hatch when fruit reach mid-level development and the larvae feed on the fruit flesh leading to premature ripening and fruit drop, ultimately resulting in economic damage and yield loss (Allwood et al. 1999; Liu et al. 2015). The larval stage is therefore considered as the most destructive life stage (Dorji et al. 2006). Heavy economic losses of USD 200 million were reported in 2008 due to the above-mentioned outbreak of *B. minax* in Guangyuan, Sichuan Province, China. Almost 1 million tons of oranges were destroyed during this flare-up. This outbreak and heavy infestation resulted in a ban on international trade of citrus commodities from China. The outbreak was reported by “The China Daily” in its headlines to highlight the serious damage to the citrus industry (Liu et al. 2015).

Similarly, *B. minax* is a major pest in the eastern Himalayan kingdom of Bhutan, where mandarin *Citrus reticulata* Blanco is one of the major fruit crops. Crop losses caused by *B. minax* infestation ranging from 35 to 75% are common in mid- and high-altitude orchards (>1100 m), and the fly is considered as one of the major barriers to citrus production (van Schoubroeck 1999; Dorji et al. 2006; Xia et al. 2018).

Figure 2. Female *Bactrocera minax* using its elongated ovipositor to lay its eggs into small green citrus fruit (20–30 mm diameter).

1.2. Taxonomy, Distribution and Host Plants

The Chinese citrus fly was for the first time described in 1920 from specimens collected from Sikkim, India by Enderlein and named *Polistomimetes minax* (Thompson 1998). In 1940, the species was also collected in Sichuan Province, China. Drew (1979) provided a detailed description of the *B. minax* based on the specimens collected in 1920 and placing the species in the subgenus *Bactrocera* (*Polistomimetes*).

Subsequently, a lectotype of *B. minax* was ascribed to the subgenus *Bactrocera* (*Tetradacus*) by White and Wang (1992), who also recorded that *Bactrocera citri* Chen should be regarded as a junior synonym of *B. minax*. The fly is currently placed in the subgenus *Bactrocera* (*Tetradacus*), a small monophyletic clade evolutionary basal to all other *Bactrocera* species (Krosch et al. 2012).

Currently, *B. minax* is regarded as present in Bhutan, China, India (West Bengal and Sikkim), Nepal, and Viet Nam (Dorji et al. 2006; Drew et al. 2006). The host range of Chinese citrus fly is almost exclusively restricted to *Citrus* species and varieties. It has been recorded in citron *Citrus medica* L., lemon *Citrus limon* (L.) Burman f., meiwa kumquat *Fortunella crassifolia* Swingle, pummelo *Citrus maxima* (Burm.) Merr., sour orange *Citrus aurantium* L., sweet orange *Citrus sinensis* (L.) Osbeck, tangerine *Citrus reticulata* Blanco, navel orange *Citrus sinensis* Osb. var. *brasiliensis* Tanaka, pyunkyul *Citrus tangerina* Hort. ex Tanaka, grapefruit *Citrus paradisi* Macfad., and trifoliolate orange *Poncirus trifoliata* L. (Nath 1972; Chao and Ming 1986; Liu et al. 2014). Among these, the preferred citrus host plant is sweet orange (Liu et al. 2014).

2. BIOLOGY AND LIFE CYCLE

2.1. Seasonal Phenology

The phenology of *B. minax*'s life cycle may vary subtly depending on local climate conditions. However, the general pattern appears quite fixed. Based on the population of *B. minax* in Guizhou, China and in Bhutan, we summarize the life cycle of *B. minax* as follows:

1. The female oviposits eggs in small unripe fruit from mid-June to mid-July. Usually it takes two months for the eggs to hatch, much longer than other species in the *Bactrocera* genus; the eggs hatch in late August, after which the larvae go through three larval instars.
2. The larval stage lasts until the end of October, which is then followed by a pupal stage.
3. To survive the cold winter temperatures, the pupae enter a six-month overwintering diapause.
4. Adult emergence usually begins in May and mating starts about 25 to 30 days after adult emergence (Wang and Luo 1995; Dorji et al. 2006).

2.2. Eggs of *B. minax*

The egg of *B. minax* is milky white, oblong and curved in shape, slightly pointed at one end and round at the other. It has a length of 1.1 to 1.5 mm and a maximum width of 0.2 to 0.4 mm (Sun 1961). Female *B. minax* lay eggs in clutches. Usually, each female oviposits about 14 to 17 eggs per oviposition event, with a maximum of 35 eggs per clutch. During its lifetime, a female can produce more than 100 eggs (Zhang 1989). After depositing eggs, the fruit surface is covered with juice around the oviposition wound. In the beginning, this juice is transparent to translucent for one to three days, then it gradually becomes yellow. The oviposition site bulges out, cracks and fruit skin around the oviposition site turns yellow to crimson-purple (Wang and Zhang 1993). Egg hatch starts in July and reaches its peak in late August (Xiong et al. 2016).

2.3. Larvae of *B. minax*

After eclosion, the larva feeds internally on the citrus flesh. The mature larva is milky white or pale yellow, 15 to 18 mm long, conical in shape and nearly transparent at one end. The mouth is equipped with sclerotized mouth hooks, and the body has 11 segments. The larval stage lasts 52 to 72 days depending on temperature, with an average of 63 days (Lu et al. 1997). Young larvae usually feed in a small group on a single fruit segment, later spreading to other segments: the average number of larvae in an infected fruit is 9.5 (Zhang 1989). Larval infestation leads to premature fruit-fall from October to November (Liu et al. 2015).

The larvae stay within the fruit for about 18-52 days after fruit drop. Such a long pre-pupal period is very unusual among the Dacinae and it suggests that long larval development occurs after fruit drop (Dorji et al. 2006). A mature, third instar larva usually leaves the fruit in the early morning and pupates within a day. Pupation starts in late October and reaches a peak in early to mid-November (Dong et al. 2013; Chen et al. 2016).

2.4. Pupae of *B. minax*

The mature third instar larva pupates in the soil at 3 to 5 cm depth (Zhang 1989; Dorji et al. 2006). The long overwintering pupal diapause in *B. minax* is highly unusual within the genus *Bactrocera* and is considered as an adaptive strategy to survive the cold winter periods which occur in its native range (Fan et al. 1994). In Yichang city, where *B. minax* is present, the mean winter temperature usually ranges from 5 to 15 °C (Dong et al. 2013). The overwintering pupal phase lasts for 160-170 days, with the emergence of adults synchronised with the early fruiting season of citrus (Wang and Luo 1995). The pupa of *B. minax* is 9 to 10 mm long with a diameter of 4 mm, weight average of 77.5 mg. It is oval in shape and yellow-brown in colour. Prior to adult emergence, the pupal case becomes slightly dark brown (Zhang 1989; Wang and Luo 1995).

2.5. *Diapause Termination in B. minax*

Research on the development of efficient and sustainable *B. minax* management techniques is still very difficult because the fly has only one generation per year, accompanied by six months of pupal diapause (White and Wang 1992; van Schoubroeck 1999). This bottleneck made the mass-production of this fly very difficult, thereby limiting research capability and its potential use in area-wide integrated pest management (AW-IPM) programmes that have a Sterile Insect Technique (SIT) component (Lü et al. 2014). Therefore, options to break the diapause are considered essential for research and the development of the SIT package for this fly (see Section 4.2.3).

Pupal diapause is usually an evolved response in univoltine temperate tephritids in order to survive harsh environmental conditions and seasonal periods of host scarcity (Teixeira and Polavarapu 2001, 2005; Ragland et al. 2009; Papanastasiou et al. 2011; Moraiti et al. 2012). In *B. minax* pupal diapause is a vital strategy to tolerate cold stress and face the seasonal adversity. Research into the underlying mechanisms in terms of diapause termination, such as major cellular shifts, protein processing, differentially expressed genes and pathways, are still ongoing (Lü et al. 2014; Dong et al. 2014a; Wang et al. 2016, 2017).

Pupal diapause in *B. minax* is influenced by both chilling temperature and duration. A higher chilling temperature, coupled with longer chilling duration, results in a shorter pupal developmental time and improves the synchronisation of adult emergence (Dong et al. 2013). Apart from providing this chilling exposure, hormonal application of 20-hydroxyecdysone (20E) is considered a faster and more efficient method to break pupal diapause in *B. minax* (Dong et al. 2014a; Wang et al. 2014; Chen et al. 2016). Either injection or topical application of 20E can trigger a rapid termination of the pupal diapause in *B. minax* and the morphological changes are observed within 1 week at 22°C. On the tenth day after 20E treatment, the head, thorax and abdomen of the insect can clearly be distinguished, and the colour of body and eyes are milky (Chen et al. 2016). The 20E early-response genes, including *ecr*, *broad* and *foxo*, are up-regulated within 72 h of 20E exposure, indicating these genes are involved in diapause termination processes and pupal metamorphosis (Chen et al. 2016).

The gene sets involved in protein and energy metabolisms vary throughout early-, late- and post-diapause insects in response to cold stress. When diapause is terminated by 20E, many genes involved in ribosome and metabolic pathways are differentially expressed, which may mediate diapause transition (Dong et al. 2014a). The variation of transcriptomic and metabolomic profiles of pupae at five stages (pre-, early-, middle-, late-, and post-diapause) suggests major shifts in metabolism and signal transduction, as well as changes in the endocrine and digestive systems. Nine metabolites significantly contribute to the variation in the metabolomic profiles, especially proline and trehalose, which are well-known cryoprotective agents (Wang et al. 2017).

2.6. Adults of *B. minax*

The adult fly is 10 to 13.2 mm long, not including the female ovipositor, with a wingspan of ~10.8 mm (Drew 1979). The female possesses a long ovipositor of approximately 6.5 mm. The flies are of a brownish colour with yellow markings, the wings have a dark band along the outer margin, and the general appearance is wasp-like (Chen and Xie 1955). A morphological description of the adult is provided in Drew et al. (2007), who also note that the fly is probably the largest of all *Bactrocera* species (Fig. 2).

3. BEHAVIOUR AND ECOLOGY

Since the Chinese citrus fly is not attracted to methyl eugenol or cue-lure as are many species of the genus *Bactrocera*, thorough behavioural and ecological studies have been carried out over the past years in order to develop effective monitoring and control strategies in the long run.

3.1. Feeding Behaviour

It is well known that for most studied tephritid species, both males and females are anautogenous and forage for sugar and protein to fuel metabolic activities and to meet reproductive requirements (Aluja and Norrbom 1999; Drew and Yuval 2000; Taylor et al. 2013). Females need a protein diet for vitellogenesis and ovarian development (Harwood et al. 2015), while males feed on protein to reach sexual maturity leading to copulation which is crucial in achieving reproduction (Lushchak et al. 2013). Adult *B. minax* forage on non-host plants for honeydew, nectar, sooty mould and fruit juices to meet their dietary requirements during sexual maturation. The flies then shift to licking sooty moulds, bird faeces and, to a lesser extent, an unknown substance (probably leaf phylloplane bacteria and plant leachates) on citrus leaves and fruits during the mating and oviposition period (Hendrichs et al. 1993; Dong et al. 2014b).

3.2. Mating Behaviour

Male aggression and territoriality have been reported as a typical behaviour in some *Bactrocera* species (Shelly 1999; Weldon 2005; Benelli et al. 2014, 2015). For most tropical polyphagous *Bactrocera* species, males aggregate at a common place on foliage to attract and court females for mating, i.e. a non-resource-based lek mating system (Emlen and Oring 1977; Maan and Seehausen 2011). However, in *B. minax*, as in many temperate and oligophagous tephritids, male courtship behaviour is absent and the mating system is a resource-based defence polygyny, consisting of two phases: 1) males defend a resource (host fruit) (intrasexual selection), 2) where copulation takes place (intersexual selection) (Opp et al. 1996). In the wild, all mating events take place on citrus fruit (Dong et al. 2014b). Territory formation and copulation usually occurs on immature green fruits. It has been suggested that male flies that try to copulate in the vicinity of the ovipositional site have more chances to encounter and court receptive females (Prokopy 1976; Smith and Prokopy 1980).

Mating behaviour in *B. minax* is closely synchronised with the host fruiting season and has been described in the field as follows: (i) the male establishes its territory close to a potential oviposition substrate (citrus fruit); (ii) the female lands on the fruit and begins inspection and ovipositor probing on the fruit surface; and (iii), the male mounts and copulates with the female (Dong et al. 2014b). In this mating system females face trade-offs associated with the cost of additional, apparently unneeded matings on each fruit in return for access to resources. Like in the case of *Rhagoletis* species the resource is assumed to be the oviposition site (Opp et al. 1996; Opp and Prokopy 2000; Prokopy and Papaj 2000).

3.3. Oviposition and Host Preference

In tephritids, fruit flies use different cues for host finding behaviour and egg-laying behaviour. Usually, long-distance volatile chemicals are important before landing on host trees, visual stimuli act as short range once on the tree and contact chemicals on and inside host fruit influence female egg-laying decision.

Bactrocera minax oviposits solely into citrus fruits (Family *Rutaceae*) (Wang and Luo 1995; Dong et al. 2013). It is a large, powerful insect with a long ovipositor adapted for piercing through the thick skin of young, green citrus fruit (Liu and Zhou 2016). Visual cues including fruit shape, colour, and size are important for host finding (Prokopy and Owens 1983; Piñero et al. 2017). On the other hand, the egg-laying behaviour is greatly influenced by chemical stimuli, for example, semiochemicals, sugar content, levels of secondary plant compounds and physical properties of fruit (Bush 1969). The preference of *B. minax* oviposition on different citrus varieties is as follows, *Citrus sinensis* cv. Navel and *C. aurantium* > *C. sinensis* cv. Bintang, Amakusa and *C. reticulata* cv. Satsuma > *C. maxima* cv. Shatian > *C. reticulata* cv. Ponka. This ovipositional preference is positively correlated with larval survival and development; while in the field greater egg-laying occurs on those citrus fruits which are close to the surrounding vegetation and trees (Liu et al. 2014).

3.3.1. Visual Cues (Colour, Shape, and Size) for Oviposition

The hardness of a citrus fruit peel has an impact on the female insect's decision to oviposit (Lin et al. 2011). On the basis of egg oviposition marks on citrus fruit, it appears that *B. minax* significantly prefers to oviposit on the distal hemisphere rather than the basal hemisphere (Liu and Zhou 2016). Apart from peel hardness, the ovipositional behaviour of tephritid female flies is also influenced by fruit colour and shape (Alyokhin et al. 2000). It has been shown that tephritid flies respond to fruit-mimics of the same colour or reflecting similar levels of light than host fruit of a particular fly species (Aluja and Norrbom 1999). For example, the Queensland fruit fly *Bactrocera tryoni* (Froggatt) showed attraction to spheres painted with cobalt blue pigments, which reflected the same UV spectrum as favoured blue-coloured host fruits occurring in its native rainforest environment (Drew et al. 2003). The oriental fruit fly *Bactrocera dorsalis* (Hendel) is attracted to white-yellow colour (Vargas et al. 1991) and the apple maggot *Rhagoletis pomonella* (Walsh), a pest of apples, is attracted to fruit-mimicking traps such as a red sphere (Duan and Prokopy 1995).

Both sexes of *B. minax* are attracted to orange or yellow/green spheres of 50 mm diameter (Drew et al. 2006). However, we found that *B. minax* adults prefer green over other colours, and this preference is significantly increased in sexually mature flies over immature flies (author submitted results).

3.3.2. Chemical Cues (Semiochemicals) for Oviposition

Chemical cues play an important role in foraging and oviposition of fruit flies (Sarles et al. 2015), and these chemicals are widely exploited in integrated pest management (Shrivastava et al. 2010). For example, the application of oviposition marking pheromone reduced *Rhagoletis cerasi* L. infestation up to 100% in cherry orchards (Katsoyannos and Boller 1976; Boller and Hurther 1998). Together with visual cues, semiochemical cues may also influence host finding and egg-laying of *B. minax*. The peel odours of different varieties of orange preferred by *B. minax* produce different volatile blends, including acids, aldehydes, alcohols, and oils. It is presumed that these volatile compounds directly influence the olfactory orientation of *B. minax* females. However, there is as yet no proof if these volatiles released by host plants have a direct impact on the oviposition preference of *B. minax* (Liu and Zhou 2016).

From the perspective of the biology, ecology and behaviour, *B. minax* is more reminiscent of flies in the temperate genus *Rhagoletis* than other pest species in the *Bactrocera* genus. Therefore, some components of pest management can be drawn from the extensive scientific literature on apple maggot fly *R. pomonella*, European cherry fruit fly *Rhagoletis cerasi*, and other fruit flies (Vargas et al. 2016). For example, the application of fruit volatiles in conjunction with visual traps may yield good results for the control of *B. minax*.

4. TOWARDS THE AREA-WIDE MANAGEMENT OF *B. MINAX*

Several different control tactics have been used to manage populations of the Chinese citrus fly. These include chemical control and “attract and kill” techniques using protein/food baits and fruit-mimicking traps. In addition, pilot trials of the Sterile Insect Technique (SIT) have been assessed for *B. minax* control (Wang et al. 1990; Wang and Luo 1995), and farmers in China and Bhutan have adopted locally developed suppression techniques.

4.1. Use of Chemicals

Pesticide applications (cover sprays) are the most commonly used conventional control practices against insects pests, especially in the case of outbreaks. Though they are effective in reducing the losses caused by fruit fly infestation, the negative impacts of pesticides on humans, the environment and non-target organisms have raised much concern. Different insecticides have been used to suppress *B. minax* populations, including phoxim, dichlorvos, chlorpyrifos, abamectin, botanically derived pesticides, and pyrethroids. Amongst these, abamectin and dichlorvos proved to have the highest and lowest toxicity, respectively. However, chlorpyrifos had the strongest effect on pupae, and phoxim had the strongest influence on emergence.

These chemicals are not recommended due to toxicity to non-target organisms and long residual effects, but they are effective against *B. minax* (Liu et al. 2015).

4.2. *Eco-friendly Management*

4.2.1. *Field Sanitation*

Field sanitation is an effective and important strategy to reduce *B. minax* populations for the next fruiting season. The collection of infested fruits from the ground every week from mid-September to late-November is essential to remove the breeding population from orchards. The protocol demands that these infested fruits are transferred into thick plastic bags (20-25 kg per bag), that can be supplemented with aluminium phosphide to facilitate the killing of larvae. However, this is not critical if the bags are kept in the field under the sun for 7~10 days. Finally, the rotting fruits serve as fertilizer. The plastic bags can be recycled and used again (Liu et al. 2011; Li et al. 2013).

In Bhutan, cultural practices such as the application of soil tillage, along with natural predation (pupae picking by birds), seem to have a role in reducing the number of pupae. However, this reduction is not significant. Thus, it is not recommended as the only control measure for reducing the overall *B. minax* population in the wild (Dorji et al. 2010).

4.2.2. *Protein and Food Baits*

Spraying a mixture of protein bait, with a small quantity of insecticide added, has proven to be an effective strategy for large-scale control of fruit fly populations (Conway and Forrester 2011). Bait sprays are effective for fruit fly population control as newly emerged females require protein to become sexually mature (Perez-Staples et al. 2007; McQuate 2009). In China, it is a widely accepted approach for the farmers to use vinegar, sugar and wine mixtures, plus detergent, as baits station/spots spray for the control of *B. minax*, which is simple and cheap (Zhou et al. 2012). Attractants such as GF-120, and other locally available commercial products, are also used for the suppression of the Chinese citrus fly. Fresh enzymatically-hydrolysed beer yeast (H-protein) liquid protein bait effectively attracted and killed more *B. minax* flies than GF-120 sprayed in the field (Zhou et al. 2012).

4.2.3. *Use of Fruit-Mimicking Traps*

Usually semiochemicals and plant derived volatiles are used to trap fruit flies (Díaz-Fleischer et al. 2014), but the males of *B. minax* are not attracted to either of the standard *Bactrocera* male lures (i.e. methyl eugenol and cue-lure) (Drew et al. 2006). Visual traps have been used as an alternative, and in Bhutan, both sexes of the fly were most attracted to green-yellow or orange fruit-mimicking spheres in the field (Drew et al. 2006). In recent years, a specific fruit-mimicking trap (spherical green sticky trap) has been developed and widely applied to monitor and control *B. minax* in China. After field deployment of spherical traps in sweet orange orchards in Zigui, China, the infested fruit rate dropped to 2.7% compared with 28.6% in untreated control orchards (Yi et al. 2015). Efficiency of the control effort was closely

associated with appropriate trap deployment density and time (Chen et al. 2017). Considering the cost of commercial traps, as well as efficacy, spherical green sticky traps with a diameter of 7 cm were recommended at a deployment rate of 20–30 traps per 1000 m² in citrus orchards (Chen et al. 2017; Gong et al. 2017).

4.2.4. Sterile Insect Technique (SIT)

The SIT has been successfully used to manage fruit flies including *Ceratitidis capitata* (Wiederman), *B. dorsalis*, the melon fly *Zeugodacus cucurbitae* (Coquillett) and the Mexican fruit fly *Anastrepha ludens* (Loew) (Calkins et al. 1994; Koyama et al. 2004; Dhillon et al. 2005; Enkerlin et al. 2017). In AW-IPM programmes that have an SIT component, the production of sterile insects in large numbers is of paramount importance (Enkerlin 2021). Sterilisation can be achieved by irradiation or genetic manipulation. Irradiating *B. minax* pupa two days before emergence with a dose of 90 Gy is recommended to ensure adult sterility (Zhang and Li 1990).

Due to the serious damage in major citrus production regions in China, a mass-release of sterilized *B. minax* flies was carried out in Guizhou, China in the late 1980s and early 1990s. In these pilot projects, mature 3rd instar larvae were collected in fruit from the field and then allowed to pupate in the lab (Wang et al. 1990; Wang and Luo 1995). Although the SIT trials in Guizhou Province resulted in a significant reduction of the pest population (Wang et al. 1990; Wang and Luo 1995), the technology is so far not integrated into *B. minax* control programmes in China. This is mainly related to problems with the development of mass-rearing methods due to technical barriers such as the extreme long pupal diapause period. This is the reason that there is currently no foreseeable plan for an SIT approach against *B. minax*, although the selection of non-diapausing strains is being explored.

4.2.5. Natural Enemies

Knowledge on the parasitoids associated with the Chinese citrus fly remains very scarce. Only one parasitoid, *Diachasmimorpha feijeni* van Achterberg, has been associated with this fly in Bhutan (van Achterberg 1999) and in China (authors' unpublished data). However, there is still no detailed information available on the parasitoid's interactions with Chinese citrus fly or the potential for parasitoid manipulation.

4.2.6. From Pilot Trials to Area-Wide Management in China

Since 2009, the Ministry of Agriculture and National Agro-Tech Extension and Service Center of China has promoted and organized annual nation-wide conferences and training courses on tephritid control for local technicians and farmers, with the aim to educate and transfer new developments and technologies. Over the years, pilot trials of AW-IPM approaches against *B. minax* have been implemented in the provinces of Guizhou, Hubei, Hunan, Shanxi, and Sichuan where *B. minax* is a serious pest. The validated demonstration practices in the main citrus producing regions led to the establishment of a *B. minax* AW-IPM programme in China.

In the beginning of every year, the Ministry of Agriculture announces and issues online management programmes for *B. minax* control, along with that for other major agricultural pests, to facilitate the sustainable management of the pests. In the case of *B. minax*, the integrated environmental-friendly measures can be summarized as follows:

- First, population monitoring in April using field traps and pupal emergence cages, aimed at accurately identifying the timing of pupal developmental state and adult emergence, is carried out by local plant protection stations annually: this is critical to guide the implementation of control practices.
- Second, “attract and kill” strategies are applied from May to July including the systematic use of spherical green sticky traps, protein bait sprays and sugar-vinegar-wine liquid in bait stations or spot sprays.
- Third, field sanitation of habitats/orchards by removing the fallen and infested fruits and weeds from September to November has proved to be an important and effective population control measure. Recyclable plastic bags are widely adopted to keep-and-kill the mature larvae by a combination of hypoxia and heat.

Thus, through years of continuous efforts in AW-IPM of *B. minax*, significant progress has been achieved in suppressing *B. minax* populations to a level resulting in less than 5% of infested commercial fruit and a reduction in the use of synthetic pesticides by 60-80%. The *B. minax* AW-IPM programme will now be complying with national standards for ‘green control’, which results in notable direct and indirect economic, ecological and social benefits for China.

5. FUTURE PERSPECTIVES

With increased understanding of the biology and behaviour of *B. minax*, effective operational AW-IPM strategies against this pest have been established in China and Bhutan. However, SIT and natural enemies have not yet been exploited due to various biological and physiological obstacles. Great efforts are required to overcome these gaps for the future sustainable management of *B. minax*:

1. Mass-rearing of *B. minax* still remains a big challenge considering its univoltine and oligophagous traits. Future work should be focused on improving (a) understanding of egg hatch and (b) artificial diet formulation for the newly hatched young larvae.

2. Little information has been published on the insect-plant interactions of *B. minax*. It is widely known that the adults have a close relationship with *Citrus* spp., but how these adults utilize visual, olfactory and tactile cues to orientate to host plants for mating and oviposition has received very little attention.

3. Symbiotic organisms, including *Wolbachia* (Stouthamer et al. 1999), that affect biology and reproduction of *B. minax* should be characterized. A thorough screening of microorganisms by culture-dependent and high-throughput technology, in combination with related functional studies, will help to better understand the complex relationship between symbionts and *B. minax*. Such knowledge may lead to potential development and application of the Incompatible Insect Technique (IIT) (Zabalou et al. 2004).

4. Natural enemies have not been used as a component of AW-IPM against *B. minax* most likely because very little knowledge is available about them and their effectiveness. The possible synchronised diapause of parasitoids and *B. minax* pupae needs to be investigated. In addition, other agents such as predators or fungi causing pupal mortality deserve to be further investigated.

5. There are currently no effective semiochemical or plant derived volatile lures to attract *B. minax* males or females available. The volatile chemicals from the host fruits, as well as a sex attractant for monitoring and mass-trapping the Chinese citrus fly, urgently needs to be identified and exploited.

6. The growing published online resources on transcriptome, proteome, and genome of *B. minax*, RNA interference and CRISPR-Cas9 technologies targeting specific gene functions, will facilitate further investigations of molecular mechanisms responsible for the biology, behaviour, physiology and evolution of the Chinese citrus fly. The comprehensive understanding of *B. minax* is the most promising way to develop sustainable management of this economically important citrus pest in the long run.

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