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Mass rearing and release of *Trichogramma* for biological control of insect pests of corn in China

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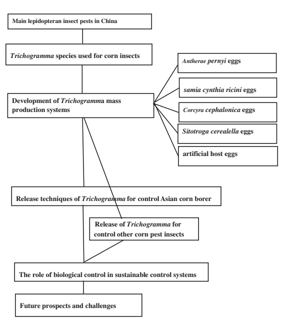
HIGHLIGHTS

- *Trichogramma* species are being used for corn pests in China.
- Technology for mass-rearing of *Trichogramma* has advanced.
- Release techniques for *Trichogramma* in China are described.
- *Trichogramma* release offers ecological and economic benefits in China.
- Future prospects and challenges for *Trichogramma* technology.

GRAPHICAL ABSTRACT



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ABSTRACT

Corn (*Zea mays* L., Poaceae) is ranked first as food crop in planting area and in total yield production in China. Asian corn borer, *Ostrinia furnacalis*, is the most destructive pest of corn in China, causing 6–9 million tons of yield loss per year on average. *Trichogramma* has been released for control of Asian corn borer at large scale since the 1970’s, partly triggered by the fact that *Trichogramma dendrolimi* can be successfully mass reared on eggs of the Chinese oak silkworm, *Antheraea pernyi*. Eggs of different hosts, such as Eri-silkworm, *Samia cynthia ricini*, *A. pernyi*, the Rice moth, *Corcyra cephalonica*, the Angoumois grain moth, *Sitotroga cerealella* and also artificial host eggs were tested and successfully used to mass-rear various *Trichogramma* species in China since then. The mass production technology and release technique of *Trichogramma* have been greatly improved in recent years making *Trichogramma* production and field application more practical and cost efficient. Nowadays, nearly 4 million hectares of corn are treated with *T. dendrolimi, Trichogramma chilonis* and *Trichogramma ostriniae* annually, mainly in North-east China. Large ecological and economic benefits have been achieved in areas where *Trichogramma* have been released continuously for many years. This includes an increase of natural populations of *Trichogramma* and other natural enemies in cornfields, the avoidance of any insecticide treatments in corn, a reduction in mycotoxin contamination and overall higher yields. The release of *Trichogramma* for controlling Asian corn borer and other lepidopteran pests became one of the key measures in corn IPM in China. *Trichogramma* applications combined with other non-chemical control measures for corn insect pests IPM began a new era in China as the new concept of “Public Plant Protection, Green Plant Protection” was put forward in 2006 as the guideline for plant protection in China. The future prospects and challenges of *Trichogramma* application are also discussed in this review.

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1. Introduction

Corn (*Zea mays* L., Poaceae) plays a very important role in grain production in China making it the second largest corn production country world-wide. Among the grain crops grown in China, corn ranks first in planting area and the total yield. In the year 2008, corn planting area in China was 29.9 million ha, total yield was 165.9 million ton, and the average yield was 5.56 ton/ha (Editoral Board of China Agriculture Yearbook, 2009). There are 6 corn planting regions in China, including the North Spring Corn Region, Huang-Huai-Hai Summer Corn Region and Southwest Hilly Corn Region which together make up for the Corn Belt of China.

In the North Spring Corn Region, corn is planted on about 12.9 million ha, which makes up 45% of the total corn planting area and also about 45% of the total grain yield of corn in China. Huang-Huai-Hai Summer Corn Region ranks second with 10 million ha of corn planted in the region, occupying about 37% of total corn planting area and about 36% of total grain yield in China (Li and Wang, 2010).

The Asian corn borer, *Ostrinia furnacalis* (Guenée), 1854 (Lepidoptera: Crambidae), is the most serious insect pest of corn that occurs in most corn-growing areas in China (All China Corn Borer Research Group, 1988). Annual loss due to this insect ranges from 6 to 9 million tons per year (He et al., 2003). The direct yield losses arise from *O. furnacalis* damage, whereby the largest impact indirectly arise from larval feeding on silks and kernels that leads to ear rot, increasing mycotoxin contamination and reducing grain quality (Song et al., 2009). Other lepidopteran pests of concern for corn in China include *Helicoverpa armigera* (Hübner), 1804 (Lepidoptera: Noctuidae) and *Conogethes punctiferalis* (Guenée), 1854 (Lepidoptera: Crambidae). The problems with *H. armigera* and *C. punctiferalis* in corn appear to be increasing as cropping systems from the 1990s have been changed. For instance the more frequent use of no-till farming results in increased survival of *H. armigera* pupae in the soil during hibernation (Wang et al., 2002a, 2006).

Parasitoid wasps of genus *Trichogramma* (Hymenoptera: Trichogrammatidae) includes several species that are frequently used as biological control agents worldwide (Consoli et al., 2010). *Trichogramma* spp. lay their eggs inside the eggs of insect pests (Consoli et al., 2010). In China, a comprehensive study of utilization of the egg parasitoid *Trichogramma* for controlling the Asian corn borer has been conducted starting in the early 1950s in Guangxi province, followed by Shandong province in 1957, and Jiangsu, Heilongjiang, Henan and Guangdong provinces in 1961 (Zhan and Liang, 1999). Different factitious host eggs have been used for rearing *Trichogramma*, such as eggs from Eri-silkworm, *Samia cynthia ricini* (Donovan), 1856 (Lepidoptera: Saturniidae) (Pu et al., 1956), Chinese oak silkworm, *Antheraea pernyi* Guérin-Mèneville, 1855 (Lepidoptera: Saturniidae) (Wang, 2001), Rice moth, *Corcyra cephalonica* Stainton, 1865 (Lepidoptera: Galleriidae) (Qiu et al., 1980), Angoumois grain moth, *Sitotroga cerealella* Olivier, 1789 (Lepidoptera: Gelechiidae) (Jia et al., 2002; Zheng et al., 2003). Artificial host eggs have also been used in rearing *Trichogramma* spp. (Liu et al., 1996). From the 1960s to 1970s, nearly 700,000 ha of Chinese corn fields were treated with *Trichogramma* totally (Gou, 1986). Many small *Trichogramma* production facilities were set up at county or even village levels, especially in Jilin, Liaoning, Hebei, Shandong, Henan, Beijing and Shanxi Provinces. Farmers were involved in *Trichogramma* rearing and releasing during the People's commune times in China.

In the early 1980s, this *Trichogramma* biological control program was affected by the transfer of government farmlands to farmers, which led to a rapid decrease in areas in which *Trichogramma* was released to control crop pests. In reaction to this, the Chinese government has funded National IPM Technique Research Projects as one of the State Key Research Programs in five successive 5 year plans. Biological Control Technique Research has been one of the research projects since the late 1980s. There have been improvements in processes and techniques for mass production and release of *Trichogramma*, especially for the Asian corn borer in recent years. *Trichogramma* release for Asian corn borer control has been become one of the key IPM components for corn, especially in North Spring Corn Region (Wang et al., 2003). The application of *Trichogramma* for Asian corn borer and other insect pests has been enhanced as part of the new concept of "Public Plant Protection, Green Plant Protection" that serves as a guideline for the management of crop pests in China since 2006.

In this paper, the mass rearing and release techniques for *Trichogramma* to control the corn insect pests, and the benefits from the *Trichogramma* releases in China are reviewed. The future and challenges of *Trichogramma* applications against corn insect pests in China are also discussed.

2. *Trichogramma* species used for corn insect pests control in China

There are 12 *Trichogramma* species that were identified from parasitized Asian corn borer eggs throughout most parts of corn growing areas in China. Among them, *Trichogramma dendrolimi* Matsumura, 1926, *Trichogramma chilonis* Ishii, 1941, *Trichogramma ostriniae* Pang et Chen, 1974, *Trichogramma evanescens* Westwood, 1833 are distributed throughout the country, while *Trichogramma leucaniae* Pang et Chen, 1974, *Trichogramma poliae* Nagaraja, 1973, *Trichogramma closterae* Pang et Chen, 1974, *Trichogramma pintoi* Voegele, 1982, *Trichogramma ivelae* Pang et Chen, 1974, *Trichogramma exiguum* Pinto and Platner, 1978, *Trichogramma forcipiformis* Zhang and Wang, 1982, *Trichogramma tielingensis* Zhang and Wang, 1982 are distributed in only some regions. *T. ostriniae* is the predominant species parasitizing the Asian corn borer eggs in most corn growing regions of China, comprising from 72.2% to 100% of all the *Trichogramma* samples collected in corn field in Beijing, Hebei and Shanxi provinces. However, *T. dendrolimi* comprises 97.3%, 28.9% and 45.1% of the total samples of *Trichogramma* found in corn in Heilongjiang, Jilin, and Liaoning provinces in Northeast China, respectively, and *T. chilonis* comprises 88.9% of all *Trichogramma* samples found in corn in Guizhou province in Southwest China (Zhang et al., 1990a). *T. ostriniae* accounts for up to 90% of the total parasitized Asian corn borer eggs by *Trichogramma* spp. in Beijing (Zhang, 1988).

O. furnacalis egg is not a preferred host of *T. dendrolimi* and laboratory studies showed that the capacity of *T. dendrolimi* to parasitize eggs of *O. furnacalis* were affected by the rearing host species and host age (Zhang, 1988; Zhang et al., 1995; Li et al., 2002). Wasps reared from eggs of *A. pernyi* showed the parasitic capacity on eggs of *O. furnacalis* on average twice as high as that of the wasps reared from eggs of *C. cephalonica*. When the age of *O. furnacalis* eggs increased from 0–6 h to 18–24 h at 26 °C, the number of *T. dendrolimi* wasps that successfully parasitized host eggs and the number of host eggs parasitized per wasp decreased by more than 50%. The number of *O. furnacalis* eggs parasitized per female *T. dendrolimi* reached 22.9 during a 24 h period. Despite *O. furnacalis* is a less preferred host, these data suggest that *T. dendrolimi* has the potential to achieve sufficient parasitism on *O. furnacalis* eggs in corn fields under suitable conditions, and could be used practically in the biological control of this pest (Liu et al., 1998a,b). Interestingly, it appears as if a comparatively good strain of *T. dendrolimi* has been chosen from parasitized Asian corn borer eggs and parasitism rates are good in the field (Zhang et al., 2004). At similar release rates, however, other *Trichogramma* species show higher efficacy than *T. dendrolimi* to control the Asian corn borer (Tan, 1999; Feng et al., 1999; Wu et al., 2001; Guo et al., 2005).

For instance, the parasitism of the Asian corn borer eggs ranges between 43.8% and 51.7% with the release of *T. chilonis* at 200,000 and 300,000 wasps/ha, whereas the parasitism is 34.9% and 35.7% with release of *T. dendrolimi* at the same wasp densities (Xu et al., 2001).

T. ostriniae is the dominant species attacking the Asian corn borer eggs in most corn producing areas of China (Zhang et al., 1990a) and has been shown to more effectively control the Asian corn borer compared to *T. dendrolimi* (Zhang, 1988). However, the application of *T. ostriniae* is practically limited because it is mass reared on eggs of either *C. cephalonica* or *S. cerealella* and production efficiency is comparatively low (Feng, 1996). In contrast, the *T. dendrolimi* production is based on mass rearing of *A. pernyi* eggs or even artificial host eggs as hosts, resulting in more cost efficient production in North-east China compared to southern parts of China where the *A. pernyi* needs to be purchased from North-east China every year for *Trichogramma* production. Consequently, *T. dendrolimi* is widely used in the Northeast spring corn region where *A. pernyi* eggs are readily available. Also *T. chilonis* can be mass reared on *A. pernyi* eggs and artificial host eggs, and is released against the Asian corn borer in some areas (Feng et al., 1999; Xu et al., 2001).

3. Development of *Trichogramma* mass production systems

In this section we aim to summarize the available knowledge on the production of *Trichogramma* on four different rearing hosts and artificial eggs, including the different advantages and disadvantages of these systems. The focus on the hosts arise from the fact that only minor differences exist in the last step of *Trichogramma* production thus the host rearing is usually the most crucial step that decides on cost efficiency and feasibility of *Trichogramma* production.

3.1. Mass production of Trichogramma using A. pernyi eggs

In North-eastern China, *A. pernyi* is traditionally mass-reared directly in the woods for silk production. This insect is reared on oak tree as sideline occupation by people living in forest regions. Shandong Academy of Agricultural Sciences successfully produced *T. dendrolimi* on *A. pernyi* in the 1960s (Wang, 2001). Pu (1979) documented the methods for *Trichogramma* mass production, which later spread to other areas in China. As the eggs of *A. pernyi* were used as host for mass rearing *Trichogramma* in 1970s, research and application of *Trichogramma* have expanded and this production system has been widely used in biological control not only for agricultural pests such as the Asian corn borer but also forest insect pests such as the Masson pine caterpillar, *Dendrolimus punctatus*, Walker, 1855 (Lepidoptera: Lasiocampidae) in pine forests and plantations (Li, 1982; Gou, 1986; Wang et al., 1998).

The cocoons of *A. pernyi* are harvested in autumn and transported to biological control stations throughout the country, and then stored in cool room for *Trichogramma* mass rearing in the following year. The cocoons of *A. pernyi* can be stored under -5 °C for 5 months. In the early summer of the following year, the cocoons are hung in the emerging room for incubation before mass rearing begins. Eggs squeezed from abdomens of female moths are better for parasitization of *Trichogramma* than naturally laid eggs. These eggs are obtained by squeezing female moth abdomen 1 or 2 days after the moth emergence and each female moth can produce around 200 eggs. Thereafter, eggs are washed and dried, then the matured eggs of *A. pernyi* are used for *Trichogramma* production (Liu et al., 2000).

Due to their large size, between 50 and 260 *T. dendrolimi* or *T. chilonis* wasps emerge from each silkworm egg. However, if too many *Trichogramma* adults emerge from a single egg, the female body size and the reproductive capacity decrease, male ratio will be high, and adult longevity is shortened. Therefore, it is very important to adjust the ratio female wasps to host eggs, as well the exposure time to avoid super-parasitism and degeneration. It was found by Wang et al. (1998) and Liu et al. (2000) that the optimum number of *Trichogramma* adults to develop in one *A. pernyi* egg is about 60–70, of which usually more than 80% are females. For *A. pernyi* eggs, the optimum ratio between the number of parent female wasps and host eggs is 2:1. The optimum time of exposure is less than 24 h and at these conditions, the parasitism of fresh eggs usually reaches or exceeds 90% (Liu et al., 2000).

Several different production components have been developed, which are basically composed of (1) collection of *Trichogramma* from field as founder population, propagation and conservation; (2) selection of host eggs and their storage, and (3) mass propagation (Piao and Yan, 1996). The selection of female cocoons which will produce host eggs, and the treatment of host eggs are processes that have been mechanized. A set of machines and devices have been designed, which include machine for collecting emerged silkworm moths, host egg washer and dryer, machine for preparing egg cards, inoculation machine for parasitization (Liu et al., 1980, 1991; Song et al., 1994). Equipment for separating healthy parasitized host eggs from unparasitized host eggs has also been developed (Wang et al., 1999). An automatic production line, with the capacity of producing 40 billion *Trichogramma* annually was established in Jilin province in early 1990s (Song et al., 1994).

The procedure for *T. dendrolimi* or *T. chilonis* mass production has been simplified in recent years. *Trichogramma* spp. are reared simply by the method of releasing wasps in a small empty room with paper cards comprising the eggs attached to glass windows or on hanging screens (room-rearing). Sometimes the parasitized host-eggs (before emergence of *Trichogramma*) are mixed with fresh unparasitized host eggs on wooden trays. When the wasps emerge, they parasitize the host egg directly. About 800–1000 million wasps have been produced in such biological stations every day (Liu et al., 2000).

Storage of *A. pernyi* cocoons in a cool room for over 6 months lowers the suitability of eggs derive from them for production of *Trichogramma* and also storing eggs directly for a longer period decreases their suitability for parasitization. This has been a major challenge in providing enough *A. pernyi* eggs to produce adequate numbers of *Trichogramma* for controlling the second generation of Asian corn borer in late of August. Preservation techniques for storing the *A. pernyi* eggs have been studied and it was shown that irradiating *A. pernyi* eggs by electron beam significantly prolonged their viability for the production of *Trichogramma*. When the irradiated eggs stored in a refrigerator at 2 °C for 89 days are used as host, the parasitism rate for *T. dendrolimi* is about 80%. Besides, the emergence rate, average number of wasps produced per host egg, as well as female wasp body size, developmental periods, and sex ratio of offspring are all normal. Irradiated eggs have no significantly adverse effects on behaviour and efficacy of *Trichogramma* in the biological control of crop pests (Ding et al., 1993).

Cold storage of host cocoons and eggs provides a more effective option for commercial rearing, transportation and inundative releases of *Trichogramma* for controlling insect pests on a large scale (Lu et al., 2005). However, storage duration, storage temperature and the developmental stage of *Trichogramma* in the host egg may negatively affect the emergence rate, longevity and the parasitism capacity of *Trichogramma* adults emerging from the host eggs. Recent studies on adult emergence rate, the optimal stage and temperature combination for cold storage of *A. pernyi* eggs parasitized by *T. dendrolimi* found that they can be best stored at 7 °C as long as *Trichogramma* is in the egg stage itself (Geng et al., 2005). When *T. chilonis* parasitize *A. pernyi* eggs for 24 h and the parasitized host eggs are transferred to 13–15 °C for another 24 h, cold storage at 2–5 °C, could not exceed 35 days, without affecting the emergence rate negatively (Tuerxun et al., 2005). Li et al. (2008) found that the optimum stage for *T. chilonis* inside eggs of *A. pernyi* is the mid-larval stage, but eggs could not be stored for more than 40 d without compromising performance of emerging wasps.

If *Trichogramma* can be forced to go to diapause long-term storage may be feasible and this procedure became highly important in mass production and biological control programmes of *Trichogramma*. First attempts indicates that eggs of *A. pernyi* parasitized by *T. dendrolimi* may enter diapause when treated at 8 °C and 10 °C for 30 days at the end of larval stage, but the diapause is not stable (Li et al., 1992). Ma and Chen (2005) investigated the diapause rates of *T. dendrolimi* in *A. pernyi* eggs under 10 alternating temperature regimes combined with 9 different exposure times and found that temperature, exposure time and their interactions have a significant influence on diapause incidence of the parasitoids. A temperature regime of 16 °C for 10 h, and 1, 4, or 7 °C for 14 h per day were the optimum temperatures regimes to induce diapause in *T. dendrolimi* (60–70% going to diapause). Under effective alternating temperature regimes, the diapause incidence increased as overall exposure time increased. However, when exposure time is >28 d, diapause incidence does not increase significantly anymore with exposure time. The sensitive age of *T. dendrolimi* for diapause induction was explored by another experiment. The optimum method to induce diapause in *T. dendrolimi* consists of exposing host eggs for parasitization at 26 °C for 8 h, and then keeping them at 26 °C for 40 h, to finally move them to 10 °C for 4 weeks. Afterwards, the diapausing *T. dendrolimi* may be stored at 1 °C for 1–4 months with high emergence rate after that period (Ma and Chen, 2006).

3.2. Mass production of Trichogramma using S. cynthia ricini eggs

The eggs of *S. cynthia ricini*, were found to be suitable factitious hosts for propagation of *T. dendrolimi* as well as some other species of *Trichogramma* in 1952. It is the first alternative host on which *T. dendrolimi* was successively reared on in China (Pu et al., 1956; Pu, 1979). One *S. cynthia ricini* egg can support the development of as much as 60 *T. dendrolimi* wasps. However, for better nutrition of the larvae of *Trichogramma* and in order to obtain more vigorous wasps, it is necessary to establish a mass rearing process which allows an average of only about 20 larvae developing in one host egg, i.e. to have approximately one *T. dendrolimi* female per host egg in the rearing.

T. ostriniae also parasitizes *S. cynthia ricini* eggs, but the parasitism rate is low for initial generations. The parasitism increases when *T. ostriniae* continuously parasitizes *S. cynthia ricini* eggs as hosts for several generations. About 17 wasps emerge per egg with 61% emergence rate at the first generation, rising to 24 wasps per egg with 71% emergence rate at the sixth generation. *T. ostriniae* produced on *S. cynthia ricini* eggs can be used successfully for controlling the Asian corn borer (Wang et al., 1982). The *S. cynthia ricini* eggs stored in ice for 6 months by quick freezing method remain viable for reproduction of *Trichogramma*. *Trichogramma* larvae in parasitized *S. cynthia ricini* eggs kept in 3–5 °C for 50 days develop normally at room temperature.

T. dendrolimi and *T. ostriniae* were both mass reared on eggs of *S. cynthia ricini* and egg cards combining both species applied to corn fields. These releases showed good control effects on Asian corn borer when released against the first, second and third generation of Asian corn borer (Feng et al., 1977; Shen et al., 1986). As the *S. cynthia ricini* has not been mass reared since the early 1980s with the reform of rural economy system, it is not used as a host for *Trichogramma* mass rearing in China anymore.

3.3. Mass production of Trichogramma using C. cephalonica eggs

C. cephalonica, an insect pest of stored grain, is commonly used as host for *Trichogramma* mass rearing and also for maintaining the *Trichogramma* species in laboratory, especially those species that can not be reared on large eggs of *A. pernyi*.

For *Trichogramma* mass production, it is very important to produce high quality host eggs in huge numbers at competitive costs. Mass rearing techniques for *C. cephalonica* have been improved and a set of machines or devices for *C. cephalonica* production have been designed in Shanxi province in the late 1970s (Qiu et al., 1980; Shi et al., 1982; Chen et al., 2000). The production line for *C. cephalonica* includes medium blender, moth collector, egg collector, egg cleaner, egg sterilizer with UV light, egg card machine, and a *Trichogramma* mass rearing facility (Shi et al., 1982).

Zhang et al. (1991) compared the effects different media on egg production by *C. cephalonica*. Among the media used including rice bran, wheat bran, rice bran/wheat bran with corn flour (9:1), the rice bran is the best for mass rearing of *C. cephalonica*. At the rearing density of 4000 eggs/kg of rice bran infested, a harvest of about 2254 adults and 378,738 eggs/kg can be attained, with a multiplication rate of 94.7 times in one generation. In north China, wheat bran is much easier to obtain than rice bran, but the survival rate and the reproduction of *C. cephalonica* reared on wheat bran is much lower than that on rice bran. When 90% wheat bran mixed with 10% soybean flour or corn flour is used as the medium for *C. cephalonica* rearing, the emergence of moth was 134% and 78% higher, and the number of eggs produced was 231% and 146% higher, respectively (Zhou et al., 1988).

In order to meet the mass rearing of *Trichogramma* with the release dates, the parasitized *C. cephalonica* eggs are usually stored in a cold room for a certain period. However, emergence rates from the parasitized eggs, the sex ratio and fecundity are affected by storage duration. To keep good quality levels in *Trichogramma* rearing, the storage period for parasitized *C. cephalonica* eggs at 4 °C should not exceed 15 days (Zhang et al., 2008).

However, emergence rate and fecundity of the parasitoid are affected by the developmental stage of the parasite, the incubation temperature and the period of the storage. When the freshly parasitized eggs are incubated for 3–6 days at alternating temperatures of 8 °C and 25 °C for 16 h and 8 h, parasitized eggs at the late egg stage can be successfully stored at 8 °C for a longer period without compromising emergence rate and fecundity. Gou (1985) showed that parasitized *C. cephalonica* eggs treated with this method showed an average emergence rate of 87.5% while emerged adults parasitized 55.8 host eggs per female wasp when the host eggs were stored at 8 °C for 90 days.

The rearing techniques for *C. cephalonica* have been studied in Taiwan, especially the thickness of diets and the rearing density (Cheng and Hung, 1996, 1997). The development of mass rearing and inundative release techniques of *T. ostriniae* for controlling of the Asian corn borer in Taiwan began in 1984 (Yu et al., 1992). Tsneg (1990) developed the egg card machine to decrease labor hours and the costs in the mass production of *T. ostriniae* by using *C. cephalonica* eggs. The egg card machine is 10 times faster in producing egg cards than human laborers and also produces a more standardized number of eggs on the paper. Egg cards irradiated by UV light for 1 h and treated with the preservative agent "Fuyolin", then stored at 4 °C was the most effective preservation. The parasitism rate on the stored egg cards stored at 4 °C for 15 days was 74.3%, not significantly different from that of egg cards not stored at low temperatures. The preservation of egg card could save the surplus of alternate host *C. cephalonica* eggs from being discarded each day and could also help adjusting the differences in time between producing and releasing egg cards in the field. For obtaining the highest egg yield in a shorter period of time, 0.25 ml of *C. cephalonica* eggs is recommended to inoculate 1 kg of crushed brown rice (Cheng and Hung, 1996).

Quality control process for *T. ostriniae* rearing with *C. cephalonica* eggs has been set up, based on the following steps: good *C. cephalonica* egg cleaning, selection of *C. cephalonica* eggs, superior breeding wasp culturing, environment of mass rearing management, parasitized eggs collection and screening and inspection. The criteria for high quality of *T. ostriniae* adults reared on *C. cephalonica* eggs include a survival (>80% of adults should survive 2 days without access to water and food at 25 °C, RH 80–90%), the ability to parasitize 50 or more *C. cephalonica* eggs per female and a 3:1 sex ratio (females:males). Survival is based on 60 female wasps while fecundity is based on 10 mated females (Chen et al., 2000).

3.4. Mass production of Trichogramma using *S. cerealella* eggs

The eggs of *S. cerealella* are mostly used as factitious host for *Trichogramma* in Europe. The mass production technique for *S. cerealella* was introduced for mass rearing *Trichogramma* in China during 2000 and subsequent years. *T. ostriniae* and some other *Trichogramma* species, such as *T. evanescens*, which can not be mass reared on *A. pernyi* eggs have been mass produced by using *S. cerealella* eggs since then. Based on the mass rearing techniques for *S. cerealella* reported by Konig et al. (1992), the Dryland Farming Institute, Hebei Academy of Agricultural & Forestry Sciences developed a modified method with adapted procedures and equipments for mass rearing *Trichogramma* on *S. cerealella* eggs has been developed in 2000. *Trichogramma brassicae* Westwood, 1833 has been mass reared since then with peak production of 1.26 million wasps/day and a maximum capacity of about 2 million wasps/day in 2001. The capacities of the mass production system of *T. brassicae* went up to 11.34 million wasps/day in 2002, while the cost of production of *T. brassicae* was reduced by 47.3% through improvement of *S. cerealella* egg collecting facilities (Zheng et al., 2003). The same techniques and equipment were also used for *T. ostriniae* mass production (Jia et al., 2002) and *T. ostriniae* became available for Asian corn borer control in Huang Huai Hai Summer Corn Region in recent years where nowadays about 10,000–15,000 ha of *T. ostriniae* are released in summer corn annually for control the Asian corn borer (Jia et al., 2010). Furthermore, this mass production system for *Trichogramma* as well as field releasing techniques developed in China has been successfully transferred to DPR Korea, with certain adaptations to account for lack of electricity in rural areas (Zhang et al., 2010).

3.5. Trichogramma in vitro on artificial host eggs

Mass rearing of factitious hosts for *Trichogramma* production is labor intensive and costly, besides being affected by abiotic and biotic factors. Studies on rearing of *Trichogramma* in vitro on artificial host eggs began in the middle of 1970s in China (Wang, 2001). Breakthroughs have been made on the rearing of *T. dendrolimi* and *T. chilonis* by means of artificial host eggs. Further research has shown that efficacies are similar to the same species reared from the factitious eggs. Oviposition stimulants that improved egg production by *T. dendrolimi* and *T. chilonis* were selected (Han et al., 1994). With the addition of tricosane in a polyvinyl alcohol hydrophilic colloid, the parasitism and pupation of *T. dendrolimi* on artificial host eggs reached 100% and 81%, respectively (Zhang, 1993). The system closest to commercial production is that developed for *T. dendrolimi* on the basis of insect hemolymph. This diet has been packaged in plastic host egg-cards. When the concentrated and dried *A. pernyi* pupal holotissues with antiseptic and protective material stored at −5 °C for 44–55 months, it still could be used for artificial host eggs in *Trichogramma* production. More than 91% of the artificial eggs made of stored *A. pernyi* pupal holotissues were parasitized, from which 76–84% adult wasps emerged. The fecundity was 70–80% and the propagation coefficient was 9.6–11.7. No significant differences were observed for the stored and fresh holotissues. This method is very convenient for storing and transporting of artificial diet due to its smaller size and availability at any season for industrial production of *Trichogramma* spp. (Liu et al., 1995). Many experiments trying to rear *T. ostriniae* on artificial host eggs were not highly successful as the rate of pupation is very low and only very few *T. ostriniae* could develop to the adult stage, (Han et al., 1999; Lian et al., 2009). The putative reasons that the *T. ostriniae* could not succeed in artificial host eggs are the narrow host range, not sufficient number of eggs to lay which may lead to parasitoid being drowned by the excessive liquid in artificial eggs (Lian et al., 2009).

Mechanized production of *T. dendrolimi* and *T. chilonis* with artificial host eggs has been successful. A prototype for producing artificial host eggs was manufactured which automatically completes all five processes, including setting-up synthetic membrane, forming the "egg-shells", injecting the artificial media into the shells, sealing the double-layered membrane, and separating into egg cards (Liu et al., 1988). Based on this prototype designed by Liu et al. (1988), the model GD-5 automatic machine for mass production of artificial host egg-cards was successfully assembled in 1995, and the *Trichogramma* mass rearing technology based on artificial host egg-cards developed. Operating rules for mechanized production of artificial host eggs for *Trichogramma* and techniques for propagating parasitoids, quality control, and releasing have been constituted (Dai et al., 1996; Liu et al., 1996). One GD-5 could produce 5000 egg cards/8 h, each with 140 eggs. Each egg could produce 50-60 wasps with good quality (Liu et al., 1998a,b). Two artificial host egg production lines for *Trichogramma* were set up in Guangzhou and Beijing in the late 1990s. The standard of quality control for mass production of *Trichogramma* in vitro has been put forward, which include the storage, backup and rearing of the seed wasps, and quality monitoring of the artificial eggs (Wang et al., 2005). *T. dendrolimi* and *T. chilonis* reared on artificial host eggs have good control effect on Asian corn borer in summer corn with parasitism rates of 65% and 68%, respectively (Feng et al., 1999). Such parasitism rates are similar to those achieved by wasps reared on eggs of *A. pernyi* (Li et al., 1997). The parasitoids from in vitro rearing have been used for control of Asian corn borer and cotton bollworm over 150,000 ha in the 1990s (Wang, 2001). A releasing container for *Trichogramma* reared on artificial host eggs was also designed and the emergence rate was over 90% (Feng et al., 1999). China was the first country worldwide to make use of in vitro rearing of *Trichogramma* for commercial production and use for insect pest control on a large scale (Feng et al., 1997; Wang, 2001). However, as the artificial host eggs can only be used for mass rearing of *T. dendrolimi* and *T. chilonis,* and also because the costs of artificial host egg production for *Trichogramma* were higher than costs of *A. pernyi* egg production, in northeast of China the former stopped in the early 2000s.

4. Release techniques of *Trichogramma* for biological control of Asian corn borer

Both, inoculative and inundative approaches have been tested and implemented for biological control of corn pests in China. Inoculative releases aim to support naturally occurring populations of the biological control agent by releasing comparatively small agent numbers before the pest populations builds up. Thus, if it works, inoculative releases can be highly cost-efficient. The most common approach of releasing *Trichogramma*, however, are inundative releases, during which massive numbers of the biological control agent are released repeatedly during the season. Shen et al. (1986) compared the inoculative release and inundative release of a mixed population of *T. dendrolimi* and *T. ostriniae* mass reared on eggs of *S. cynthia ricini* to control the Asian corn borer in spring cornfields. The parasitoid wasps were released at a rate of 7500 and 15,000/ha in the inoculative plots during late April during two releases with 10 days interval. Two times 150,000/ha were released in the inundative plots during late May, again with 10 days in between. No significant difference was observed in parasitization rate of eggs of the first and second generation of the Asian corn borer between the two treatments, but both treatments showed significantly higher rates compared to the non-release area. The parasitization rate of the Asian corn borer was 14.1%, 17.6% and 6.7% for first generation, and 69.0%, 75.0% and 16.7% for the second generation of Asian corn borer in inoculative, inundative and non-release plots set up in 1979. Field experiments last for 4 years from 1978 to 1981 with similar results in Henan province (Shen, 1987). Zhou et al. (1997) conducted a 2-years study to test inoculative releases of *T. ostriniae* at 15,000/ha in combinations with a single cross of corn partly resistant to Asian corn borer inter-planted with creeping type of mung bean, *Vigna radiata* (Fabaceae). In both, 1992 and 1993, the parasitism of Asian corn borer egg masses mass reared and attached to corn plants in the field with inter-planted mung beans, the inoculative release showed between 26% and 28% parasitism while hardly any parasitism rates were observed in the other treatments. The parasitism rate of naturally laid egg masses observed in 1993 reached 69%, which is close to rates expected under commercial conditions. The number of holes per hundred plants was 54.8, 102.6 and 277.2 in resistant single cross fields inter-planted with mung bean and with inoculative releases, in the field without inter-planting but with inoculative releases and in the susceptible single cross field, respectively. The results indicated that inoculative releases of *T. ostriniae* at 15,000/ha in corn partly resistant to Asian corn borer together with interplanting creeping mung bean and have a good effect in controlling the Asian corn borer.

Field application techniques for inundative releases of *Trichogramma* have been greatly improved since the 1980s. Release sites have decreased from 90 to 45 per ha based on the dispersal distance of *T. dendrolimi* in corn field. The release frequency has decreased from 3 to 2 releases for one generation of Asian corn borer control. The total number of *Trichogramma* wasps released range from 150,000–300,000 wasps/ha per pest generation according to the occurrence of the Asian corn borer. In recent years in Heilongjiang, Jilin and Liaoning provinces, the provincial or local governments started to provide special funds to provincial plant protection station for supporting *Trichogramma* releases. Provincial plant protection station funds order *Trichogramma* from specialized companies and organise the distribution of *Trichogramma* egg cards to the plant protection stations in county level. Since these *Trichogramma* egg cards provided by the local governments only meet 25–50% of the *Trichogramma* release requirements in the areas where the Asian corn borer are serious, some farmers buy and release additional *Trichogramma* themselves. Release time is determined by monitoring of Asian corn borer pupation rate in corn stubbles by local plant protection station in county level. When the pupation rate of the overwintering generation is 15–20%, the first release will be conducted 10 days later. The second release is usually done 6 days after the first release (Liu et al., 1990). In addition, egg cards have been developed on which the parasitized eggs of different developmental stages of *Trichogramma* are mixed, thereby staggering emergence. This ensures that there are always some females actively searching for the Asian corn borer throughout the oviposition period and allows a reduction of the number of releases (Zhang et al., 1993). Using these approaches, *Trichogramma* releases have demonstrated consistent parasitism ranging between 60% and 85% during the 1990s, with reductions in crop damage of 65–92% (Piao and Yan, 1996).

In Jilin Province, releases were made in 4.1 million ha of corn totally from 1990 to 2002 with good biological control efficacy: the parasitism of Asian corn borer eggs by *T. dendrolimi* ranged from 73.4% to 87.8%, while stalk damage decreased by 92.5% (Liu et al., 2000). In two-generation areas, additional *Trichogramma* release is needed when the egg masses of the second generation of Asian corn borer are observed, leading to an average reduction of 46.3–73.6% for the overwintering population. The strategy for controlling the Asian corn borer in two-generation areas is based on inundative releases for the first generation at 150,000 wasps/ha, and 75,000–150,000 wasp/ha for the second generation. This strategy has been explored on a large-scale in Liaoning province where it has resulted in sustainable management of Asian corn borer with good control efficacy (Cong et al., 2000; Yang et al., 2011).

5. Release of *Trichogramma* for the biological control of other corn insect pests

H. armigera has become one of the major insect pests attacking corn ears in summer corn region since the cropping system transformed in the late 1980s (Wang et al., 2002a). *T. chilonis* is the dominant egg parasitoid of *H. armigera* in North China, and showed a good control effect for this pest (Ji et al., 1994; Feng et al., 1997). A 3-years study between 1998 and 2000 in Hebei province showed that intercropping corn with creeping type mungbean could increase the parasitism of *H. armigera* eggs by *T. chilonis* and reduced damage to corn ears (Wang et al., 2002b). Similar results were achieved by inoculative releases of 150,000 *T. chilonis* wasps per ha. However, even in control fields, *T. chilonis* parasitism rates were found to be around 50%. Nevertheless, Wang et al. (2002b) suggested that intercropping might augment for the efficacy of *Trichogramma* wasps and serve as a cost-efficient management tool for *H. armigera* in summer corn fields. A study conducted in Shandong province showed that *T. chilonis* reared on artificial host eggs has a good biological control effect on *H. armigera* in summer corn field with 71.1% parasitism rate, which was much better than parasitism by *T. dendrolimi* (Feng et al., 1999).

Also, the Yellow peach moth *C. punctiferalis* has become an important corn pest as it attacks the corn ear and occurs increasingly in Chinese corn growing areas (Wang et al., 2006). Since 2010, *T. dendrolimi* has been mass released to provide biological control of this insect pest in Miyun County in Beijing.

6. The role of *Trichogramma*-based biological control in sustainable pest management systems

Large ecological and economic benefits have been achieved in areas where *Trichogramma* have been released continuously for many years. Before *Trichogramma* was released in large numbers for biological pest control in China, a monitoring conducted from 1963 to 1971 in Lankao County, Henan province, revealed on average 30.4, 14.4 and 230 Asian corn borer egg masses per hundred plants for the first, second and third generation, respectively. When *Trichogramma* were released on a large scale in corn fields for over twenty years, the number of Asian corn borer egg masses decreased gradually year by year from about 100 in 1974 to 24 in 1981. Furthermore, other pests in other crops were reduced by *Trichogramma* releases in corn fields. For instance, the parasitism of the eggs of Green semi-looper, *Anomis flava* (Fabricius, 1775) (Lepidoptera: Noctuidae) in cotton fields in *Trichogramma* release areas averaged 30% compared to 9% in non-release areas and parasitism of eggs of *Leucoma salicis* (Linnaeus 1758) (Lepidoptera: Arctiidae) in willow trees along the roads within *Trichogramma* release areas was 61%

compared to 34% in non-release areas. Since 1983, the number of egg masses of the first generation of the Asian corn borer increased to 264 when releases of *Trichogramma* had been stopped (Shen, 1987). In Xifeng and Xiuyan, Liaoning province, where *Trichogramma* releases for control of the first generation of Asian corn borer continued for 17 years in the1970s and 1980s, the natural parasitization of *Trichogramma* on first generation Asian corn borer egg masses have increased year by year and reached 70%. The parasitism rates of parasitoids attacking larval and pupal stages of Asian corn borer also increased year by year, and the combined effect of increased numbers of *Trichogramma* and other parasitoids decreased damage rates of corn and stabilized it at about 10% since 1980. The population of Asian corn borer in these regions maintained at a low dynamic equilibrium level for a long period (Zhang et al., 1990b). In Miyun of Beijing, where *Trichogramma* has been released for more than 20 years since 1977, the populations of natural enemies in corn fields have increased. This helps keeping other insect pests under control without application of pesticides (Shi, 1996). Parasitism of first generation Asian corn borer egg masses by naturally occurring *Trichogramma* in non-release corn fields increased from 1% in 1980 to 33% in 1991 (Shi, 1996). In Xifeng County, Liaoning province, where *Trichogramma* was released continuously on a large scale for over 30 years, the number of overwintering larvae has been reduced to 5.6 larvae/per hundred stalks with a corresponding yield of 7500 kg/ha, compared to 193.6 larvae/per hundred stalks and 5250 kg/ha in surrounding counties where an outbreak of the Asian corn borer was observed in 1997 (Cao and Sun, 2002). In Wafangdian city, Liaoning province, *T. dendrolimi* were mass released on a large area of corn during the first generation of Asian corn borer for 3 years. The results showed a mean parasitism rate of *T. dentrolimi* of 75.1% and a damage reduction of 62.3% for first generation of Asian corn borer. Without further releases, parasitism rate of second generation of Asian corn borer was 48.3% with the number of holes in the stem and the number of stalks broken decreasing by 44.6% and 51.9%, respectively. The average number of larvae, assessed at harvest, decreased by 46.2%. This indicated that first as well as second generation of Asian corn borer could be controlled by only one *Trichogramma* release against first pest generation (Yang et al., 2011).

In Gongzhuling City, Jilin Province, mean number of holes, the number of larvae per hundred stalks and the percentage of damaged plants decreased between 63% and 73% in areas where the *Trichogramma* have been released from 1990 to 2008. Furthermore, average natural parasitism of corn borer eggs by *Trichogramma* increased from 14.8% in 1989 to 30.6% in 2007 for consecutive 19 years of *Trichogramma* releases in Gongzhuling, Jilin province (Yu et al., 2009).

The release of *Trichogramma* for control of the Asian corn borer has become one of the key techniques of IPM of corn pests in China (Wang et al., 2003; Yang et al., 2011). It has been commonly adopted by the farmers in northeastern provinces in China because of its easy use and good control efficacy. No insecticides are applied in fields where *Trichogramma* is applied. *Trichogramma* releases for control Asian corn borer comprise 1 to 1.3 million ha each year. With the Chinese government paying more attention to grain production and environmental protection, *Trichogramma*-based biological control has been expanded to the Huang-Huai-Hai summer corn region and the Northwestern corn region in recent years. Jilin, Liaoning and Heilongjiang provincial governments have provided some subsidies for controlling the Asian corn borer with *Trichogramma* since 2002. The total release area for *Trichogramma* control of the Asian corn borer between the years 2009 and 2011 was 3.23 million ha in Jilin province. In Heilongjiang province, the *Trichogramma* release area expanded from 113,000 ha in 2010 to 500,000 ha in 2011.

7. Future prospects and challenges in the use of *Trichogramma* for biological control

Trichogramma release for control Asian corn borer and other lepidopteran insect pests is one of the key IPM components for corn in China. The new concept of "Public Plant Protection, Green Plant Protection" was put forward in 2006 as the guideline for plant protection in China. The Ministry of Agriculture of China released a notification about the Instruction of Green Control for Corn Borer in China on March 8, 2010. The Ministry of Agriculture of China and local governments in Jilin, Liaoning and Heilongjiang have provided special funds to renovate the facilities and techniques for mass production of *Trichogramma*. This has improved the efficiency and product quality of *Trichogramma* in recent years. *Trichogramma* application combined with other non-chemical control measures began a new era for IPM in corn. As the efficacy of *Trichogramma* species was not desirable and stable when the ears and stalks of corn were heavily infested by corn borers besides adverse effects of climate, green pest control technologies, including *Trichogramma* releases integrated with other green control techniques have been developed in northeastern spring corn region since 2009. In Heilongjiang province where the Asian corn borer occurs in one generation per year, projection-type moth-killing lamps are set up outside the villages where the corn stalk piles are located. Traps are set up during the period of corn borer emergence after overwintering to kill the moth immediately and to reduce the number of egg masses laid in corn field. Subsequently, *T. dendrolimi* or *T. chilonis* is released to parasitize the egg masses oviposited by the remaining corn borer moth in the early oviposition period. If the damage by the Asian corn borer still reaches the economic threshold after the *Trichogramma* release in an outbreak year, *Bacillus thuringiensis* (*Bt*) biopesticide is sprayed by self-propelled high clearance sprayer in late whorl stage. In Jilin and Liaoning provinces where the Asian corn borer generally occurs in two generations, besides using projection-type moth-killing lamp to kill the overwintering Asian corn borer, the piles of infested corn stalks are treated with a biopesticide based on *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae), to control the overwintering generation in early May. If there are still many *O. furnacalis* adults observed during the overwintering generation oviposition period, *T. dendrolimi* or *T. chilonis* are released. The percentage of infested corn plants decreased by 75.1% when the infested corn stalk pile was treated with *B. bassiana* plus subsequent releases of *Trichogramma*, while the percentage of infested corn plants decreased by 69.7% when only *Trichogramma* was released. To control the second generation, the *Trichogramma* are released early to parasitize the first laid egg masses, resulting in a 66.1% reduction of infested corn plants in Jilin province. In some areas, the first generation of Asian corn borer is controlled by *Trichogramma* releases, and the second generation is sprayed by self-propelled high clearance sprayers using *Bt* applications during the blister stage with 78.35% decrease of infested corn plants. These green control technique systems have been extended in all corn growing regions. Total control acreage of corn applied by these green control techniques for corn borer expect to be 6.67 million ha in the end of 2015, equivalent to 40% and 20% of corn in one and two generation areas, respectively, according to the Instruction of Green Control for Corn Borer in China released by Ministry of Agriculture of China (Xia, 2010).

Transgenic crops resistant to several insect pests have been commercialized for a number of years, including *Bt* corn which also targets lepidopteran pests such as Asian corn borer in USA and some other countries. The recent approval of *Bt* corn provides a challenge to the application of *Trichogramma*, as no other measurement against lepidopteran pests is generally required in *Bt* corn fields. However, there may also be chances, particularly since *Bt* corn have no direct effects on *Trichogramma* (Wang et al., 2007). Most importantly, *Trichogramma* releases could play a major role in resistance management of *Bt* corn. The latter requires refuges which can grow to considerable dimensions if a larger proportion of corn fields in China would be planted with *Bt* corn in near future. In addition, naturally occuring *Trichogramma* and inoculative releases could help controlling the late-season lepidopteran insect pests or secondary target insects.

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