DEVELOPMENT AND AREA-WIDE APPLICATION OF BIOLOGICAL CONTROL USING THE PARASITOID *Aphidius gifuensis* AGAINST *Myzus persicae* IN CHINA

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SUMMARY

Ecologically safe and environment-friendly pest control strategies and technologies are important to ensure the quality of Chinese agricultural products and sustainable agricultural development. Aphids are among the world's major agricultural and forest pests, and *Myzus persicae* Sulzer (Hemiptera: Aphididae) is one of the main agricultural pests in China, transmitting various viral diseases and causing reductions in crop yield and quality that regularly triggered applications of synthetic insecticides. *Aphidius gifuensis* Ashmead (Hymenoptera: Braconidae) is an important endoparasitoid of many aphids. Starting in 2000, the Yunnan Tobacco Company has developed methods for large-scale rearing of this parasitoid on this aphid, and technological systems for augmentative releases of *A. gifuensis*. The augmentative use of this parasitoid has achieved area-wide suppression of *M. persicae* in tobacco and other crops in China. This approach is being applied on large areas, covering more than 3 million ha between 2010 and 2015. This programme is currently the largest biological control programme in China. Over 500 mass-rearing facilities were constructed in 16 provinces with a total surface area of 420 000 m² and a breeding capacity of 24 000 million parasitoids per breeding period. This technology has effectively controlled the aphid on tobacco, while other beneficial insects have increased in the absence of insecticide applications, further protecting biodiversity in the fields and providing long-term ecological benefits. The use of this technology has also been expanded to other crops, solving problems of insecticide resistance in the targeted aphids, reducing pesticide residues and environmental pollution, and yielding benefits for society, the economy, and the environment.

Key Words: Aphididae, aphids, augmentative biological control, tobacco, technology research, technology transfer, large-scale parasitoid breeding, Braconidae, release technique, training, extension, environment-friendly pest control, Yunnan

1. INTRODUCTION

Yunnan Province, China is one of the most important tobacco growing regions in the world with 469 000 ha under cultivation, representing 35% and 20% of tobacco production in China and the world, respectively. Yunnan province is also famous for producing tobacco of high quality, and its tobacco industry has provided a sustainable livelihood and alleviated poverty for more than 800 000 farming families. Tobacco in Yunnan province is usually planted in mountainous areas, of high scenic value, that have fragile ecosystems. The Yunnan tobacco-planting region has five characteristics: (1) a wide distribution of planting sites, (2) planting in a variety of ecological regions, (3) dominance by smallholder farmers, (4) farmers of widely different backgrounds, and (5) a mosaic of factors influencing tobacco leaf yields and quality.

Aphids are among the most destructive pests, are often highly polyphagous and impact a broad range of agricultural crops worldwide. Plant sap-sucking and honeydew production by the tobacco aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae) directly injures the host plant, causing significant yield reduction (Kulash 1949; Starý 1970). In addition, damage from *M. persicae* is exacerbated by its ability to transmit over 100 viral diseases to more than 400 host plants (Mackauer and Way 1976). Most of these viral diseases cause a decline in tobacco yield and quality.

In Yunnan, control of this aphid pest has largely been dependent on insecticides (Zhao et al. 1980), leading to problems of resistance, difficulty of control, destruction of natural enemies, decrease of biodiversity, excessive pesticide residues, reduction in product quality due to repeated applications, inappropriate application methods and incorrect application rates (Gao et al. 1992; Han et al. 1989; He 2013).

In view of these problems, there was a strong need to develop and apply biological control tactics to replace the chemical control systems applied against *M. persicae*, taking into account the population dynamics of the pest, herbivore-natural enemy interactions, and the economic relationship between pest infestation and crop yield loss (Guan et al. 2016).

Aphidius gifuensis Ashmead (Hymenoptera: Braconidae) is one of the most important natural enemies of aphids and is found in many habitats (Chen 1979). This natural enemy is widely distributed all over the world, for example Canada, China, India, Japan, and USA, where a good basis of ecological knowledge has been accumulated for wider application. Also, many biological and ecological studies of this insect have been conducted in China (Bi and Ji 1993; Lu et al. 1993; Lu et al. 1994).

After mating, females of *A. gifuensis* search for hosts and lay their eggs inside aphid bodies. The eggs develop by absorbing the nutrients from the aphid, and the development of the parasitoid results in the death of the aphid (Ohta et al. 2001) (Fig. 1).

Figure 1. Life cycle and biological stages of *Aphidius gifuensis* (A.G.) (starting clockwise from top left).

There are two main challenges with respect to the biological control of aphids by using *A. gifuensis* on a large scale. The first is to develop methods for the large-scale production of the parasitoid and its release. The second is to find mechanisms to transfer this technology to technicians and farmers so that these smallholders can benefit from the technology.

Therefore, after carrying out the research, we have developed and established two systems for effective, economic, and convenient high-density mass-rearing technologies for different application areas, named *adult-plant breeding* and *seedling breeding*, published an industry-level standard, constructed a “one plus two” model of technology extension, and achieved area-wide application of the technology for sustainable aphid control by *A. gifuensis*.

2. MASS-REARING SYSTEMS

The large-scale mass-rearing of *A. gifuensis* is mainly divided into three parts: (1) cultivating of host plants for *M. persicae*, (2) breeding large populations of *M. persicae*, and (3) high-density mass-rearing of *A. gifuensis* on *M. persicae* (Gu et al. 2015). The following summarizes the optimal conditions and procedures for these technologies (Deng et al. 2010, 2011).

2.1. Adult-Plant Breeding System

2.1.1. Cultivating Host Plants

Tobacco plants that are highly resistant to tobacco mosaic virus (TMV) were selected for mass-rearing of the aphids, i.e. tobacco variety *Yunyan 203*, as well as white radish and Chinese cabbage variety *Chinese 82*. The host plants are seeded and transplanted after 70 to 80 days and grown for 25-30 days till the 6-8 leaves stage for further use.

2.1.2. Mass-rearing of *Myzus persicae*

Each tobacco plant, at the 6-8 leaves stage, is inoculated with 20 healthy aphids (nymphs and adults), followed by rearing of the aphids for 15-20 days at 17-27°C and 50-80% RH in a greenhouse (50 m x 12 m x 4.6 m) (Wu et al. 2000; Deng et al. 2006; Yang and Zhao 2009; Yang et al. 2009) (Fig. 2).

2.1.3. High-density Mass-rearing of *A. gifuensis* on *Aphids*

When the tobacco leaves are incubated with aphids for 15-20 days, *A. gifuensis* are released at a parasitoid to aphid ratio of between 1:50 and 1:100. After *A. gifuensis* females have laid their eggs, the parasitized aphids form mummies, from which a new generation of *A. gifuensis* emerges. After 10 to 15 days, parasitism rates of > 90% are obtained (Wei et al. 2003, 2005; Yang et al. 2009).

Each tobacco plant can produce 6000 – 10 000 *A. gifuensis* adults, and each small greenhouse (3 m x 3 m x 2 m) containing 28 plants, can produce 160 800 *A. gifuensis* individuals (Fig. 2).

Figure 2. Breeding process of *Aphidius gifuensis* in greenhouses (starting clockwise from top left).

2.2. Seedling Breeding System

2.2.1. Cultivating Host Plants

Tobacco variety *Yunyan 203* with high resistance to TMV is bred according to the China National Standard GB / 25241 (Liu et al. 2010). Tobacco seedlings with 5 leaves and 1 heart can be used to breed aphids by the “Method of breeding aphid and *Aphidius gifuensis* separately”.

Alternatively, tobacco seedlings in the 3rd - 4th leaf stage can be used to breed aphids by the “Method of breeding aphid and *Aphidius gifuensis* at the same time” as described below.

2.2.2. Rearing of Large Populations of *Myzus persicae*

Mass-rearing of aphids is done using two alternative methods:

Breeding aphid and A. gifuensis separately: On tobacco plants with 5 leaves and 1 heart, leaves are inoculated at a rate of 10 aphids per plant. After 10 to 12 days, at 20 to 30°C and 60 - 80% RH, when the aphid density reaches 200 per plant on average, the population of aphids is ready for use for rearing natural enemies (Fig. 2).

Breeding aphid and A. gifuensis at the same time: On tobacco plants in the 3rd - 4th leaf stage, leaves are inoculated with 2.5 aphids per plant (aphids with a parasitism rate from 40% to 60% are used for the inoculation, or a parasitoid-aphid ratio of between 1:20 and 1:10), at 20 - 30°C and 60 - 80% RH.

2.2.3. High-density Breeding of *A. gifuensis* on Aphids

Parasitoid mass-rearing on aphids is done following two alternative methods:

Breeding aphid and A. gifuensis separately: According to the population of aphids per single plant, *A. gifuensis* or parasitised aphids are inoculated onto leaves in the greenhouse. After 17 days, the population of parasitised aphids will reach one 100 000 per square meter (Fig. 2).

Breeding aphid and A. gifuensis at the same time: *A. gifuensis* parasitoids are inoculated onto leaves at the same time as aphids. After the *A. gifuensis* parasitoids emerge from the parasitised aphids, they will parasitize other aphids. If the parasitism rate is too high, more aphids need to be added; alternatively, if the parasitism rate is too low, more *A. gifuensis* will need to be added. After 23 days, the population of parasitised aphids will reach 49 000 per square meter (Fig. 3) (Chen et al. 2009).

Figure 3. Breeding process of aphids and *Aphidius gifuensis*.

2.3. Collection and Storage of *A. gifuensis*

There are two methods for collection of *A. gifuensis*:

Manual collection: Home-made, simple collection devices or automatic collection systems (aspirators) are used to collect *A. gifuensis* in the tents, and *A. gifuensis* are stored in containers (Fig. 4).

Automatic collection: Collection bags are placed in the breeding screen-houses (nylon-net covered cages or tents) in tobacco fields, and *A. gifuensis* adults will fly into the bags as a result of their phototaxis (Fig. 4).

2.4. Release of *A. gifuensis*

Different methods are used to release *A. gifuensis*:

1. Release of parasitised aphids
2. Release of *A. gifuensis*, and
3. Self-dispersal in the field.

When releasing parasitised aphids, leaves or seedling with parasitised aphids are brought to the field and hung onto plants.

When releasing *A. gifuensis*, parasitoids are taken to the field in collection bags or bottles and released before 12 o'clock in the absence of any rain; total transport time should be less than three hours.

For self-dispersal of parasitoids in the field, they are bred in the screen-houses or breeding tents in the field. The tents are opened when parasitism reaches 90%, and the *A. gifuensis* will disperse naturally to find aphids (Fig. 4).

Figure 4. Collection and release processes for *Aphidius gifuensis* parasitoids.

The density of aphids needs to be assessed to determine the parasitoid release time and numbers to be released. When the population of aphids per plant reaches an average of 1 to 5 individuals, *A. gifuensis* parasitoids are released at a rate of 3 000 to 7 500 per ha. The subsequent second and third release are adjusted according to aphid densities.

When the population of aphids per plant reaches an average of 6 to 20, *A. gifuensis* parasitoids are released at a rate of 7 500 to 15 000 per ha. When the population of aphids per plant is more than 20, *A. gifuensis* parasitoids are released at a rate of 15 000-18 000 per ha.

2.5. Conservation of Aphid and *A. gifuensis* Colonies in Winter

2.5.1. Conservation of Aphids in Winter

The main method to maintain a colony of aphids in the winter is by conserving the host plants in greenhouses. Aphids are collected from the wild and inoculated onto healthy tobacco seedlings, cabbage, radish or other host plants. The breeding conditions are held at temperatures between 17 and 27°C and 50 and 80% RH. The status of aphids and host plants is observed, and old and diseased aphids and host plants infected with virus are removed at three different times. Healthy aphids are obtained and used to reinvigorate the colony.

2.5.2. Conservation of *A. gifuensis* in Winter

Holding-over host plants with aphids in the greenhouse or cold storage of parasitised aphids are the two methods used for storing *A. gifuensis* in winter. *A. gifuensis* are collected from the wild and used to parasitize aphids several times. To obtain healthy *A. gifuensis*, the colony needs to be maintained at a temperature of 17-27°C and 50-80% RH.

When in cold storage, parasitised aphids are collected using a brush or other tools to directly collect them from host plants, placing aphids into tubes, which are then held at 4-5°C. The seedlings or larger plants with parasitised aphids can also directly be placed into 4-5°C. Parasitoid emergence rates of up to 90% are obtained after cold storage for 20 days.

3. TECHNOLOGY TRANSFER AND APPLICATION SYSTEMS

3.1. Technical Standard

An industrial standard named “Technical specification for *Myzus persicae* biological control with *Aphidius gifuensis* (YC/T 437-2012)” was published (Yun et al. 2012), including conservation, colony rejuvenation, large-scale breeding, collection and release.

A total of 506 breeding facilities were built in 16 provinces (regions, cities) in China, with a total surface area of 420 000 m² and a breeding capacity of 24 000 million parasitoids per breeding period.

3.2. Training

To spread and transfer this technology, a training system was developed and implemented on four levels, i.e. industrial, provincial, municipal and county level. The training programme was developed based on research conducted by the tobacco industry, and also based on field experience by extension services of the government (Fig. 5).

Figure 5. Extension system for biological control of tobacco aphid by parasitoid augmentative releases.

We developed the training platform, carried out theoretical and practical training for the technical experts, technicians, extension workers and farmers, covering step-by-step the key points and difficulties for technicians during programme operation (Fig. 6).

Figure 6. Training system for biological control of tobacco aphid Aphidius gifuensis by parasitoids.

Overall, we have trained more than 3000 core experts and more than 20 000 technicians, and also provided more than 1200 thousand copies of technical training books and booklets (Fig. 7). More than 120 000 farms benefited from this training and extension work at all levels (Fig. 8).

Figure 7. Technical materials on the use of *Aphidius gifuensis* parasitoids.

Figure 8. Four-level training for biological control of tobacco aphid by mass-release of *Aphidius gifuensis* parasitoids.

3.3. Goal Setting

Depending on the densities of the aphids in the farms with tobacco fields, *A. gifuensis* are released following five release densities, i.e. 7500, 12 000, 15 000, 18 000 and 22 500 parasitoids per ha. Also, depending on the aphid infestation pressure in different areas, application rates are 30%, 50%, 80% and 95% of the total tobacco-planting area.

3.4. Matching of Funding

The funding for the technical application is contained within technical project funds. Each provincial tobacco company matches the obtained funding of USD 11.3-22.6 per ha according to set application areas.

3.5. Evaluation

The programme evaluation is carried out to check implementation, supply, and application scale, and to control efficiency of the application of this technology at different levels. To promote the tobacco aphid biocontrol technique, a series of rewards and penalties were established, with the outcomes of the evaluation results directly tied to the salary of technicians and also to the funding for application in the next year.

4. IMPACT ASSESSMENT

Each small greenhouse containing 28 plants can produce ca. 17 000 *A. gifuensis* individuals for release that can protect six ha of tobacco plants. Compared to chemical control of aphids, the cost of aphid control by *A. gifuensis* mass-releases is much lower. The cost of biological control is estimated at about USD 13.3 per ha, compared to USD 244.8 per ha for the chemical control applying insecticides. Table 1 compares the sub-item costs for both treatments.

Table 1. Comparison of costs of biological and chemical control of aphids

Treatments	Total Cost (USD/ha)	Sub-items	Sub-items costs (USD/ha)
Insecticide	244.8	Cost of insecticides	13.6×3 times = 40.8
		Cost of labour	68.0×3 times = 204.0
Biocontrol	13.3	Cost of facilities	3.8
		Cost of mass-rearing	5.0
		Cost of releasing	4.5

In addition, aphids were well controlled because of the sustained, long-term release of *A. gifuensis* (Yang et al. 2010) (Fig. 9 and 10).

Meanwhile, populations of other beneficial insects, such as the predators *Coccinella septempunctata* (L.), *Harmonia axyridis* (Pallas), *Episyrphus balteatus* (De Geer), *Chrysopa sinica* (Tjeder), and *Lycosa pseudoamulata* Boes. et Str. obviously increased in the absence of insecticide applications, further protecting biodiversity in the fields and providing long-term ecological benefits resulting from the biological control (Shen et al. 2018).

Application of this technology was started in 2000 in the tobacco-planting area of Yuxi, Yunnan Province (Yunnan Tobacco Yuxi City Company 2010), and by 2010 the entire tobacco planting area of Yuxi had been covered (AERET 2011; Yang et al. 2011).

Figure 9. Aphid population control results without *A. gifuensis* between 1998-2001 (A) and with *A. gifuensis* between 2000-2007 (B).

Figure 10. The population dynamics of aphids in tobacco fields treated by insecticides or the release of parasitoids (left y-axis), or not treated (control) (right y-axis), in tobacco fields in Yuxi, China in 2005.

By 2013, 90% of all the Yunnan tobacco fields had been covered by the augmentative biological control programme. Starting in 2014, this technology has been applied throughout China. Step by step, this technology has expanded to cover 100% of the Yunnan tobacco-planting area and 90% of the Chinese tobacco-planting areas.

Effectiveness of aphid control has reached 80%, better than in pesticide-treated fields (Li et al. 2006). The total application area in China has reached 3 017 547 ha over six years (2010 to 2015), total decrease of pesticide use reached 1 966.05 tons, and total decrease of control costs reached USD 230 million. A financial loss of USD 1 326 million was avoided and 1 million farm households have benefited from this technology.

The use of *A. gifuensis* against *M. persicae* is the biological control technology that is most widely adopted in China. Aphid population control has been transformed from mainly insecticide- to largely biological control-based, promoting a pest control strategy that changed from passive, reactive and chemical insecticide-based to active, preventive and ecologically-founded, with significant social and environmental benefits.

The biological control of aphids by the mass-release of *A. gifuensis* parasitoids in some other crops (Fig. 11) has also reached high efficacies on a large scale (Shen et al. 2018).

Figure 11. Examples of biological control of aphids by *Aphidius gifuensis* augmentative releases on other crops in China.

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