Area-Wide Integrated Pest Management

Development and Field Application

Edited by

Jorge Hendrichs, Rui Pereira and Marc J.B. Vreysen

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PREFACE

The concept of area-wide integrated pest management (AW-IPM), in which the total population of a pest in an area is targeted, is central to the effective control of such populations through the integration of genetic, biological and other pest suppression technologies. Insect movement, occurring sometimes over long distances, is generally underestimated. As a consequence, most conventional pest management is implemented as a localized or field-by-field, un-coordinated action against segments of a pest population, not taking in consideration insect movement, resulting very often in an unsustainable spiral of insecticide application and eventual resistance of the pest against the used insecticides. On the other hand, an AW-IPM approach adopts a preventive rather than a reactive strategy, whereby all individuals of the pest population are targeted in time and space and selecting a time when the pest populations are more vulnerable (e.g. during certain times of the year when the population densities are naturally low), requiring in the longer term fewer inputs and resulting in more cost-effective and sustainable pest management. It involves a coordinated effort over often larger areas, including not only agricultural, but also natural and other areas with pest presence. By addressing these sources of re-infestation in the surroundings of the agricultural areas, satisfactory pest control is achieved in the whole area and fewer control actions are required.

This new textbook on AW-IPM assembles a series of selected papers that attempts to address various fundamental components of AW-IPM, e.g. the importance of relevant problem-solving research, the need for essential baseline data, the significance of integrating adequate tools for appropriate control strategies, and the value of pilot trials, etc. Of special interest are the numerous papers on pilot and operational programmes that pay special attention to practical problems encountered during the implementation of insect pest control programmes. A significant number of contributions to this book resulted from oral and poster presentations at the Third FAO/IAEA International Conference on "Area-wide Management of Insect Pests: Integrating the Sterile Insect and Related Nuclear and Other Techniques", which was successfully held from 22-26 May 2017 at the Vienna International Centre, Vienna, Austria. The conference was attended by 360 delegates from 81 countries and six international organization. However, the book contributions were selected beyond the work presented at the conference and a number of experts dealing mainly with action programmes were invited to present their work in this publication.

The book is a compilation of 48 papers that are authored by experts from 30 countries. Each paper was peer-reviewed by two or more independent, outside experts and edited for the English language. In addition, the editors subjected each paper to an in-depth technical quality control process. As a result, we trust that the information provided is accurate, up-to date and of a high international standard. This process of peer-review, editing and formatting has taken considerable time and we appreciate the patience of the authors.

The Editors
August 2020

FOREWORD

The latest report of the UN Intergovernmental Panel on Climate Change concludes that the world faces serious threats to its food supply. Reaching the Food and Agriculture Organization (FAO)'s goal to eradicate hunger and ensuring food security is only possible if we work together in partnership. Only through effective collaboration with governments, civil society, private sector, academia, research centres and cooperatives, and making use of each other's knowledge and comparative advantages, can our goal of "nourishing people while nurturing the planet" be achieved.

There could hardly be a less efficient use of resources than to invest in land, water, fertilizer, seeds, labour, and energy to produce agricultural commodities, only to have these investments partially or totally destroyed by insects and other pests. Pre-harvest losses in developing countries are currently estimated at more than one third of attainable crop production, while post-harvest losses add at least another 10–20%. Insects, followed by pathogens and weeds, cause the largest portion of these losses.

The availability of effective and persistent synthetic organic insecticides immediately after the Second World War marked the onset of chemically-based insect pest control. The availability and easy accessibility of these "off-the-shelf", relatively cheap and often subsidized chemicals offered farmers the freedom and flexibility to implement insect pest control measures on their property at any time. But, as we know today, chemically-based insect pest control also came at a heavy cost. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target species, air, water and soil. In addition, insecticide use reduces biodiversity, contributes to pollinator decline, destroys habitat, and threatens endangered species. And insect pests develop quickly resistance to insecticides, necessitating new formulations or application of higher doses to counteract the resistance, which exacerbates the pollution problem. The World Health Organization estimates that each year, three million workers in agriculture in the developing world experience severe poisoning from insecticides, about 18 000 of whom die.

Integrated pest management (IPM) has been endorsed by the FAO since 1966 and has remained the dominant paradigm of pest control for the past 50 years. IPM offers a strategic approach to solving pest problems in an ecosystem context, while reducing insecticide use and guarding human health and the environment. The integration of a number of different insect pest control methods into the IPM approach, to facilitate the achievement of these goals, is still primarily done at the local field-by-field, orchard-by-orchard or herd-by-herd level, which is often very ineffective as many insects travel freely between commercial, back-yard and abandoned properties, and between wild hosts and cultivated areas.

A much more effective application of the IPM approach is on an area-wide (AW) or population-wide basis, which aims at the management of the total population of a pest within an often larger but delimited area. This requires close coordination among the numerous stakeholders, a centrally managed approach and strong community involvement.

AW-IPM is now increasingly practiced, especially for mobile insect pests, where management at a larger scale is more effective and preferable to the uncoordinated field-by-field approach. For major livestock pests, vectors of human diseases and pests of high-value crops with low pest tolerance, there are compelling economic reasons for participating in AW-IPM.

Nevertheless, issues around public participation, financing of public goods and free riders, all play a significant role in AW-IPM implementation. These social and managerial issues have, in some cases, severely hampered the positive outcome of AW-IPM programmes and emphasise the need for contemplation not only of ecological, environmental and economic aspects, but also of the social and management dimensions.

For more than five decades the FAO and the International Atomic Energy Agency (IAEA), through their Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, have expanded the use of the sterile insect technique (SIT). This has involved both applied research to improve the technique and to develop it for new pest insects, as well as the transfer of the SIT package to member countries so that these can benefit from improved plant, animal and human health, cleaner environments, increased crop and animal production and accelerated economic development.

Today, the Joint FAO/IAEA Programme supports some 35 field projects that integrate the SIT to manage populations of major insect pests, including several species of tsetse flies and fruit flies, screwworm flies and moths. And endeavours are currently underway to develop the SIT for the control of mosquitoes, important vectors of major diseases such as malaria, dengue, chikungunya and Zika, and a main hindrance to economic development in endemic areas and a serious threat to as yet non-endemic areas. The development and validation of effective and sustainable approaches to managing pests and diseases will be essential to meet major future challenges.

Ren Wang

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SECTION 1

OPERATIONAL AREA-WIDE PROGRAMMES

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 m^2 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.

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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).

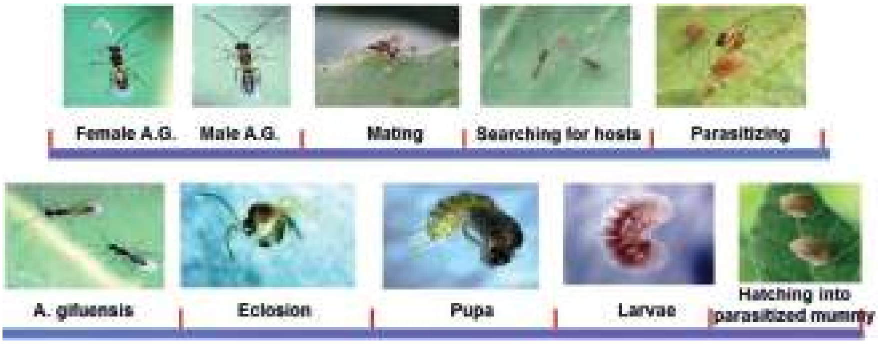

Figure 1. Life cycle and biological stages of Aphidius gifuensis (*A.G.*) (*starting clockwise from top left*).

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).

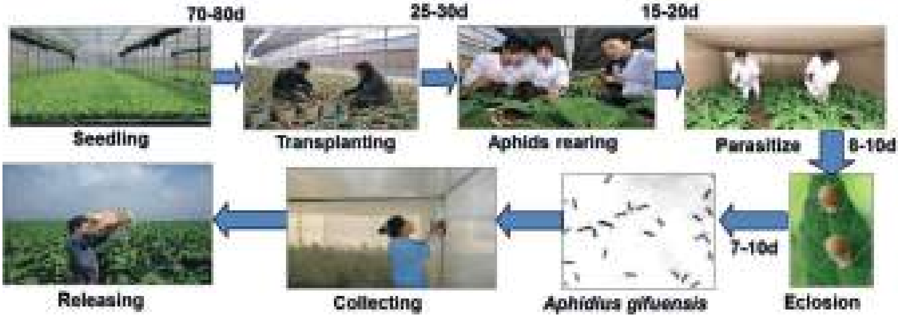

Figure 2. Breeding process of Aphidus gifuensis *in greenhouses* (*starting clockwise from top left*).

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 *Aphidus gifuensis* separately".

Alternatively, tobacco seedlings in the 3rd - 4th leaf stage can be used to breed aphids by the "Method of breeding aphid and *Aphidus 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).

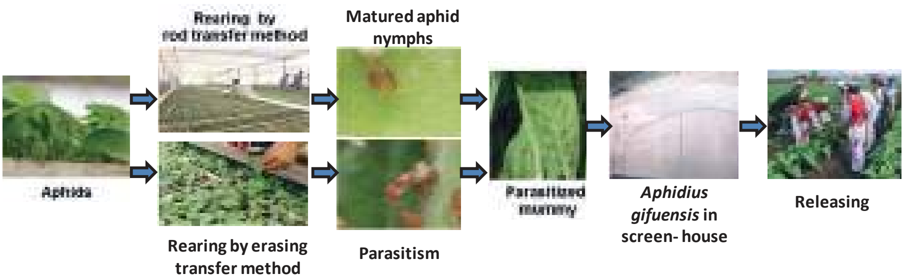

Figure 3. Breeding process of aphids and Aphidius gifuensis.

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).

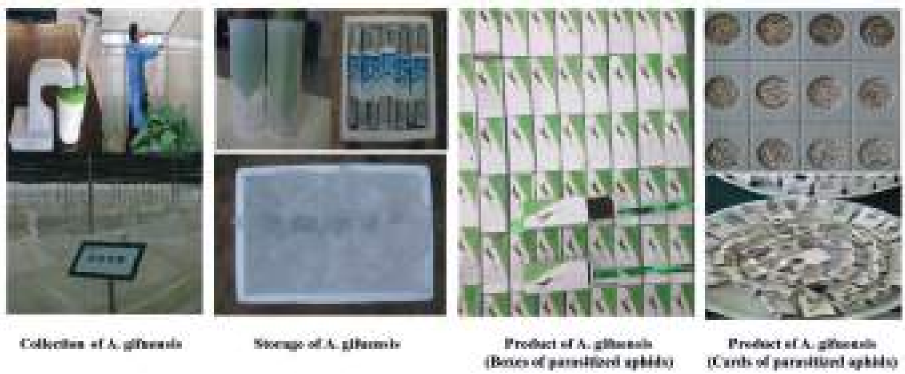

Figure 4. Collection and release processes for Aphidius gifuensis *parasitoids*.

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 m^2 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).

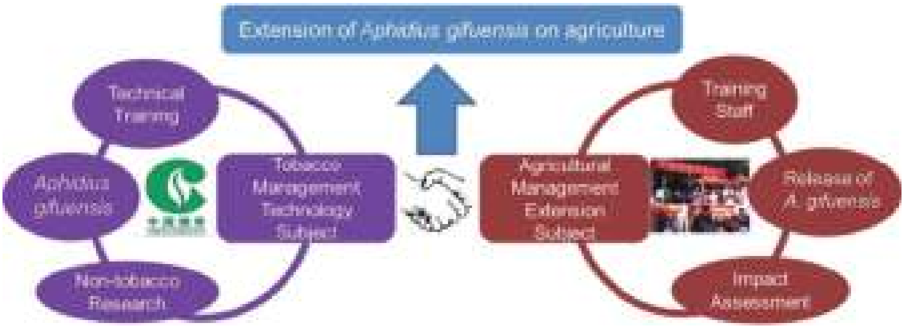

Figure 5. Extension system for biological control of tobacco aphid by parasitoid augmentative releases.

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).

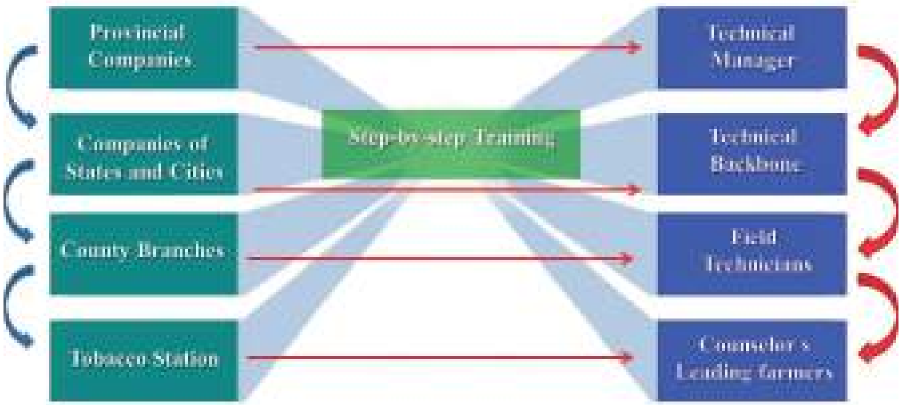

Figure 6. Training system for biological control of tobacco aphid Aphidius gifuensis *by parasitoids.*

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*.

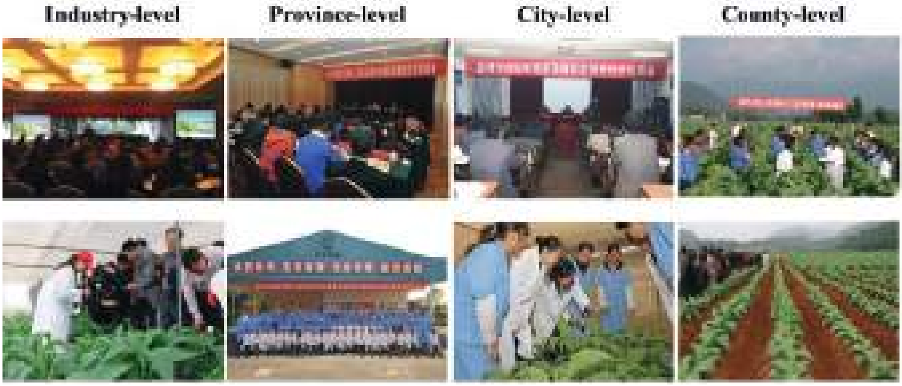

Figure 8. Four-level training for biological control of tobacco aphid by mass-release of Aphidius gifuensis *parasitoids.*

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).

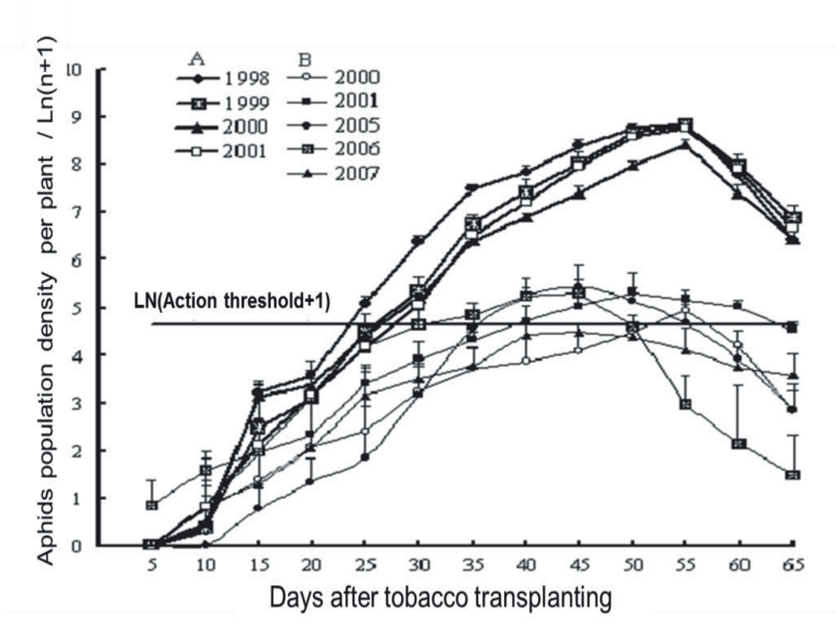

Figure 9. Aphid population control results without A. gifuensis *between 1998-2001 (A) and with* A. gifuensis *between 2000-2007 (B).*

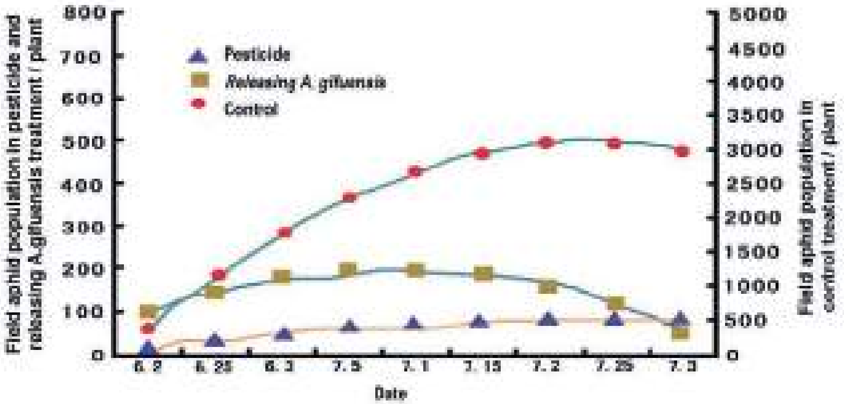

Figure 10. The population dynamics of aphids in tobacco fields treated by insecticides or the release of parasitoids (left y-axis), or not treated (control) (right y-axis), in tobacco fields in Yuxi, China in 2005.

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.*

5. ACKNOWLEDGEMENTS

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BIOLOGICAL CONTROL: CORNERSTONE OF AREA-WIDE-INTEGRATED PEST MANAGEMENT FOR THE CASSAVA MEALYBUG IN TROPICAL ASIA

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SUMMARY

The cassava mealybug *Phenacoccus manihoti* Mat.-Ferr. (Hemiptera: Pseudococcidae) is a globally important pest of cassava (*Manihot esculenta* Crantz), a crop that is cultivated on nearly 25 million ha across the tropics. Following its continent-wide invasion of Africa during the 1970s and early 1980s, *P. manihoti* was inadvertently introduced to Southeast Asia in late 2008, where it caused important yield drops in local crops. Guided by the widely-acclaimed biological control successes against this mealybug in Africa, the endophagous parasitoid *Anagyrus lopezi* De Santis (Hymenoptera: Encyrtidae) was introduced to Thailand in 2009. Subsequent introductions of *A. lopezi* were made into neighbouring countries, and an integrated campaign was launched to scale-up mealybug biological control. Multi-country field surveys were carried out to map *P. manihoti* geographic distribution, field-level abundance and extent of parasitoid-mediated suppression, and innovative extension programmes were deployed to raise farmer awareness of mealybug pests and associated natural enemies. Survey work from nearly 600 fields throughout mainland Southeast Asia revealed that *P. manihoti* occurred at abundance levels of 14.3 ± 30.8 individuals per tip in the dry-season, and *A. lopezi* parasitism averaged at 38.9%. An applied research programme yielded critical insights into various determinants of *A. lopezi* establishment, spread and biological control efficacy. In close collaboration with national partners, research was carried out on the eventual effects of soil fertility and plant nutrition, landscape composition, and a plant's phytopathogen infection status, amongst others. Our work shows how the host-specific *A. lopezi* effectively suppresses the cassava mealybug across a range of agro-climatic, biophysical and socio-economic contexts in tropical Asia, and constitutes a central component of area-wide integrated pest management (AW-IPM) for this global pest invader. This study also underlines the need for holistic, transdisciplinary approaches to (invasive) pest management, and the tangible yet (largely) untapped potential of coupling social and biological sciences to address crop protection problems in the developing-world tropics.

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Key Words: Classical biological control, ecosystem services, invasive species management, food security, landscape diversity, natural enemy, trophic ecology, tropical agriculture, *Manihot esculenta, Phenacoccus manihoti, Anagyrus lopezi*

1. INTRODUCTION

Cassava mealybug *Phenacoccus manihoti* Mat.-Ferr. (Hemiptera: Pseudococcidae) is a prominent herbivore on cassava (*Manihot esculenta* Crantz) and one of the world's most notorious invasive species. Endemic to the Paraguay River basin in South America, *P. manihoti* was inadvertently introduced into Africa during the early 1970s and rapidly spread across the continent's extensive cassava belt (Herren and Neuenschwander 1991; Bellotti et al. 2012). Capable of inflicting yield losses up to 58-84% (Nwanze 1982; Schulthess et al. 1991), *P. manihoti* devastated local cassava production and caused widespread hunger for farming families across sub-Saharan Africa.

In late 2008, this same pest was detected in Thailand's eastern seaboard, where it caused an 18% drop in aggregate crop yield of cassava, a yearly loss of over 8 million ton fresh root in Thailand alone, and more than 2-fold surges in prices of cassava starch (Muniappan et al. 2009; Wyckhuys et al. unpublished). By 2011, *P. manihoti* had spread extensively in Thailand and had inflicted economic losses on the country's cassava sector of over USD 30 million nationally (TTTA 2011).

In 2014, *P. manihoti* had also entered prime cassava growing areas in neighbouring Cambodia, Indonesia, Lao PDR, Malaysia, and Viet Nam (Sartiami et al. 2015; Graziosi et al. 2016). Climate-based niche modelling revealed that other key cassava production areas in eastern Indonesia and the Philippines are also at risk to *P. manihoti* (Yonow et al. 2017). As Southeast Asia accounts for nearly 95% of the world's cassava exports and is home to a multi-billion-dollar cassava starch industry (Cramb et al. 2017), *P. manihoti* was expected to inflict major socio-economic impacts at a regional level.

1.1. Control of Cassava Mealybug in Africa

Though this mealybug invader evidently posed an immediate threat to the rural economy of several Asian countries, a nearly tailor-made management solution had been successfully developed in Africa more than thirty years ago. In fact, after the 1980 discovery of *P. manihoti* in Paraguay by A. Bellotti (International Center for Tropical Agriculture, CIAT), one of the world's best-known and successful insect classical biological control programmes was initiated (Bellotti et al. 1999; Neuenschwander 2001). In 1981, the Centre for Agriculture and Bioscience International (CABI) and the International Institute of Tropical Agriculture (IITA) teamed up to carry out foreign exploration in the presumed region of origin of *P. manihoti*, ultimately resulting in the collection and subsequent shipment of the *Anagyrus lopezi* De Santis (Hymenoptera: Encyrtidae) (Löhr et al. 1990).

Following its 1981 release in western Nigeria, *A. lopezi* promptly established and suppressed *P. manihoti* population levels from more than 100 to fewer than 10–20 individuals per cassava tip (Hammond et al. 1987). In less than three years following its release, *A. lopezi* had effectively dispersed over 200 000 km^2 in south-western

Nigeria. It had also been mass-reared and distributed across multiple release points in several African countries (Herren et al. 1987). Though multiple endemic primary parasitoids and hyperparasitoids were recorded in mealybug-invaded areas in Africa (Neuenschwander et al. 1987; Neuenschwander and Hammond 1988), these largely did not impede the success of *A. lopezi* as a biological control agent.

Overall, the parasitoid wasp successfully established in 26 African countries, prevented wide-spread famine and generated economic benefits of USD 9400-20 200 million (Zeddies et al. 2001). Moreover, across the highly diverse and vast African continent, no agro-ecological conditions were found under which *A. lopezi* was unable to establish and attack its mealybug host (Neuenschwander 2001).

1.2. Cassava Mealybug in Southeast Asia

Soon after its detection in Asia, Thailand's late Amporn Winotai of the Department of Agriculture (DoA) solicited assistance from CIAT's Anthony Bellotti to tackle the fast-spreading mealybug pests in her country. Well aware of the accomplishments in Africa, A. Bellotti rightly pointed Thai colleagues to G. Goergen and P. Neuenschwander at the IITA station in Cotonou, Benin. In late 2009, *A. lopezi* was then effectively introduced from West Africa into Thailand, and rearing labs were established in different parts of the country, through a joint endeavour between the Food and Agriculture Organization of the United Nations (FAO), centres of the Consultative Group on International Agricultural Research (CGIAR), and Thailand's Royal Government (Winotai et al. 2010).

The *A. lopezi* releases received ample public attention and quickly culminated in an unprecedented, nation-wide campaign to mass-rear and distribute wasps, in which government institutions, grower associations and private sector actors, such as the Thai Tapioca Development Institute (TTDI), all joined forces. Within the context of a regional technical cooperation project, mass-releases of *A. lopezi* were carried out across Thailand, some of which by airplane, and were followed by FAO-led introductions into Cambodia, Lao PDR and Viet Nam. The 2014 release of several hundred *A. lopezi* pairs into Indonesia were enabled by A. Rauf at Bogor Agricultural University (Wyckhuys et al. 2015). All these parasitoid introductions and capacity building interventions were implemented with the Royal Thai Government Ministry of Agriculture and Cooperatives' technical assistance, most notably from key IPM experts in its Department of Agriculture (DoA) and Department of Agricultural Extension (DoAE).

Other methods promoted for *P. manihoti* control include neonicotinoid stake dips (e.g. Parsa et al. 2012) and mass-releases of laboratory-reared predators and entomopathogens (Saengyot and Burikam 2012; Sattayawong et al. 2016). Despite the above efforts to promote a wide range of chemical and biologically-based management tactics, it is widely thought that it is *A. lopezi* that suppressed cassava mealybug populations across mainland Southeast Asia.

In this paper, we provide an in-depth assessment of *P. manihoti* population pressure and Asia-wide distribution, report on the establishment and spatial spread of the introduced *A. lopezi*, and examine biophysical, agro-climatic, and social factors that might enhance or impede mealybug biological control.

2. MAPPING MEALYBUG DISTRIBUTION

Until the appearance of *P. manihoti* in Asia's cassava fields, there was only scant and scattered information about the nature, distribution, etiology, epidemiology and ecology of the primary phytosanitary constraints of this crop in Southeast Asia (Bellotti et al. 2012; Graziosi and Wyckhuys 2017). In late 2013 though, an ambitious surveillance programme was set up together with international and national partners in Cambodia, Lao PDR, Myanmar, southern China, Thailand, and Viet Nam. With backstopping through the late Prabat Kumar at the Asian Institute of Technology, this programme intended to map the geographic distribution of *P. manihoti*, assess its pest pressure in local cassava fields, chart its potential invasion pathways, and understand its relative importance in relation to other arthropod pests and plant diseases. Ultimately, the programme sampled more than 572 cassava fields over 2 years, covering areas as diverse as Viet Nam's Central Highlands, the Ayeyawaddy delta of Myanmar or the remote uplands of southern Lao PDR.

Survey protocols are described in detail in Graziosi et al. (2016). In brief, we randomly selected older fields (>5-6 months old) in the main cassava-growing provinces within each country, with separate plots located at least 1 km apart. Surveys were carried out in January-May 2014 (dry season), October-November 2014 (late rainy season) and January-March 2015 (dry season). Location and elevation of each field were recorded using a handheld GPS unit (Garmin Ltd, Olathe, Kansas, USA). Per field, five linear transects were randomly chosen, with each transect covering 10 plants. By doing so, a total of 50 plants per field were assessed for *P. manihoti* infection status and associated 'bunchy top' symptoms (Neuenschwander et al. 1987), and per-plant mealybug abundance. In-field identification of mealybugs was based on morphological characters such as colour and length of abdominal waxy filaments. Following transect walks, we computed average *P. manihoti* infestation pressure (number of individuals per infected tip) and estimated field-level incidence of this pest (proportion of *P. manihoti*-affected tips, or 'bunchy tops') for each field.

Mealybugs are the most widespread group of arthropods on cassava crops in Southeast Asia, occurring in 70% of cassava fields (Graziosi et al. 2016). In some countries, such as Myanmar and Thailand, mealybugs were found in 95 and 100% of the fields, respectively. In infested fields, mealybugs were found on 27 ± 2% of plants, this representing the highest incidence among cassava-associated arthropods. The resident mealybug community on cassava was composed of 4 non-native species: (1) *P. manihoti*; (2) the papaya mealybug *Paracoccus marginatus* Williams & Granara de Willink; (3) *Pseudococcus jackbeardsleyi* Gimpel & Miller; and (4) the striped mealybug *Ferrisia virgata* Cockerell.

Within this mealybug community, *P. manihoti* represented 19.8% of the species complex (n= 572 fields, across dry and rainy season), and was recorded from 37% of fields during the 2014 dry season. The cassava mealybug was recorded from fields across Cambodia and Thailand, and it was also recorded in southern parts of Lao PDR and Viet Nam (Fig. 1). Across sites and sampling events, *P. manihoti* was recorded at average incidence rates of 7.4 ± 15.8% and dry-season abundance of 14.3 ± 30.8 individuals per infected tip. Maximum incidence rates were 100%, and maximum field-level abundance was 366.6 mealybugs per tip. Field-level abundance and incidence rates were highly variable between settings and countries (Table 1).

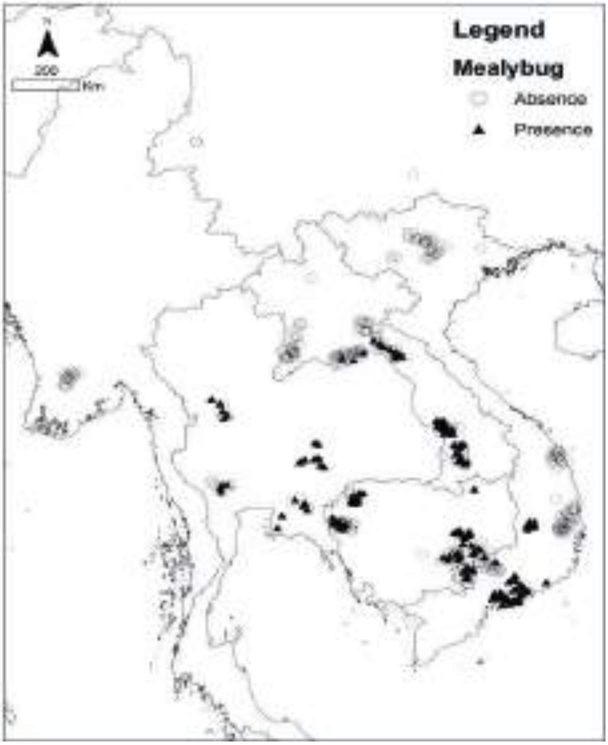

Figure 1. Geographic distribution of Phenacoccus manihoti, *as recorded during 2014-15 surveillance across the Greater Mekong subregion.*

Overall, in Asia's primary cassava cropping areas, current mealybug pest pressure was nearly identical to that in Africa during the mid-1980s. At the time, *P. manihoti* populations collapsed soon after parasitoid introduction and stabilized at incidence rates of 23% and abundance levels below 10 individuals per tip (Hammond and Neuenschwander 1990).

3. PARASITOID ESTABLISHMENT, SPREAD AND INCIDENCE

To assess *A. lopezi* establishment patterns and parasitism rates, and to delineate the parasitoid community associated with *P. manihoti*, we conducted two observational studies. First, over the course of two consecutive growing seasons, bi-monthly sampling was carried out in Tay Ninh, Viet Nam to characterize mealybug-parasitoid population dynamics (Le et al., unpublished). Second, dry-season sampling was done during 2014-2016 at a number of mealybug-invaded sites in eastern Cambodia (n = 15), eastern Thailand (n = 20), and southern Viet Nam (n = 19, 40). In each experiment, sampling consisted of collecting a total of 20 mealybug-infested tips or 'bunchy tops' from local cassava fields that were transferred to the laboratory for subsequent assessment of parasitoid emergence.

Table 1. Average incidence (percentage mealybug-infested plants per field; mean ± SD) and abundance (number of individuals per infected tip) of Phenacoccus manihoti *as recorded during multi-country surveillance in the 2014-15 growing seasons*

Country	Province	Date	Season	Sample size (*n*)	Incidence (%)	Abundance (# / tip)
Thailand	Nakhon Ratchasima	Mar 2014	Dry	10	22.6 ± 16.1	16.3 ± 18.5
	Kampheang Phet	Mar 2014	Dry	9	3.8 ± 5.2	9.2 ± 8.8
	Chachoengsao	Mar 2014	Dry	9	2.9 ± 4.7	6.5 ± 5.4
	Kanchanaburi	Mar 2014	Dry	11	6.6 ± 13.2	37.0 ± 58.9
Lao PDR	Borikhamxay	Feb 2014	Dry	27	0.5 ± 1.9	1.7 ± 1.5
	Vientiane capital	Feb 2014	Dry	22	0.1 ± 0.4	1.0 ± 0.0
	Xiengkhuong	Jan 2014	Dry	6	0.0	0.0
	Xayabuli	Feb 2014	Dry	20	0.0	0.0
	Champasak	Feb 2014	Dry	25	4.2 ± 11.1	3.4 ± 4.2
	Salavan	Feb 2014	Dry	25	13.6 ± 18.3	6.3 ± 9.6
Cambodia	Banteay Meanchey	Feb 2014	Dry	20	13.8 ± 11.9	7.7 ± 4.4
	Kampong Cham	Feb 2014	Dry	20	6.7 ± 18.2	14.4 ± 14.4
	Pailin	Feb 2014	Dry	19	9.5 ± 17.9	5.5 ± 3.9
	Battambang	Mar 2014	Dry	20	0.3 ± 0.9	1.2 ± 0.3
	Kratie	Feb 2014	Dry	20	16.3 ± 14.2	24.7 ± 26.5
Myanmar	Ayeyawaddy	Apr 2014	Dry	20	0.0	0.0
Viet Nam	Dong Nai	Feb 2014	Dry	20	43.7 ± 19.7	32.5 ± 14.6
	Binh Phuoc	Apr 2014	Dry	21	3.0 ± 13.4	7.7 ± 0.0
	Ba Ria-Vung Tau	May 2014	Rainy	20	35.9 ± 29.4	11.1 ± 17.0
	Tay Ninh	May 2014	Rainy	21	5.0 ± 8.2	5.0 ± 2.3
	Phu Yen	Apr 2014	Dry	19	0.0	0.0
	Dak Lak	Mar 2015	Dry	10	11.6 ± 6.4	3.7 ± 2.4
	Quang Ngai	Apr 2014	Rainy	20	0.0	0.0
	Binh Thuan	Mar 2015	Dry	10	9.2 ± 14.5	7.1 ± 12.8
	Yen Bai	Oct 2014	Rainy	20	0.0	0.0
	Phu Tho	Oct 2014	Rainy	19	0.0	0.0
China	Yunnan	Nov 2014	Rainy	25	0.0	0.0

Sampling procedures were adapted from Neuenschwander and Hammond (1988) and consisted of breaking off 20-cm ‘tips’ of infested plants and placing these in sealed paper bags. Next, bags with plant material were transferred to the laboratory, where each tip was carefully examined and the total number of *P. manihoti* was counted. Next, cassava tips were individualized within transparent polyvinyl chloride (PVC) containers and covered with fine cotton fabric mesh. Over the course of 3 weeks, containers were stored at ambient conditions and inspected on a daily basis for emergence of parasitoid wasps. Next, parasitoids and potential hyperparasitoids were collected by aspirator and stored for subsequent identification.

In the first study, *P. manihoti* occurred at an average incidence of 24.8 ± 17.7% and abundance level of 5.6 ± 5.3 individuals per tip across both growing seasons. In general, mealybug populations built up during the second half of the dry season and remained at low levels during the rainy season. High *A. lopezi* parasitism levels were recorded during each year, at average levels of 50.3% and 43.9% in rainy and dry season, respectively. Though rainfall does indeed cause high mortality of *P. manihoti*, it is believed that *A. lopezi* accounts for the sustained low mealybug population levels across seasons, through direct *P. manihoti* parasitism and host-feeding. The primary parasitoid community was entirely composed of *A. lopezi*, yet three potential hymenopterous hyperparasitoid species were also found from sites in Tay Ninh (Viet Nam): *Chartocerus* sp. near *walkeri* (Signiphoridae), *Promuscidea unfasciativentris* Girault (Eriaporidae) and *Prochiloneurus* sp. (Encyrtidae). Hyperparasitism levels were on average 2.8 ± 5.4%, with maximum rates of 26.4%. In smallholder cassava fields in eastern Cambodia, the hyperparasitoid community was found to be more diverse and species-rich, though locally-recorded species remain to be identified (Wyckhuys et al. 2017c).

In the second study, *A. lopezi* was found in *P. manihoti*-affected fields in Cambodia, Thailand and Viet Nam at parasitism levels of 10-57%, with an overall average of 38.9% (Wyckhuys et al. 2017b). Both studies exemplify how the introduced parasitoid has effectively colonized cassava fields in at least three Asian countries, attaining medium to high parasitism rates and contributing to *P. manihoti* control under a variety of agro-ecological conditions.

4. MULTI-FACETED DETERMINANTS OF BIOLOGICAL CONTROL SUCCESS

Field surveys and observational studies across the tropical Asia region have shown relatively low *P. manihoti* infestation levels, yet highly variable *A. lopezi* parasitism rates. For example, while *A. lopezi* attains dry-season parasitism of 16.3 ± 3.4% in coastal Viet Nam, it attains rates of 52.9 ± 4.3% in intensified cropping systems in the Tay Ninh province (Fig. 2).

To gain a better appreciation of potential constraints to *A. lopezi* success, we examined *P. manihoti* biological control through a number of different lenses, drawing on disciplines such as landscape ecology, plant pathology and soil science. Other factors, such as access to floral nectar and interference through tending ants are being investigated by A. Rauf and students in Indonesia, but they are not reported in this paper.

4.1. Soil-Mediated Effects on Mealybug Biological Control

Soil fertility and structure can determine plant health and shape overall resistance to pests (Amtmann et al. 2008), however, the impact of below-ground processes on above-ground interactions varies and is particularly difficult to predict. Also, alterations in plant nutrients are readily transmitted through trophic chains and affect the relative role of resource ("bottom-up") versus consumer ("top-down") forces in the structuring of ecological communities (Hunter and Price 1992). The success of both native and invasive herbivores has been explained through a range of theories and hypotheses, some of which simultaneously account for the role of the above plant resource availability and natural enemies (Blumenthal 2005; Center et al. 2014). Hence, understanding how certain herbivores (such as *P. manihoti*) and their associated parasitoids such as *A. lopezi* interact and respond to soil fertility and plant nutrient status is extremely valuable.

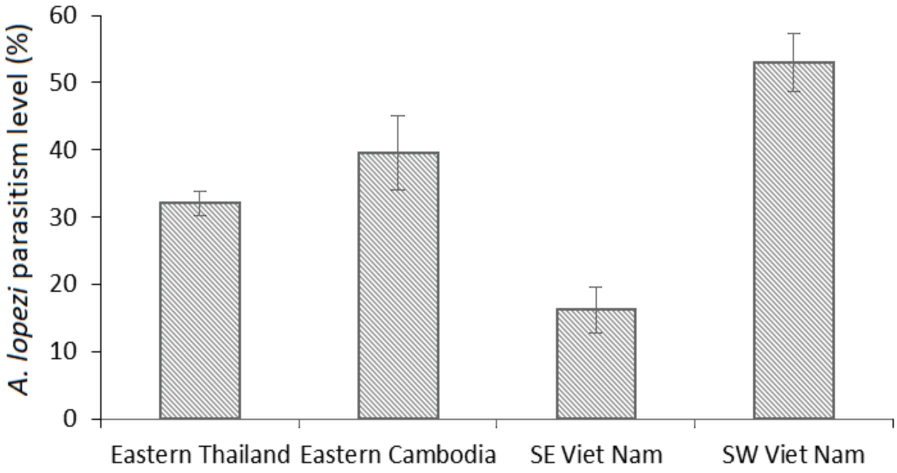

Figure 2. Parasitism levels (mean ± SD) by Anagyrus lopezi*, as recorded from selected fields in the invaded range of* Phenacoccus manihoti *(respective sample size: 20, 15, 19, 40).*

During 2015-2016, a set of manipulative trials and observational studies were carried out to illuminate how soil fertility affects *P. manihoti* x *A. lopezi* interactions (Wyckhuys et al. 2017b). More specifically, potted-plant fertilizer trials were combined with a regional survey of 65 cassava fields with varying soil fertility. Pot trials revealed strong bottom-up effects for *P. manihoti*, with nitrogen and potassium addition equally boosting development and fitness of *A. lopezi*.

Field surveys indicate that mealybug performance is highly species-specific and context-dependent. For *P. manihoti*, in-field abundance is associated with soil texture, i.e. silt content, and mealybug populations are disproportionately favoured in low-fertility conditions. Parasitism by *A. lopezi* varied greatly with field and soil fertility conditions and was highest in soils with intermediate fertility levels and where management practices include the addition of fertilizer supplements.

These findings on the field context show how deficient soil management can further exacerbate mealybug pest problems and ultimately push farmers into ‘poverty traps’. On the other hand, our work can help target additional biological control measures and inform management practices, such as mulching, organic matter addition, or corrective nutrient supplementation, to enhance or restore mealybug biological control.

Similar impact across several trophic levels had been documented also in Africa. In Malawi, for instance, biological control despite the presence of *A. lopezi* only failed in the few (10%) fields on sandy, un-mulched soils that did not sustain leafy plants (Neuenschwander et al. 1991).

4.2. Plant-Microbe-Insect Interactions

In recent years, scientific interest in plant-insect-microbe or ‘cross-kingdom’ interactions has boomed, steadily revealing the multiple, intricate ways in which micro-organisms mediate plant-herbivore interplay (Ponzio et al. 2013; Tack and Dicke 2013). Phytopathogens regularly alter whole repertoires of plant phenotypic traits, and they bring about shifts in key chemical or morphological characteristics of plant hosts (Tack et al. 2012; Biere and Tack 2013). Though largely overlooked, pathogens can also cause cascading effects on higher trophic levels and eventually shape entire plant-associated arthropod communities.

In an observational study in early 2016, I. Graziosi and Cambodian colleagues investigated whether a *Candidatus* Phytoplasma causing cassava witches' broom (CWB) is altering relative abundance and species composition of different invasive mealybugs and determines success of their associated parasitoids, including *A. lopezi*. The CWB is an emerging phytopathogen that occurs at near-pandemic levels in several parts of Southeast Asia, and which causes leaf discoloration, extensive proliferation of leaves and stems, and stunted growth. In their study, samples were taken from multiple sites of CWB-symptomatic and asymptomatic plants (Wyckhuys et al. 2007c). From each plant, the apical part or ‘tip’ was collected and transferred to the laboratory for further processing. After counting and identifying all mealybug individuals, each cassava tip was transferred individually to transparent PVC containers, closed with fabric mesh. Over a period of 14 days, containers were checked for emergence of parasitoids or hyperparasitoids. Parasitoids were identified to morpho-type and stored in ethanol for subsequent species-level identification. CWB infection was found to positively affect overall mealybug abundance and species richness, and to disproportionately favour the generalist *Paracoccus marginatus* over *P. manihoti* (Fig. 3).

Moreover, CWB phytoplasma infection was positively correlated with an increased parasitoid richness and diversity. Though overall parasitism rate did not differ among CWB-infected and uninfected plants, lower numbers of *A. lopezi* were obtained from infected plants. Also, CWB-infection status affected *A. lopezi* sex ratio, with more male-biased sex ratios on CWB-infected plants (Wyckhuys et al. 2017c). This possibly could be explained by smaller ‘undernourished’ mealybugs which are more often selected by females for male eggs.

This work underlines how systemic plant pathogens such as CWB do impact parasitoid establishment and efficacy, and how they could influence *P. manihoti* biological control. Hence, entomologists need to work across disciplines and take into consideration plant pathology aspects when assessing field-level parasitism rates and biological control success.

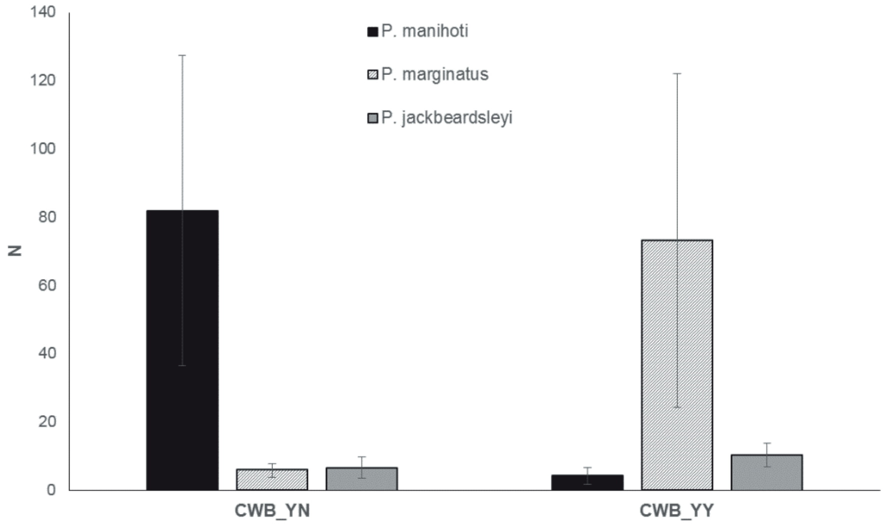

Figure 3. Plant-level abundance of different mealybug species on plants with characteristic symptoms of asymptomatic plants (CWB_YN) and cassava witches' broom (CWB_YY).

4.3. Landscape-Level Drivers

As exemplified in the above Sections, patch-level characteristics, such as soil fertility or plant disease pressure, greatly impact *A. lopezi* abundance and performance. On the other hand, landscape-level variables may equally affect biological control of invasive pests such as *P. manihoti*, though have rarely been taken into consideration.

The impact of landscape structure on natural enemy abundance, diversity, and activity in temperate cropping systems such as grains, canola or cabbage crops is fairly well documented (Bianchi et al. 2006; Chaplin-Kramer et al. 2011; Schellhorn et al. 2015), though much less is known about its overall impact on pest pressure or natural biological control. However, for specialist parasitoids such as *A. lopezi*, landscape simplification could be particularly disruptive (Cagnolo et al. 2009). Also, landscape complexity differentially benefits hyperparasitoids, which potentially could derail biological control of *P. manihoti* (Rand et al. 2012).

In mid-2013, T. T. N. Le and collaborators from Viet Nam's Plant Protection Department (VPPD) embarked upon a two-year study to assess mealybug x parasitoid population dynamics under varying landscape context (Le et al. 2018). Over the course of two consecutive cropping seasons, insect populations were surveyed under small-field and high-diversity or large-field and low-diversity landscape settings. In certain areas, cassava fields are small (1–2 ha in size), embedded within relatively complex and diverse landscape settings (here termed 'high-diversity' sites). Other landscape sectors are primarily made up of larger fields, ranging between 4 and 8 ha (here termed 'low-diversity' sites). Overall, *P. manihoti* colonized fields earlier and attained higher incidence in small plots within high-diversity landscapes as compared to large fields in simplified landscapes (Fig. 4).

Landscape type, however, significantly affected hyperparasitism rate at certain crop ages, but did not impact *P. manihoti* abundance or *A. lopezi* parasitism rate. Also, a slightly more pronounced density-dependent response of *A. lopezi* was found within low-diversity settings, at a scale of both individual cassava tips and entire fields. These landscape-dependent impacts likely directly relate to dispersal modalities and other ecological traits of *A. lopezi*, including its supreme ecological plasticity, exceptional dispersal capacity and ability to equally host-feed and consume cassava extra-floral nectar (Neuenschwander 2001).

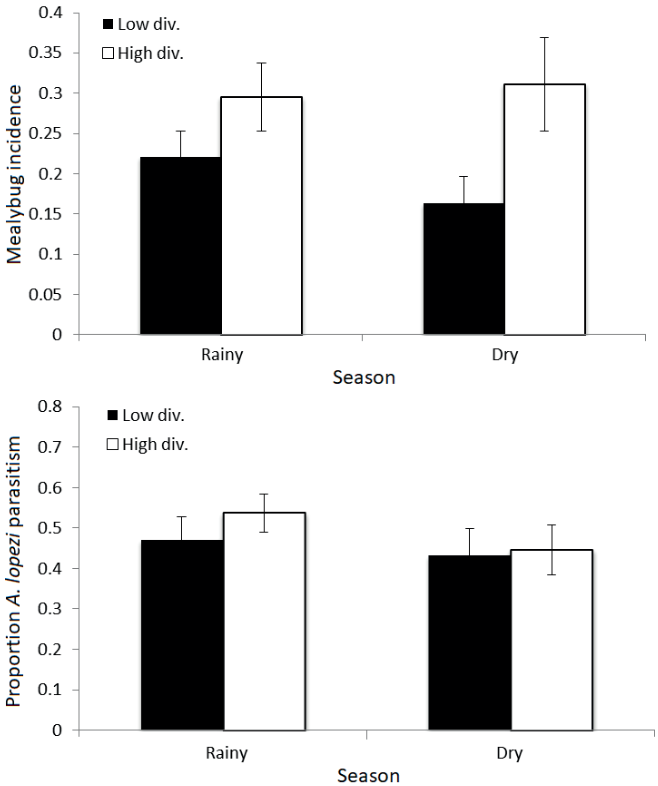

Figure 4. Phenacoccus manihoti *incidence (proportion infected plants) and* Anagyrus lopezi *parasitism rate during dry and rainy season, for fields within high- and low-diversity landscape settings (see Le et al. 2018).*

5. SOCIAL SCIENCE: COMPASS AND PUBLIC AWARENESS FOR A REGIONAL BIOLOGICAL CONTROL CAMPAIGN

Though regularly overlooked or roundly disregarded, social science is of paramount importance for the successful promotion of IPM, and particularly for effective biological insect control. More so, only a fraction of biological control studies over the past 25 years explicitly address social science or technology transfer aspects (Wyckhuys et al. 2017a).

A retrospective analysis of social science studies from the developing-world tropics showed that most farmers have highly-deficient knowledge of natural enemies, and routinely express doubts about the exact value of biological control services on their farm. More so, farmers' knowledge of (fast-spreading) invasive pests and their control is considered to be exceptionally weak. These trends are worrying and could stifle farm-level adoption and subsequent diffusion of knowledge-intensive technologies such as biological control (Catalini and Tucker 2017).

Though classical biological control - as in the case of *A. lopezi* - is largely considered to be self-propelling and requires little or no intervention by farmers (Andrews et al. 1992), it is critical to build and strengthen farmers' agro-ecological knowledge in light of the active promotion of systemic insecticides for mealybug control. At the time of writing this chapter, hundreds of thousands of smallholder growers in one particular Asian country had embraced the use of prophylactic dips with neonicotinoids and considered those as a central component in their cassava crop management. These products cause long-term negative impacts on *A. lopezi* survival and fitness (Lankaew, Tan, Nguyen and Wyckhuys, unpublished), and as such they could hamper biological control.

In late 2014, a two-country survey was started to characterize agro-ecological knowledge, attitudes and pest management practices of local cassava growers (Uphadyay et al. 2018). A parallel study was done by A. Rauf and N. Wardani in Indonesia. In Lao PDR and Viet Nam, farmers had limited awareness of recent invasive pests, such as *P. manihoti*, and their knowledge was highly context- and locality-specific, i.e. shaped by the invasion history of the mealybug. Only the occasional farmer was aware of the existence of natural enemies on his/her farm, and even charismatic and important guilds such as lady beetles, spiders or lacewings were recognized by <10% local growers. Overall, a minority of farmers used preventive tactics and in recently invaded fields frequently resorted to drastic measures such as overhead sprays of insecticides and burning of entire fields. In southern Viet Nam, women guide household-level IPM decision-making (Uphadyay et al. 2018), yet their weak agro-ecological knowledge base could further enable local diffusion of insecticide use.

To counteract some of the above trends and ease obstacles in farmer learning about biological control, a video-mediated extension campaign was launched. Farmer-to-farmer video is particularly suited to transfer complex concepts such as parasitism and insect predation, and it can help secure *P. manihoti* biological control in Asia's cassava systems. A farmer to farmer video was developed by carefully selecting farmers from the FAO-deployed Farmer Field Schools in eastern Thailand and documenting some of their successes with *P. manihoti* control. This allowed production of a multi-lingual video entitled '*Managing Mealybugs in Cassava*' by the Belgium-based company AgroInsight. This video is available for streaming in English and multiple Asian languages through Access Agriculture (2019) or YouTube channels. This farmer-to-farmer video was subsequently distributed through multiple means, including private sector actors, national television and rural extension bureaus, reaching >200 000 growers in a matter of months in Viet Nam alone.

6. CONCLUSIONS

This chapter aims at providing a comprehensive overview of the multi-institutional biological control programme against the invasive mealybug *P. manihoti* in cassava crops across mainland Southeast Asia. Two-year population surveys and area-wide pest surveillance reveal how *P. manihoti* has effectively spread to at least six Asian countries, yet occurs at low to intermediate incidence rates and at abundance levels of 10-20 individuals per tip. Though rainfall and humidity are responsible for important levels of mortality, *P. manihoti* is deemed to be under effective biological control by the introduced *A. lopezi*. Five years after its introduction in eastern Thailand, *A. lopezi* is presently recorded at high though variable population levels in most mealybug-invaded fields in the region. Although not covered in this chapter, chemical and physical exclusion assays have shown how the resulting *P. manihoti* infestation rates only have minor impact on cassava crop yield or harvest indices. Hence, the minute wasp that was originally sourced in southern Brazil and Paraguay in 1981, and released across Africa during the 1980s, now also brings relief to cassava farmers in tropical Asia. The impact of this biological control programme on farmer livelihoods, national economies and rural agro-industries still needs to be assessed, but the economic benefits are expected to equal and probably surpass the multi-billion dollar benefits that were estimated in Africa (Zeddies et al. 2001; Wyckhuys et al. 2018).

Some of the factors that underpinned the outright success of this tropical Asia-wide biological control campaign are the following:

1. Globe-spanning collaboration between FAO, CABI and CGIAR institutions, plus ready access to insect biodiversity in countries such as Brazil and Paraguay, allowed the necessary foreign exploration and effective identification of suitable natural enemies. Next, a swift mobilization of government institutions in Thailand and the strong support from private sector actors such as TTDI proved to be of crucial importance in ensuring establishment and country-wide spread of *A. lopezi*.

2. Extension campaigns that were built upon a sound appreciation of farmers' knowledge, attitudes and practices across farming contexts and sites. Though conventional extension initiatives were effective in Thailand, a farmer-to-farmer educational video proved to be key to transferring growers' experiences, perceptions and innovations from early-adopters and Farmer Field School groups in invaded areas. This undoubtedly boosted preparedness and prevented certain detrimental practices — such as unguided use of insecticides — from gaining a foothold.

3. A near-exclusive focus on herbivore-natural enemy interactions at the level of a single plant is often adopted in today's biological control or IPM studies. Though this yields valuable insights into those particular trophic interactions, it regularly obscures other equally important mechanisms at different trophic, spatial or organizational scales. Hence, we advocate holistic, systems-level approaches that draw upon disciplines beyond conventional entomology or insect ecology.

4. Gaining a thorough understanding of the various factors that shape variability in parasitism not only is valuable from an ecological perspective, but it can equally guide efforts to improve biological control efficacy (Rosenheim 1998). Our assessment of determinants of *A. lopezi* parasitism pointed to options to enhance mealybug pest control through interventions targeting soil nutrients, landscape composition, plant diseases, or crop management scenarios (for the latter, see Delaquis et al. 2018).

5. In September 2015, Nature Magazine (Volume 525) boldly stated that:

> "*to solve the grand challenges facing society and to save the world, scientists and social scientists must work together*" (Nature 2015).

Interdisciplinary science matters (see also Brondizio et al. 2016), and our work underscores that fully collaborative, integrative research is central to effectively solve invasive pest problems and to advance AW-IPM and biological control in developing-world agriculture.

7. ACKNOWLEDGEMENTS

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HOLISTIC AREA-WIDE APPROACH FOR SUCCESSFULLY MANAGING CITRUS GREENING (HUANGLONGBING) IN MEXICO

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SUMMARY

The General Directorate of Plant Health (DGSV in Spanish) is recognized as the National Plant Protection Organization of the Federal Government of Mexico that acts under the Plant Health Federal Law. Some relevant plant protection programmes that Mexico is implementing include: The Huanglongbing (HLB) - Asian Citrus Psyllid (ACP) Programme, Mediterranean Fruit Fly Programme, National Fruit Fly Campaign, Pink Hibiscus Mealybug Programme and a permanent Phytosanitary Epidemiological Surveillance Programme to prevent the introduction and spread of regulated non-native pests. HLB or citrus greening is caused by the bacterium, *Candidatus* Liberibacter spp., and considered the most devastating citrus disease in the world. Once infected, it causes the death of orange, mandarin, grapefruit and lemon trees within 3 to 8 years. HLB is transmitted by the ACP, (*Diaphorina citri* Kuwayama), an insect vector widely distributed in most citrus producing regions of the world, including the citrus areas of Mexico. Until 2004, the disease only existed in Asia and Africa. It was first reported to occur in the Americas in 2004 (São Paulo, Brazil) and 2005 (Florida USA). In 2009, it was detected for the first time in Yucatán, Mexico. During that year, the national Mexican citrus production was 6.82 million tons (SIAP 2017). The economic impact evaluation by Salcedo-Baca et al. (2010) indicated that without the intervention of the Federal Government, HLB would be responsible for a reduction of the Mexican citrus production by 2.7 million tons in five years (39.6%). In spite of the spread of HLB, the citrus production in Mexico increased 11% to 7.56 million tons in 2015 (SIAP 2017). Today Mexico has 573 406 hectares (ha) of citrus compared to 545 947 in 2009, an increase of 5%. The first phytosanitary actions implemented on an area-wide basis were: (1) timely detection of HLB in agricultural and urban areas; (2) systematic elimination of infected trees in areas under surveillance; (3) control of the *D. citri* vector and (4) protection of propagative material in nurseries to avoid its infection. As a result of the successful HLB Programme implemented since 2008, adverse effects of the disease have largely been avoided. Management of HLB is organized through Regional Areas of Control (*Areas Regionales de Control* or ARCOs), which implement the following area-wide measures: epidemiological surveillance and monitoring of psyllids based on criteria associated to climate and host presence in urban

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and cultivated areas, and chemical and biological controls. From 2010 to 2015, 31 million parasitoid wasps *Tamarixia radiata* (Waterston) were produced and released in commercial citrus and backyard host areas of Yucatán, Quintana Roo, Campeche, Tabasco, Chiapas, Oaxaca, Hidalgo and Guerrero. The ARCOs are public-private organizations jointly operated by federal and state governments together with citrus grower associations. In 2016, the Mexican government allocated almost USD 8.5 million to the HLB Programme. With these actions, Mexico has largely mitigated the adverse effects of the disease while at the same time slightly increased citrus production. In addition, research programmes have been established together with scientific institutions to generate vegetative material with tolerance or resistance to the disease. Although the government has successfully implemented area-wide strategies for regional control, it is necessary to develop new and improved technologies to eliminate the vector, following the example of the Mediterranean fruit fly Programme in Mexico.

Key Words: Asian citrus psyllid, *Diaphorina citri*, *Candidatus* Liberibacter, economic impact, Mexican states, citrus production, *Tamarixia radiata*

1. INTRODUCTION

Huanglongbing (HLB) or citrus greening is a disease native to China and is considered one of the most destructive citrus diseases in Asian countries where its occurrence was originally reported more than a century ago (Bové 2006). More recently this bacterium reached the American continent together with its insect vector, the Asian citrus psyllid (ACP) (*Diaphorina citri* Kuwayama), capable of infecting and causing considerable damage to plants in the family Rutaceae (Alemán et al. 2007; Bové 2012).

The current geographic distribution of HLB extends to 12 countries in Asia, several islands in the Indian Ocean, Iran, portions of Africa, the Arabian Peninsula, Argentina, Brazil, Paraguay, Central America (except Panama), Barbados, Belize, Cuba, Dominican Republic, Guadeloupe, Jamaica, Martinique, USA (including Puerto Rico and the U.S. Virgin Islands) and Mexico (da Graça and Korsten 2004; Halbert and Manjunath 2004; NAPPO 2005; Bové 2006; Manjunath et al. 2008; Collazo et al. 2009; Trujillo-Arriaga 2011). In 2009, HLB was detected in Mexico for the first time in the state of Yucatán, and now is known to occur in 24 Mexican states (SENASICA 2017a).

The HLB-causing bacterium mainly attacks sweet orange and mandarins (da Graça and Korsten 2004) although all citrus varieties have shown varying degrees of susceptibility to infection as well as other members of the Rutaceae family. Currently, there is no successful control method to cure this disease, and as a result, infected trees die in the course of a few years.

This disease is caused by Gram-negative bacteria of the genus *Candidatus* Liberibacter (Bové 2006). There are three species of HLB-associated bacteria: *Ca*. L. asiaticus, *Ca*. L. africanus and *Ca*. L. americanus. All three have been described as the cause of HLB in different countries and climates world-wide (da Graça and Korsten 2004; Halbert and Manjunath 2004; Bové 2006; Wang et al. 2009), along with its insect vectors: ACP and the African psyllid, *Trioza erytreae* (Del Guercio). The first is the vector of *Ca*. L. asiaticus and *Ca*. L. americanus; while *T. erytreae* is the vector of *Ca*. L. africanus. A fourth species, *Ca.* L. caribbeanus, recently was identified from samples of ACP and *Citrus sinensis* (L.) Osbeck in Córdoba, Colombia. Efforts are underway to determine its pathogenicity and if it causes HLB symptoms (Keremane et al 2015).

The main symptoms of HLB are asymmetrical blotchy mottle yellowing in leaves, chlorosis, fruit drop and foliar loss and tree death. In addition to reducing the size and quality of fruit, the disease causes malformations and bad taste of fruit (Schwarz et al. 1973; Bové 2006). Although infected trees can remain productive for 5 to 8 years, the fruit are of poor quality (Halbert and Manjunath 2004).

To date, no control strategy is available that allows the immediate elimination of the pathogen, so its management in commercial citrus production areas is limited to the control of psyllid vectors through the application of insecticides, elimination of symptomatic trees with the aim of reducing levels of inoculum, isolation of affected areas by quarantine enforcement, and certification of pathogen-free propagative material (da Graça and Korsten 2004; Bové 2006; Manjunath et al. 2008; Gottwald et al. 2012). Because infected orchards become economically non-viable after 7 to 10 years, efforts focus on elimination of infected trees that have caused losses worth billions of dollars on a global basis (Gottwald et al. 1991; Vojnov et al. 2010).

In Florida, introduction of HLB resulted in major changes to pest management practices and corresponding costs. According to Singerman and Burani-Arouca (2017), average annual pest control consisted of two sprays for processed juice fruit and six sprays for fresh market grapefruit mostly to control several minor diseases, mites and weevils. Following a series of hurricanes in 2004 and 2005, that resulted in the catastrophic spread of citrus canker and forced abandonment of eradication actions, the number of sprays increased to 3–4 for processed juice oranges and 10 for fresh market grapefruit. After the discovery of HLB in August 2005 and citrus black spot in 2010, the number of treatments rose to 8–9 sprays for processed juice fruit and 14 sprays for fresh market grapefruit aimed at both disease and ACP control combined with additional fertilizer treatments.

Costs per acre of foliar sprays for producing processed oranges in south-western Florida rose from USD 185.63 in 2003/2004 to USD 666.00 (+240%) in 2014/2015 while fertilizer treatments went from USD 207.69 to USD 486.96 (+134%) during the same timeframes. By comparison, costs per acre of foliar sprays for fresh market grapefruit in the Indian River area increased from USD 493.08 in 2003/2004 to USD 1300.40 (+164%) in 2014/2015 while fertilizer treatments rose from USD 190.56 per acre in 2003/2004 to USD 452.55 (+137%) per acre in 2014/2015. These totals do not include other cultural control costs nor cost for tree replacement. It was found that area-wide control of ACP through Citrus Health Management Areas provides an estimated differential gross economic benefit of USD 714 (USD 1218) for 2012/2013 (2013/2014) (Singerman and Page 2016).

Over the period from 2012/2013 to 2015/2016, HLB caused a cumulative loss of USD 1672 million in grower revenues (average of USD 418 million annually) resulting in average annual economic impacts to the Florida economy of -7945 jobs, -USD 658 million in value added, and USD 1098 million in industry output (Court et al. 2017). Citrus bearing acreage in Florida diminished from 576 400 acres in 2005/2006 to 410 700 (-29%) in 2016/2017 while production value dropped from USD 1 491 136 in 2005/2006 to USD 1 032 227 (-30%) in 2016/2017 (USDA-NASS 2006, 2017).

2. THE HLB IN MEXICO

Mexico is the fourth largest producer of citrus in the world, with a total of 572 000 ha in production, yielding 7.8 million tons annually (Table 1).

Table 1. Areas under citrus production for Mexican states - 2015 (SIAP 2017)

State	Area (ha)	%
Veracruz	246 750	43.13
Michoacán	50 276	8.79
Tamaulipas	44 432	7.77
San Luis Potosí	37 505	6.56
Puebla	32 067	5.61
Nuevo León	31 789	5.56
Oaxaca	25 469	4.45
Colima	19 748	3.45
Yucatán	18 189	3.18
Tabasco	15 532	2.72
Sonora	8 523	1.49
Guerrero	7 135	1.25
Jalisco	6 841	1.20
Hidalgo	5 680	0.99
Campeche	4 731	0.83
Chiapas	4 725	0.83
Quintana Roo	3 089	0.54
Baja California Sur	2 870	0.50
Sinaloa	2 729	0.48
Nayarit	2 469	0.43
Morelos	610	0.11
Baja California	383	0.07
Querétaro	253	0.04
Zacatecas	246	0.04
Total	**572 051**	**100.00**

The major commercial citrus varieties grown in Mexico are orange (*Citrus sinensis*), Key lime (*Citrus aurantifolia*), Persian lime (*Citrus latifolia*), mandarin (*Citrus reticulata*) and grapefruit (*Citrus paradisi*) (Table 2). The value of this crop was estimate at USD 862 million (SIAP 2017).

HLB was first reported in samples of psyllids in July 2009 in Yucatán. In subsequent years more detections occurred in other states of the country (Table 3) (Trujillo-Arriaga 2014; SENASICA 2017a; SENASICA 2017b; SENASICA 2017c). In addition, infected psyllids were detected in Coahuila and Tamaulipas, where mechanisms of control and eradication of the disease vector have been implemented.

Table 2. Production and value of principal citrus varieties in Mexico - 2015 (SIAP 2017)

Citrus crop	Production (million tons)	Value (USD million)
Limes (Key & Persian)	2.33	461
Orange	4.52	345
Grapefruit	0.42	33
Mandarin	0.29	23
Total	**7.56**	**862**

Table 3. HLB detections in Mexico, after initial detection in the state of Yucatán in the month of July 2009 (Trujillo-Arriaga 2014; SENASICA 2017a; SENASICA 2017b; SENASICA 2017c)

State	**Detections of HLB**	**State**	**Detections of HLB**
Quintana Roo	August, 2009	Tabasco	December, 2012
Jalisco	December, 2009	Guerrero	March, 2013
Nayarit	December, 2009	Puebla	September, 2013
Campeche	March, 2010	Zacatecas	September, 2013
Colima	April, 2010	Coahuila	December 2013*
Veracruz	June, 2010	Oaxaca	April, 2014
Sinaloa	July 2010*	Tamaulipas	June 2014*
Michoacán	December, 2010	Querétaro	October, 2015
Morelos	December 2010*	San Luis Potosí	October, 2015
Chiapas	March, 2011	Nuevo León	December, 2015
Sonora	April 2011*	Veracruz	December, 2015
Hidalgo	April, 2011	Tamaulipas	December, 2015
Baja California Sur	April, 2011	Baja California	January, 2016
Nuevo León	August 2011*	Morelos	August, 2016
San Luis Potosí	September 2011*	Sonora	March, 2017

* *Infective psyllids*

As of September 2017, HLB is recorded to infect plant material in all 24 citrus-producing states of Mexico (Fig. 1), where it has been detected in a total of 450 municipalities (SENASICA 2017a).

In Persian lime production in Yucatán, the presence of *Ca.* L. asiaticus caused a reduction in weight of the fruit (17.3%) and a decrease in the volume of juice (18.6%) (Flores-Sánchez et al. 2015). In Key lime, experts estimated a reduction of 183 168 tons should HLB become established throughout Mexico (Salcedo-Baca et al. 2010).

3. RESPONSE TO HLB IN MEXICO

The Mexican citrus industry, federal and state plant protection agencies responded to this situation with the following area-wide phytosanitary actions to manage the HLB disease: epidemiological surveillance in commercial orchards, urban areas, and sentinel gardens; and, chemical and biological controls of the insect vector in both backyards and commercial orchards in those regions where the weather conditions favour infection.

Management of HLB is organized through Regional Control Areas (ARCOs), which implement the area-wide measures, and allow for coordination of monitoring, biological and chemical control actions across hundreds of ha; thereby preventing outbreaks from expanding to thousands of ha (SENASICA 2012).

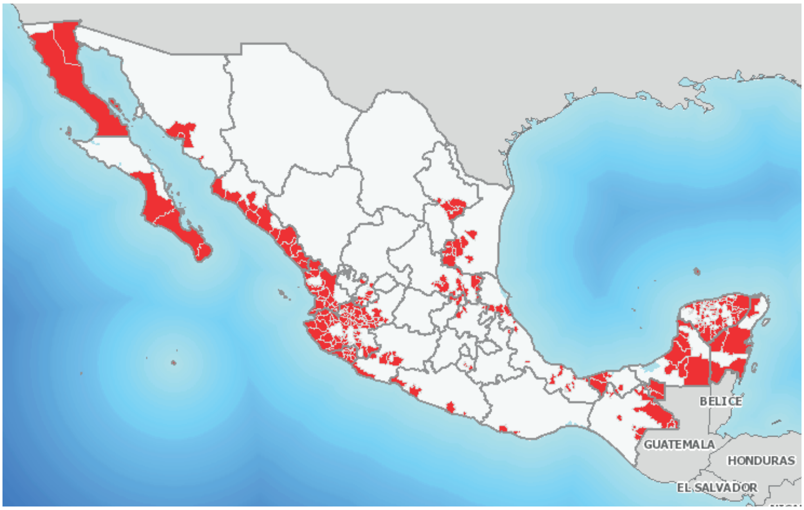

Figure 1. Areas in 24 states of Mexico with presence of HLB as of September 2017 (source SIRVEF 2019).

Since 2002, the vector of HLB has been present in Mexico, posing a significant risk for spreading HLB disease. Following the detection of HLB in Belize during 2008, the National Health, Food Safety and Food Quality Service (SENASICA in Spanish), of the General Directorate of Plant Health (DGSV in Spanish), initiated in 2008 a national priority campaign in 24 citrus states of the country. The aim of the programme was to detect the disease in a timely fashion and provide protection to more than 570 000 ha of citrus. A series of protocols were developed and revised by DGSV with input from State Plant Health Committees and the affected industry (SENASICA 2008a, 2009).

In 2009, the programme received an allocation of USD 2.56 million that allowed for monitoring the disease in 77 192 ha of commercial citrus and backyard trees (Sanchez 2013). As a result of these actions, the first detection of HLB found in infected ACP occurred in July 2009 in the town of Cuyo in the municipality of Tizimín, Yucatán (Trujillo-Arriaga 2011). In that same year and the following years, there were more detections in other states of the country (Table 3).

Due to the importance of the HLB to the Mexican citrus industry, efforts also were made at the federal and scientific levels to involve national universities and research centres to develop better management strategies, as well as better methods for the timely phytosanitary surveillance of the disease and the insect vector. To this end, SENASICA-DGSV established the legal bases for action as follows:

3.1. Phytosanitary Regulation

With the detection in 2009 of HLB in Tizimín, Yucatán, the federal government issued as a matter of emergency, the Mexican Official Emergency Norm NOM-EM-047-FITO-2009, which outlined and established phytosanitary actions to mitigate the risks of introduction and dispersal of HLB in Mexico (DOF 2009). It outlined the phytosanitary actions to implement a monitoring programme that included sampling, diagnosis, inspection and surveillance to assess any new introduction and further spread of HLB in the country and, where appropriate, the application of phytosanitary measures for its management. These included the delimitation of infected areas under phytosanitary control, the removal of infected material, the application of vector control methods, sampling, inspections, and restriction of the movement of vegetative material. The same actions are established in the "*Protocol of Action to the Emergency by the Detection of HLB*" and are supported under the agreement that discloses the phytosanitary measures which should be applied for the control of HLB and its vector (DOF 2010). In addition, the DGSV and the State or Regional Technical Working Group issued the Protocol to establish the ARCOs (SENASICA 2012) for HLB and ACP.

3.2. Sampling and Monitoring to Assess HLB Distribution

With the presence of HLB in Mexico, one of the main activities carried out in all citrus-producing states of the country was an assessment of the presence of the disease through the collection of plant material and adult *D. citri*. Priority was given to the commercial areas of Key lime, Persian lime and orange. When symptomatic plant material was found, photographs were taken and sent through the Digital Diagnostic System (SIDIADI), so the trained technical staff, based on the visual symptoms, determined whether the material was suspect of HLB infection and further sampling was required. In commercial orchards, sampling of *D. citri* was done mainly in trees located along the periphery of the orchards, where 1 to 100 adults were collected and analysed by molecular techniques for presence of HLB. Urban areas (parks, tree-lined boulevards, harbours, etc.) were also sampled.

3.3. Phytosanitary Diagnosis

Given the need for timely diagnoses, four official laboratories were used where plant material and psyllids could be examined: 1) the National Phytosanitary Reference Center (CNRF) in Tecámac, Mexico state, 2) the National Quarantine Station of Epidemiology and Plant Sanitation in Querétaro; 3) State Committee of Plant Protection of Yucatán, and 4) State Committee of Plant Health of Colima. In addition, eight private laboratories were approved to assist with these diagnoses. Furthermore, the DGSV has a mobile phytosanitary diagnostic unit with adequate equipment required for HLB *in situ* detections such as bioclimatic chambers, real-time PCR and End-Point PCR. This excellent infrastructure reduced the response time to a minimum and allowed speedy decision-making to apply local or regional phytosanitary strategies as described in 3.4 through 3.6 below.

3.4. Cultural Control

After an initial find of HLB positive vegetal material in a new area, the cultural control consisted of removing infected plants to avoid further spread from or possible resurgence of infection from new outbreaks. The following phytosanitary measures were implemented:

- All trees with symptoms of HLB should be eliminated within 5 days. For detections in commercial orchards, the owners were responsible for surveying all trees within their groves for the disease. Plants with symptoms were marked with a plastic tape indicating the branch(es) exhibiting symptoms. Plants located on the outer rows of orchards where the symptomatic plants were detected, were also marked with tape. Technical personnel checked each suspect orchard and confirmed whether or not the symptoms were caused by HLB. All positive cases were georeferenced. It was not considered necessary to sample these plants and technical personnel immediately removed the plants based upon a visual diagnosis. After tree removal, herbicides were applied to the stump. It is important to note that pruning cannot be used to manage HLB control and that replanting is not recommended as new plants are more susceptible to the disease (SENASICA 2010a; DOF 2010).
- In 2010 the Mexican government performed these activities in the following states with presence of HLB: Campeche, Colima, Jalisco, Michoacán, Nayarit, Quintana Roo, Sinaloa, and Yucatán. In these entities the exploration was carried out in 1553 localities, in which 1 127 275 citrus plants and 306 138 plants of lakeview jasmine (*Murraya paniculata* (L.) Jack) were inspected in search of suspicious symptoms and psyllids carrying the bacteria. Likewise, 17 539 citrus trees in orchards and 77 522 of lakeview jasmine in backyards were eliminated, as well as 5037 trees of Mexican lemon, Persian lemon and orange, in commercial orchards and 1 360 626 nursery plants (SAGARPA 2011).
- The presence of HLB in all the plants was determined by searching for symptoms and sending samples to the laboratory for diagnosis by molecular techniques, including those in the proximity of citrus nurseries. All hosts present in nurseries without anti-aphid mesh protection were removed. When a detection occurred in a backyard, it was the responsibility of trained Auxiliary Plant Protection Organization staff to identify HLB symptoms (SENASICA 2010a; DOF 2010).
- In orchards where at least 28% of plants showed HLB symptoms and where the clinical diagnosis was positive for the disease, all trees in the orchard were removed within a period of no more than five calendar days. The review of orchards in an outbreak area is being implemented permanently, with a review of such orchards done every 3 weeks to know the status of the disease (SENASICA 2010a; DOF 2010).

Currently HLB control is still based on eradicating sick trees in the states with new detections. In areas with high incidence of HLB disease, the growers have opted to implement intensive nutrition programmes to extend the productive life of the affected plantations. The strategies of the federal government campaign are directed towards the control of the ACP, through the establishment of the ARCOs, which implement the biological and chemical control activities in their respective regional areas (SENASICA 2017d).

3.5. Biological Control of the Vector

Augmentative biological control is a strategy that plays an important role in reducing the population density of *D. citri*, and its area-wide use in ARCOs has significantly contributed to reducing adverse environmental effects and minimizing interference with natural control of agricultural pests as a result of using agrochemicals (DGSV 2016).

In response to the detection of HLB and its vector *D. citri* in Mexico and to mitigate its threat for citriculture, SENASICA-DGSV and the CNRF established a *Biological Control Programme for Asian Citrus Psyllid*, as a complementary strategy to the integrated management of the HLB vector. The main activities in this programme have been:

- Search and selection of biological control agents of *D. citri*
- Mass-production of the species-specific ectoparasitoid *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae)
- The release of adult parasitoids in specific areas not subject to insecticide application (such as urban areas, inaccessible areas, organic orchards, abandoned vegetable gardens, backyard host trees, orchards adjacent to urban areas, areas under integrated pest management, and protected natural areas or reserves)
- Assessment of the effectiveness of biological control agents in the laboratory and in the field
- Training and public education on the recognition and use of biological control agents of ACP
- Advice on the design of rearing facilities for *T. radiata*
- Optimization of the mass-production process of the parasitoid
- Research on different strains of *T. radiata* present in Mexico and their regional impact
- Research on the use of strains of entomopathogenic fungi, and finally
- Obtaining national and international support on parasitoid rearing (FAO-SENASICA 2013).

Therefore, the DGSV through the CNRF, established collaborative agreements with the State Committees of Plant Protection in the states of Colima and Yucatán in 2009. The first agreement with the Entomophagous Insects Department of the National Reference Center for Biological Control (CNRCB), based in Tecomán, Colima, aims to generate technology (basic and applied) for the use of biological control agents of *D. citri*, as well as the production of *T. radiata* in Tecomán, Colima; while the second agreement with the Regional Mass-Rearing Laboratory of *Tamarixia radiata* in the Southeast has as its sole objective the mass-production of the parasitoid in Merida, Yucatán; both agreements are coordinated by the CNRCB (FAO-SENASICA 2013).

From 2010 to 2015, 31 million *T. radiata* were produced and released in citrus orchards and abandoned, urban and backyard areas of Yucatán, Quintana Roo, Campeche, Tabasco, Chiapas, Oaxaca, Hidalgo, and Guerrero. These release activities were supported by the State Plant Health Committees, who are responsible for transporting and release of parasitoids to the infested areas (CNRCB-CNRF-DGSV 2016). Many of these areas began with a parasitism rate ranging between 3-26% that increased to 70-85% after augmentative releases (SENASICA 2016).

The recommended release rate is 100 parasitoids every 50-100 linear meters depending on the level of ACP infestation and density of host plants. If on average more than 20 *D. citri* nymphs were observed per tree shoot, 100 parasitoids were released every 50 meters (SENASICA 2015). The releases are carried out with a minimum interval of one month and a maximum of 3 months (DGSV 2016). These releases directly and indirectly benefit hundreds of growers.

With respect to the use of entomopathogenic fungi as a complement to the control of *D. citri* populations, the following research activities were established in support of the Biological Control Programme of the ACP:

- Exploration of entomopathogenic fungi
- Selection of isolates of entomopathogenic fungi candidates for the control of immature and adult stages of *D. citri*
- Evaluation of conidia production
- Evaluation of types of entomopathogenic fungi formulation
- Evaluation of fungal formulations in the field
- Evaluation of application equipment; and
- Biosafety tests (FAO-SENASICA 2013).

By 2016 three strains of *Isaria javanica* (formerly *fumosorosea*), CHE-CNRCB 303, 305 and 307, formerly Pf15, Pf17 and Pf21, respectively, as well as one of *Metarhizium anisopliae* (CHE-CNRCB 224, formerly Ma59), had been identified. Laboratory tests achieved 93-100% mortality in nymphs and up to 95% in adults of *D. citri*, respectively. Applications of entomopathogenic fungi in preliminary field trials reduced psyllid populations from 48 to 90% (FAO-SENASICA 2013). In 2012 and 2013, the application of two strains of entomopathogenic fungi (Ma59 and Pf21) was carried out on 15 932 ha in the states of Colima, Hidalgo, Jalisco, Nayarit, San Luis Potosí and Veracruz (CNRCB-CNRF-DGSV 2016).

3.6. Chemical Control

In other countries, HLB disease has been managed mainly through the suppression of vector populations using synthetic insecticides. In Mexico, only the use of insecticides authorized by COFEPRIS (DOF 2010) are recommended in accordance with the use of products that have been approved and shown to be efficacious in other countries. Cortéz-Mondaca et al. (2010) conducted tests of the effectiveness of conventional synthetic and organic insecticides with different modes of action, including botanical extracts, mineral oils, soaps, entomopathogenic detergents and growth regulators. Based on these results, the National Campaign against HLB has been rotating the use of the following active ingredients according to the specific local situations: thiamethoxam, imidacloprid + beta cyfluthrin, mineral oil, bifenthrin, tricarboxyls, chlorpyrifos, imidacloprid, dimethoate, thiamethoxam + lambda cyhalothrin, bifenthrin + zeta cypermethrin, azadirachtin, bifenthrin + abamectin, detergent and lime oxide (SENASICA 2012).

In Mexico, chemical control is performed by the ARCOs. The application time is determined by the population dynamics of *D. citri* and the phenology of the citrus in each region. The spraying is done 2-3 times in all the orchards that are part of an ARCO within a two-week period of time. The State Plant Health Committees responsible for HLB disease management oversee the actions taken by ARCOs and inform growers about the timing of annual applications, and the overall pesticide management programme. ARCOs are responsible for any pesticide applications (DGSV 2016). In 2015 and 2016, 216 566 and 273 318 commercial ha of citrus were sprayed, respectively (SENASICA 2017b).

3.7. Vegetative Material

The Mexican Official Standard NOM-079-FITO-2002, "Phytosanitary Specifications for the Production and Mobilization of Propagation Material Free of Citrus Tristeza Virus and Other Pathogens Associated with Citrus", and the agreement that discloses the phytosanitary measures to be applied for the control of HLB and its vector (DOF 2002, 2010; SENASICA 2010a) establishes requirements for propagation and certification of citrus nursery stock through Certified Production Units as a means of providing disease-free trees for commercial sale and planting purposes.

3.8. Training and Outreach

Two international workshops on ACP and Citrus Huanglongbing were held in Mexico during 2008 and 2010, respectively, with the objective of providing training on disease diagnostics to technical staff in charge of field monitoring and sampling. Experts were invited from infected countries such as Belize, Brazil, China, Cuba and the USA, to share their experiences on management and disease prevention practices (SENASICA 2008b, 2010b). In addition, three events on quarantine pests of citrus were organized in 2009, 2011, and 2013 (SENASICA 2013).

In order to create greater awareness among the general public about HLB and the risks posed, an additional 34 667 training events were organized between 2008 and 2011 in different regions of the country. Attendees were encouraged to participate under the motto "All against HLB of citrus and its vector".

In addition, billboards along roadways and avenues in rural villages and information disseminated using printed triptychs, posters, flyers, technical files, postcards, radio spots and videos were used to educate all involved. These materials invited the public to be on the alert for symptoms of HLB in their commercial and backyard orchards, and to immediately report HLB symptoms to the local plant health boards in their region (Trujillo-Arriaga 2011).

4. HLB IMPACT ON CITRUS PRODUCTION FROM 2009 TO 2015

In 2009, the year when HLB was first detected in Mexico, the area planted with lime, orange, mandarin and grapefruit comprised 523 321 ha yielding a total of 6.82 million tons of fruit (SIAP 2017). That same year, SENASICA and the Inter-American Institute for Cooperation on Agriculture (IICA) commissioned a study to estimate the expected impact of HLB in Mexico (Salcedo-Baca et al. 2010).

Various scenarios were developed to assess potential impacts to the industry and economy with and without any governmental intervention. Under the low impact scenario, the study estimated that within five years following the establishment of the disease a loss of 2.7 million tons of citrus fruit would occur nationwide with an overall reduction of 39.6% in orange, 33% in grapefruit, 17% in mandarin, and 10% in lime production respectively. Under the high impact scenario, losses would increase to 3 million tons of fruit equivalent to 41% of orange, 53% of grapefruit, 26% of mandarin, and 18% of lime production.

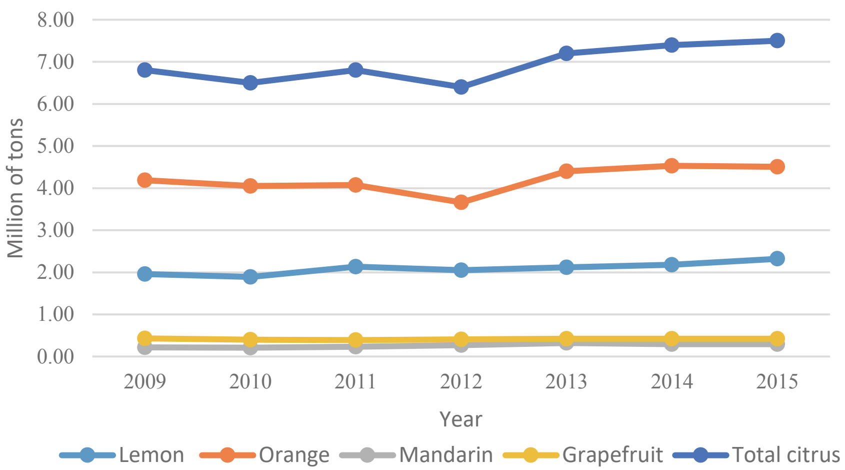

Figure 2. National citrus production in Mexico after HLB arrival in 2009 (SIAP 2017).

Six years after HLB was first detected in Mexico, however, with governmental intervention, the estimated losses in citrus production have yet to occur. Although citrus production in 2010 and 2012 decreased slightly, annual yields have remained fairly stable and have slightly increased by 0.7 million tons above the 2009 level (Fig. 2 and Table 4).

In 2016, the Mexican government allocated approximately USD 8.5 million to the HLB Programme. As a result of the successful application of the area-wide actions outlined above, Mexico largely mitigated the adverse effects of the disease, while at the same time slightly increasing citrus production. In addition, research programmes have been promoted among scientific institutions to generate propagative material tolerant or resistant to the disease.

Table 4. *State-by-state comparison of citrus production in Mexico - 2009 and 2015 (SIAP 2017)*

State	**Production (tons) 2009**				**Production (tons) 2015**			
	Orange	**Lime**	**Mandarin**	**Grapefruit**	**Orange**	**Lime**	**Mandarin**	**Grapefruit**
Aguascalientes	23	0	0	0	14	46	0	0
Baja California	3, 499	2,978	192	96	3,260	1,171	0	183
Baja California Sur	29, 303	144	0	66	49,995	309	22	495
Campeche	37, 018	5,953	215	15,014	25,122	10,325	332	18,955
Chiapas	15,983	4,185	452	0	16,027	7416	404	0
Colima	3,977	423,040	0	0	4,496	191890	0	158
Durango	804	856	0	612	785	702	0	577
Guanajuato	0	3	0	0	0	12	0	0
Guerrero	4,189	78,404	230	66	4,435	71,867	138	9
Hidalgo	45,481	2,589	262	0	59,041	2,231	239	0
Jalisco	6,779	30,351	6	1,310	6,386	81,198	0	740
México	327	1,227	0	0	234	1,190	39	0
Michoacán	4,374	414,562	75	59,559	3,489	670,613	0	49,566
Morelos	4,430	3,660	82	160	4,857	4,438	190	144
Nayarit	1,576	14,093	41	5	712	18,423	19	0
Nuevo Leon	296,973	0	35,892	17,734	313,439	0	45,751	26,201
Oaxaca	60,626	176,182	0	5,440	56,290	245,137	0	1,161
Puebla	254,841	48,352	680	11,160	214,175	28,211	51,746	5,067
Querétaro	1,345	47	24	0	2,446	26	5	0
Quintana Roo	32,289	1,107	32	0	16,841	26,222	112	0
San Luis Potosí	431,567	8,599	20,358	55	337,717	21,986	22,212	62
Sinaloa	16,970	2,173	1,420	5,195	19,320	1,648	446	2,887
Sonora	167,371	988	581	17,500	142,445	1,564	3,059	16,689
Tabasco	81,519	80,939	380	850	81,451	83,141	387	845
Tamaulipas	539,526	46,411	54,235	29,083	668,935	121,200	11,618	46,544
Veracruz	2,058,040	514,728	102,046	256,064	2,336,427	659,034	147,345	248,927
Yucatán	94,534	104,777	6,441	11,704	147,107	74,463	6,885	5,106
Zacatecas	128	0	72	0	74	1,605	130	0
Total	**4,193,484**	**1,966,345**	**223,718**	**431,671**	**4,515,520**	**2,326,068**	**291,078**	**424,315**

Although the government has successfully implemented area-wide strategies for regional control, it is necessary to further develop new and improved technologies. Mexico, Belize and USA have formed a Tri-National Working Group for purposes of technical exchange, information sharing, planning, coordination, and identification of research priorities.

Mexico currently has 573 406 ha of citrus corresponding to 335 019 ha of oranges, 180 209 ha of limes (Key and Persian), 21 297 ha of mandarin, 17 590 ha of grapefruit, 12 736 ha of tangerine, 5238 has of tangelo and 1317 ha of sweet limes, with an estimated annual production of 8 million tons per year, and whose production value is approximately USD 20 424 million pesos (approximately USD 1.12 billion dollars) (SIAP 2017).

The first phytosanitary actions implemented on an area-wide basis were: timely detection of HLB in citrus and urban areas, elimination of infected plants in areas under control, suppression of the *D. citri* vector, and protection of propagative material within enclosed or screened nurseries.

5. CONCLUSION

In conclusion, as a result of the successful HLB Programme, citrus losses have been largely avoided in Mexico despite the fact that the disease has now been detected in all states of the country where commercial citrus is produced. Six years after the disease first appeared, the surface for the four principal citrus varieties actually increased by 5%, from 523 321 to 553 671 ha (Fig. 3) (SIAP 2017).

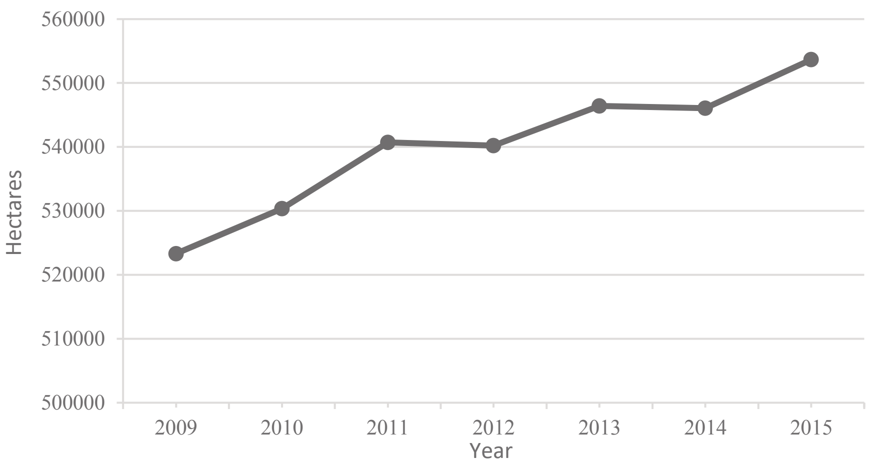

Figure 3. Expanding national areas of the four principal Citrus *spp. produced in Mexico after the HLB arrival in 2009 (SIAP 2017).*

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TECHNOLOGY USED BY FIELD MANAGERS FOR PINK BOLLWORM ERADICATION WITH ITS SUCCESSFUL OUTCOME IN THE UNITED STATES AND MEXICO

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SUMMARY

Pink bollworm, *Pectinophora gossypiella* (Saunders), has been eradicated over a 7-state area in northern Mexico and the southern USA. Over this region, pink bollworm has been a key pest of cotton for 50+ years. The bi-national eradication programme grew out of a long-standing Sterile Insect Technique (SIT) containment/exclusion programme to protect cotton in the San Joaquin Valley of California, as well as numerous area-wide research and demonstration projects in southern California, Baja California, and Arizona. It included all contiguous infested production areas of the states of Chihuahua, Sonora, and Baja California in Mexico. It also included all contiguous generally infested areas of the states of Texas, New Mexico, Arizona, and California in the USA. In this chapter we provide descriptions and key references for the technologies that were integrated in this multi-tactic, area-wide programme over its extensive geographic range. Technology described and used includes state programme-based central data management. The programme covered all activities including extensive GPS mapping, pheromone trap monitoring for adult populations, and the integration of all control operations. Operational information and data were shared among all participants as needed. Control tools included *Bt*-cotton, the release of sterile moths, pheromone mating disruption, cultural control, and on a very limited basis conventional insecticide application. Critical area-wide resistance management using sterile moth release, rather than planting susceptible cotton in refugia, was pioneered in this programme. Success as documented was possible over an enormous and diverse cotton production area because the technologies used were heavily researched, broad-based, and could be tailored to fit each major area. Uniform management within each state was coordinated bi-nationally. This programme was conducted sequentially over time. Summaries for each state provide measurements of progress, success, and experiences gained through time of operation.

Key Words: Pectinophora gossypiella, pink bollworm trap, area-wide management, integrated pest management, *Bt*-cotton, resistance management, gossyplure, mating disruption, Sterile Insect Technique, SIT, pest detection survey, okra

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1. INTRODUCTION

Introduction of the pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), to the south-western USA and north-western Mexico irrigated cotton growing regions was first reported in 1916 near Torreón, in Coahuila, Mexico (Noble 1969). This infestation was presumed to originate from seed shipments from Egypt into Mexico in 1911. It quickly became the key pest of cotton.

With the exception of the San Joaquin Valley of California, no other pest species was as dominant or detrimental to the fortunes and survivability of the cotton farmers of these regions. This was particularly true in the cotton growing areas of the Colorado River Basin. Insecticide use for this pest was extensive for a period of more than 45 years.

Early attempts at management or eradication with conventional insecticides were expensive and difficult. All control efforts resulted in some short-term success and frequent frustration. This was true both when control was on a field-by-field basis and also in the case of a coordinated, insecticide-driven state-wide programme in Arizona (Anonymous 1961; Schmitt Jr. 1967).

The emphasis of this chapter will be to record the technology used and the success of the bi-national Pink Bollworm Eradication Programme to assist others in the development of bio-rational approaches to the pest. We expect such knowledge will be critical in mitigating any seed-borne movement of PBW back to the USA and northern Mexico. The programme herein described evolved only after in-depth research (over 3000 references in a CPHST-APHIS-USDA data base and Naranjo et al. 2002) and numerous large-scale field trials. All this investment in R&D is frequently oversimplified and overlooked when the positive results of the programme are considered.

The programme operations started on a sequential basis in the generally infested areas of the south-western USA and northern Mexico (Fig. 1). This "rolling carpet" approach (Hendrichs et al. 2021), followed as programme phases, was necessary due to the physical limitations of the production output of sterile PBW moths by a single mass-rearing facility located in Phoenix, Arizona.

In this summation we identify the most important tools commonly used by the programme and provide relevant references and accounts of experience used in the design of the programme. Important summary data utilized to measure programme progress are also provided. Units of measurement were those used in the respective countries (metric system in Mexico, "U.S. customary unit system" in the USA). The data came from the records used in day-to-day management and are provided in Sections on a state-by-state basis in both countries.

Each of these Sections starts with a listing of the state managers who were responsible for day-to-day operations and ultimately were the backbone of the programme's achievement. The success of this programme grew out of a long-standing containment/exclusion programme in the San Joaquin Valley of California (Staten et al. 1993) and numerous area-wide research and demonstration projects in geographically-defined locations particularly in southern California, Baja California, and Arizona (Walters et al. 2000). Many of these trials were reported through proceedings of the National Cotton Council Beltwide Cotton Production Conferences.

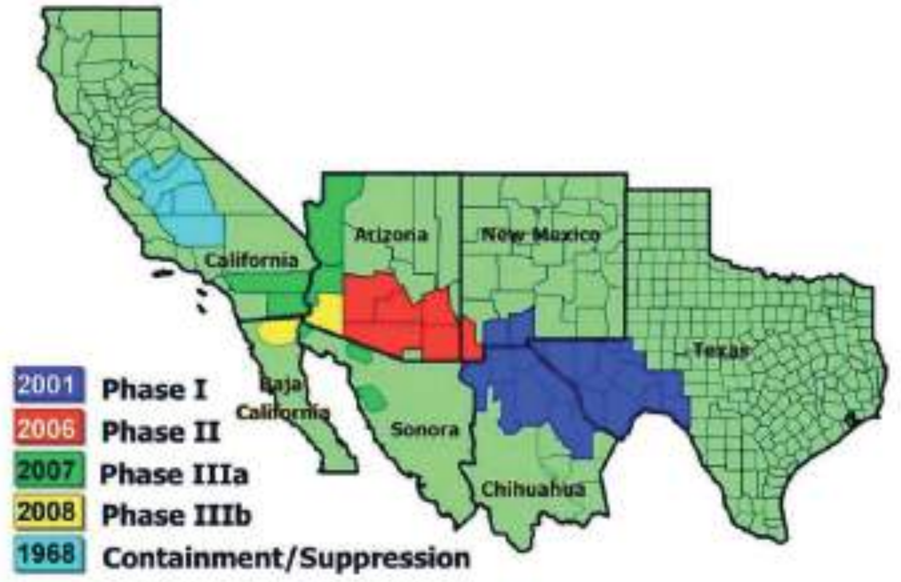

Figure 1. Pink bollworm eradication phases, dates, and areas in south-western USA and north-western Mexico (updated from Grefenstette et al. 2009).

2. MANAGEMENT

Standardized management and organization are critical in integrated area-wide insect pest eradication programmes (Vreysen et al. 2007; Suckling et al. 2014). As this was not a "voluntary" programme, all in-season applications of suppression treatments (insecticides, sterile insect release, and pheromones) for PBW were under a central management and coordinating authority in each state. This area-wide concept of the bi-national programme had to be agreed upon by the majority of all cotton growers in referenda held before the programme could be initiated in each of the states. In Arizona, for example, the programme could only be started after a second grower referendum passed in 2005 with more than a 66 % grower approval (Grefenstette et al. 2009).

A brief outline of management entities in the USA and Mexico involved in the PBW eradication programme are shown in Tables 1 and 2.

Table 1. Brief outline of management entities involved in the USA and their contributions to the pink bollworm eradication programme

ENTITIES IN USA	CONTRIBUTIONS
USDA-APHIS	All sterile insect production, USA release cost, and USA regulatory enforcement
The producer communities:	Within-state cost of all non-SIT[2] in-field treatments and operations (includes *Bt*-cotton, pheromone mating disruption, and insecticides)
1. Texas Boll Weevil Foundation (TBWF)[1]	All field management of treatments, monitoring, evaluation and reporting
2. New Mexico PBW and BW Foundation[1]	All field management of treatments, monitoring, evaluation and reporting
3. Arizona Cotton Research and Protection Council (ACRPC)[1]	All field management of treatments, monitoring, evaluation and reporting
4. California Cotton Pest Control Board (CCPCB), funds managed by CDFA[1]	All field management of treatments, monitoring, evaluation and reporting

[1] *All funds were raised via local assessments through organizations 1-4, and from legislative support to USDA via the National Cotton Council*
[2] *SIT = Sterile Insect Technique*

3. TECHNOLOGY USED

The successful PBW eradication was dependent on a multi-tactic approach in which the authors will not designate one control technology as most critical for its success. All technologies, integrated in different ways in the different areas, were essential and born from in-depth research and development efforts over a 100-year time frame (.

This programme was fortunate in following an ongoing Boll Weevil Eradication Programme (Knipling 1971; Allen 2008), which established the benefits of a standardized area-wide approach to programme success. Lessons learned and organizational basics were of extreme importance. El-Lissy et al. (2002) used a simple classification for all programme activities. This paper was the foundation for all field operations used by state organizations for PBW.

All activities were sub-divided into three activities: 1. mapping and data management, 2. surveying (trapping and larval sampling), and 3. control. The authors will generally follow these three critical components, adding detail to each to fully elucidate their scope and interaction. It should be noted that a separate Section is included dedicated to transgenic cotton. Despite being an essential part of the programme's control components, unique issues related to the incorporation of transgenic cotton require additional discussion.

Table 2. Brief outline of management entities involved in Mexico and their contributions to the pink bollworm eradication programme

ENTITIES IN MEXICO	CONTRIBUTIONS
SAGARPA (Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food), SENASICA (National Service of Health, Food Safety, and Agriculture Quality)	Leadership, Technical and managerial support, critical funds (varied year to year dependent on needs and availability at national level)
USDA-APHIS-International Services and Plant Protection and Quarantine (PPQ)	Technical and information technology support, logistical support, bi-national coordination, coordination with USA embassy for security, procurement of some supplies, and some field personnel and SIT[2] coordination
1. Comité Estatal de Sanidad Vegetal (state plant protection committee) de Chihuahua[1]	State level management of operations (treatment, survey, and control), funding via grower assessments and direct contributions
2. Comité Estatal de Sanidad Vegetal (state plant protection committee) de Sonora[1]	State level management of operations (treatment, survey, and control), funding via grower assessments and direct contributions
3. Comité Estatal de Sanidad Vegetal (state plant protection committee) de Baja California[1]	State level management of operations (treatment, survey, and control), funding via grower assessments and direct contributions

[1] Authors were not involved in funding decisions, but they understand that sources of revenue varied according to available resources from the various entities in the different states

3.1. Mapping and Data Management

Eradication requires complete control of needed technology over broad or well-defined geographic areas, over which uniform management is of paramount importance. This starts at the beginning of each growing season with the process of finding and mapping of all fields with cotton. Each state's programme had to be able to monitor all these fields. Managers required rapid access to all mapping, survey and treatment data. PBW populations are clustered and non-uniform in distribution within a field and within subsites and definable geographic areas. Management must be able to operate within a spatial context allocating resources where they are most critically needed without regard to ownership or political constraints.

When Texas initiated the first surveys of PBW populations, it modified its boll weevil data management system to include all PBW management needs. This was then made available to all participating states. Details of how this data management system is used today can be found under TBWEF (2019). It was adopted by all state programmes except California, which already had a long-standing data management system in place.

Within the above context each state management had complete access to its mapping and data including the following:

1. Precise GPS locations of all fields with unique identification numbers for every field and its trap or traps
2. Barcoded identification of all traps with GPS location within the programme
3. Storage and access to all trap and capture data for sterile and non-sterile specimens
4. Precise location of all *Bt* and non-*Bt* cotton (*Gossypium hirsutum* L.) fields, including a distinction for Pima cotton, *Gossypium barbadense* L.
5. Access to detailed information on all programme-applied pheromone mating disruption treatments, conventional insecticides, and sterile moth releases – this included access to needed regulatory notifications within each state and flight recordings for all spray and sterile release aircraft, and
6. Reports generated from complete data by servicing date or any other needed time interval and geographically-defined parameter.

The use of this harmonized system expedited communication within and between state programmes.

3.2. Survey Technology

3.2.1. Trap Selection and Use

The eradication of PBW has long relied on the use of the delta trap for surveys. This trap and the modified Frick trap it replaced are fully illustrated by Foster et al. (1977). The trap is deltoid in shape and is 7 inches (17.8 cm) long and 3.5 inches (8.9 cm) on each of its three sides. The inverted triangular opening found on each end is one inch (2.54 cm) on each of the three sides that form the opening.

The delta trap was first used on an area-wide basis in the San Joaquin valley of California in 1976. Staten et al. (1993) reported on multiple years of data from very large numbers of this trap in over one million acres (> 400 000 ha) of cotton each year. The delta trap will overload in high PBW populations when traps quickly exceed 50 moths per service interval. It is however the most sensitive trap known to the authors in detection and monitoring of lower density PBW populations. The most important need in this programme was its ability to successfully find low level populations before they reached levels only allowed in pre-eradication pest management scenarios.

The following are four key requirements of traps needed for operational success:

1. Superiority as a detection tool with the best capture rate in low population densities
2. The trap must facilitate accurate identification, preserving the specimen intact enough for dissection and/or, with special servicing, DNA analysis
3. The trap must be durable enough to withstand “normal” extremes such as wind, rain, handling and routine field and operational hazards, and
4. The trap must be cost- and operationally-effective to use. This includes unit cost, storage, installation, recovery, and replacement.

Throughout the PBW programme, trap density standards were set at one trap per 80 acres (32.4 ha) in the USA and one trap per 20 ha in Mexico for all *Bt*-cotton (cotton genetically modified to express the endotoxins of *Bacillus thuringiensis* Berliner) (*Bt*). All cotton fields which did not express these resistant traits were trapped at one trap per 10 acres (4.05 ha) in the USA or one trap per 4 ha in Mexico. Trap placement at these numbers required 2 considerations. The trap must be in a position from which its emitted pheromone attractant would have a high probability of intersecting the casting flights of the male moths. It must also be reachable by servicing personnel quickly and efficiently.

Studies by Leggett et al. (1994) resolved many questions involved in management of trapping. Traps were placed on field margins preferably where they will not be destroyed in normal field cultivation activities. Trappers are trained to look at such factors as prevailing air movement patterns as fields cool down after sunset. Male moths typically become active in search of females as temperatures decline with an 80°F (26.7°C) threshold (Lingren1989). Traps were serviced or changed at least weekly.

3.2.2. Trap Lure Formulation

The discovery and development of the female sex attractant of the PBW was the single most important entomological breakthrough of the mid 1970's with respect to PBW control. The name "gossyplure" and its characteristics were first published by Hummel et al. in 1973. Bierl et al. (1974) published detailed data illustrating its importance and the role of its specific components. Gossyplure is a near 50/50 ratio mixture of (ZZ) and (ZE)-7,11 hexadecadien-1-ol acetate isomers. This paper also noted the detrimental effect of the EE and EZ isomers of this molecule in reducing attraction. The introduction of a controlled release formulation of gossyplure had a profound impact on the San Joaquin Valley exclusion programme being implemented at that time for PBW in California. Previous adult surveys had relied on the para-pheromone hexalure. Hexalure required 20+ times the attractant for much lower capture rates and detection efficacy.

A number of controlled release formulations have been used in PBW traps. Flint et al. (1974) provided the first published paper showing the advantages of a controlled release trap lure formulation for PBW. Flint used the red rubber septa and a version of this lure was used throughout the programme at 4 mg per lure. There are other formulations which have a flatter emission release rate over longer periods of time, but they are more expensive.

Throughout the eradication programme covered in this publication, lures were replaced with every trap service or at least every two weeks, even though the septa have excellent properties for a longer period. This lure is currently produced for all state programmes at cost by the Arizona programme.

The quality of the gossyplure is as or more important than the substrate used. Staten et al. (1988) illustrated the importance of using trap bioassays in the procurement of gossyplure for surveys. Important differences of PBW attraction still cannot be explained with known chemical analysis alone; traces of an alcohol are suspected. The programme in Arizona maintains a supply of "technical" grade gossyplure for all post-eradication survey in this programme.

3.2.3. Moth Identification

The programme had to face two critical issues, namely species identification (taxonomic) and separation of sterile from native insect specimens. From the first sterile moth releases in the San Joaquin Valley in 1968, moth taxonomic identification used labial palp bands, and genital clasper characteristics to separate *P. gossypiella* males from other species. The survey traps and lures used, with rare exceptions, only attracts the male moth. The trap is not absolutely species-specific and will sometimes capture a few accidental "contaminant" specimens. Most of these do not resemble PBW. There are a few similar-sized moths which may have an attractant similar to gossyplure. If these captures were confused with PBW, they could affect treatment decisions adversely. Good dissection techniques and microscopic examination were used for specimen identification. This was considered sufficient throughout the years of the programme. Late in the programme, DNA signatures were under development at the University of Arizona.

Separation of mass-produced release moths from native moths in the cotton fields was accomplished using a dye incorporated in the sterile moth larval production diet. Calco Red oil food dye was used as a diet induced marker (Graham and Mangum 1971). Marking was very accurate as non-marked moths could not be found among laboratory-produced moths even when extreme searches were conducted periodically throughout the Phoenix rearing facility's history. Searches involved thousands of aged moths crushed on white filter paper. In these searches, moths were routinely taken from discarded egg production cages. This, however, cannot completely represent moths under field conditions.

A simple paper chromatograph technique was in use in support of sterile moth releases as early as 1970 in the Coachella Valley of California, when the first author worked with that trial programme. It has endured through all sterile moth releases. The technique involves the use of a small straight sided vial at or near 25 x 10 mm; exact size is not critical. A moth is crushed in the bottom with an uncontaminated rod, preferably glass. The vial plus moth then receives 1 ml of hexane and a strip of chromatographic paper cut to 9 x 30 mm. The strip is cut to a point at its terminal end so that as the solvent moves upward it concentrates the dye in the tip as it dries, facilitating identification.

As the eradication programme reached completion, the potential for misidentification became extremely critical. As each state programme reached this point, the importance of absolute accuracy increased. If a 1:1 000 000 rate of error was possible, a second independent marker or analysis with high levels of confidence would, in terms of probability, make a missed detection of a non-marked sterile virtually impossible.

Burns et al. (1983a) used an inductively coupled plasma-atomic emission spectrometer to test possible use of 13 elemental markers. Out of those, Strontium (Sr) proved to be the most viable candidate as a second marker, independent from the Calco Red dye. Burns also illustrated its potential in sterile Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) analysis (Burns et al. 1983b).

PBW larval diet preparation had undergone major changes in the mass-rearing facility (Miller et al. 1996), so some re-testing with Sr was required. In this effort in 2011, a 540-ppm level of Sr was found to have excellent retention in moths as old as

45 days (Walters, unpublished reports). This technology was used starting in 2012 for all the sterile moth production.

3.2.4. Larval Sampling

Pre-eradication pest management scouting procedures usually used larval PBW populations at 2-5% as the action threshold to trigger insecticide ground and aerial applications of conventional insecticides. When populations reached this level, non-selective insecticides were considered the only workable solution. All treatment decisions were on a field-by-field basis. In many areas that resulted in frequent (5-15) treatments for PBW alone or in combination with insecticides for other pests in a growing season.

In the case of the PBW eradication programme, protocols used for suppression were designed to prevent development of populations high enough for normal detection of larvae. Boll larval sampling was used most extensively in the first 2-3 years of the programme. Some states used a random selection of non-*Bt* fields for boll collection to assess larval populations. Conversely, during the first years of the Arizona programme all non-*Bt* fields were sampled. This was logistically possible due to a high ratio of *Bt* to non-*Bt* fields. In instances where pockets of higher native moths were detected in cotton fields, targeted searches were also used as the programme progressed.

Two different sampling methods were used. Bolls collected from the field could be processed within boll holding boxes (Fye 1976) or by direct examination of bolls cut open immediately after field collection. When the data are needed for immediate operational decisions, boll cutting is critical. In this case data could trigger an immediate conventional insecticide treatment. This became extremely rare as the programme progressed. For resistance monitoring, or when some assessment of reproduction was desired, boll boxes were used. The detection threshold of a trap is always better than any larval assessment.

3.3. Control Technologies

Throughout the eradication programme, PBW control was the responsibility of state-wide programme management (Tables 1 and 2). These organizations controlled all treatment activity except for the type of cotton to be grown. Producers chose not to plant or to plant *Bt*-cotton, although the latter option was encouraged. Individual growers were responsible for adhering to Environmental Protection Agency (US-EPA) regulations in terms of respecting seed contracts and label compliance. In Texas, USA and Chihuahua, Mexico (Phase I), the PBW programme ran simultaneously with active boll weevil eradication, where boll weevil treatments were concurrent. In the USA, the grower who chose to use *Bt*-cotton contracted with the seed provider to pay the technology fee for that resistant cotton. Where *Bt*-cotton was not in use, producers paid a higher assessment for programme-applied pheromone mating disruption and other control actions needed for suppression. Base costs covered other programme aspects.

3.3.1. Transgenic Cotton

Cotton genetically modified with genes from *B. thuringiensis* (*Bt*) provided the single most important change in PBW control in the late 20th century. In 1990, Wilson et al. (1992) conducted the first tests of experimental lines "that carry an altered version of the insect-controlling protein gene from *B. thuringiensis kurstaki". These lines were* not commercially available at that time. The technology subsequently developed to a commercial state quickly.

The PBW mortality levels which commercial *Bt*-cotton varieties produced were unprecedented at >99% (Flint et al. 1995; Watson 1995). Staten et al. (1995) noted its potential importance as an eradication tool, proposing its integration with other "soft" technologies because it targeted only the larval stage as it fed within or on the plant. Because *Bt*-cotton varieties did not affect adult PBW it would, in effect, provide an excellent synergistic tool when combined with the inverse density-dependent action mode of the SIT and mating disruption.

The immediate concern for the *Bt* technology in all control contexts, however, was that its extreme efficacy would lead to overuse and thus ultimately to resistance development. This has proven to be a realistic concern. Tabashnik et al. (2013) offered an excellent review "after the 1st billion acres of use". Resistance to both common endotoxins (*Cry 1Ab* and *Cry 2Ac*) found in commercial cotton resulted in major losses or shifts in strategies in India and China (Tabashnik et al. 2013; Wan et al. 2017).

Currently there is discussion of major losses in *Bt*-cotton from PBW in Pakistan (Shahid 2014). Losses from resistant PBW have been reversed by using hybrid cotton in China (Wan et al. 2017). In this case $\pm$25 % of all cotton plants would not express the *Bt* traits. This provides "in the bag refugia" as described by Head and Greenplate (2012).

Within the eradication zones, the commercial use of *Bt*-cotton always had label restrictions requiring resistance management by providing plantings of susceptible cotton (refugia). These enforced EPA label restrictions required that an individual grower entity use one of two choices. The grower could plant at least 20% of his cotton with a non-*Bt* variety, which could be treated with any labelled conventional insecticide. Alternatively, the 2nd choice was that at least 5% of the surface would be of a susceptible variety of cotton, but the grower could not use a long list of conventional insecticides on those refugia.

Additional restrictions published in 2005 for the 2006 growing season added mating disruption and sterile moth release to this list. These restrictions in the use of *Bt*-cotton precluded eradication. Under this scenario, simple calculations could place production of a diapausing PBW population in a 5-acre field at > 500 000 insects in one late-season generation. This is a very conservative number. Late-season cotton produces susceptible bolls for more than one generation, thereby laying the groundwork for a large overwintering population.

Staten et al. (1999) had noted the potential use of sterile moth release over *Bt*-cotton in lieu of structured untreated refugia as part of a resistance management strategy. Sterile insect distribution is more reliable than non-directed capricious movement found in nature from native populations which are non-randomly distributed.

Arizona immediately sought a section 24C special local needs label to utilize a grower choice of up to 100% *Bt*-cotton as long as this acreage would receive an average of 10 or more sterile insects/acre/day (24.7/ha/day). This application ultimately required an extensive formal review before an EPA Science Advisory Panel in 2006 (Antilla and Liesner 2008). This strategy with some variation is in use today. Tabashnik et al. (2010) published an excellent review and assessment of this strategy in Arizona.

The choice of what cotton is to be grown has always been left to the individual producer in this programme. As part of the field mapping procedures all growers are canvassed in early spring for an inventory of expected fields to be planted. The inventory includes a separation of *Bt* and non-*Bt* types. All fields are then checked and tested with an ELISA test after germination (AGDIA Inc. Elkhart, Indiana, USA), as a safety check to ensure accuracy of *Bt*-cotton distribution maps.

3.3.2. Mating Disruption

Within this eradication programme, mating disruption was used on all non-*Bt* cotton during at least the first four years of each state's operations. The hand-applied PBW Rope (Shin-Etsu Chemical Company) was preferred. Aerially applied NoMate Fiber, NoMate Mec (Scentry Biologicals) and Check Mate (Suterra), were also used when circumstances required. These latter formulations had an effective disruption time of 8 to 14 days. A review with product details is found in Staten et al. (1997). The use of gossyplure for mating disruption for PBW represents the most successful early application of this technology (Cardé et al. 1997; Cardé, this volume). A hollow fibre-controlled release formulation was the first EPA registered disruptant (Brooks et al. 1979).

The use of pheromones for mating disruption is fundamentally different than any conventionally applied insecticide (Cardé, this volume). A treatment of a controlled release pheromone does not kill the intended target. In the case of PBW, treating an already reproducing or mated population in even moderate levels is therefore futile. There was an "attract and kill" system (Staten and Conley patent 4671010 now expired), which involved adding very small traces of a pyrethroid insecticide to the adhesive in the NoMate fibre system. It appeared to be of assistance, but its value was not great.

Staten et al. (1997) characterized both low and high-rate systems. Low-rate systems (applicable by air) require frequent reapplication with escalated error potential as each treatment timing decision is made to achieve constant disruption for a 30 to 60-day time frame. The PBW eradication programme used in the aerial spraying the three low-rate formulations described above.

The first high-rate system known as PBW Rope (Flint et al. 1985) was field tested in the Imperial, USA and Mexicali, Mexico valleys in 1986 (Staten et al. 1987). This PBW Rope provided continuous disruption and efficacy over a much longer time frame than 4-8 applications of low-rate systems applied by air. This was true even on a field-by-field basis. From that time to the present, the formulation has only had one major change. Its application, when correctly done, maintains complete trap shut-down for a 50-70-day time frame in low to moderate populations.

PBW Rope was designed to be tied on an individual plant. The programme began to pre-wrap it on a bamboo stake in 2006 in Arizona (Antilla and Liesner 2008). The current formulation was applied at 200/acre or 500/ha. For maximum efficacy in upland cotton, it must be in the field at or before cotton reaches the 6-leaf stage. This is before a female can mate and live long enough to successfully oviposit. In rare cases a second application has been justified.

Area-wide (mandated) use of pheromone has a long history. Baker et al. 1990 reported on a one-year project in the Imperial Valley of California using low-rate systems. It covered > 40 000 acres and targeted the first two generations. Low rate pheromone systems were required before first square (first flower bud) formation. Its goal was to suppress PBW long enough to reduce conventional insecticide use and secondary whitefly problems.

The aforenoted review by Staten (1997) covered two separate, later area-wide trials. These trials were in the Coachella Valley of southern California and the Parker Valley of Arizona. The most important trial in Parker, Arizona is covered in detail over a 5-year period by Antilla et al. (1996). Both trials strongly illustrated the importance of an area-wide approach with pheromone disruption. For the first time, after the first or second year, season-long control without major reliance on conventional insecticides became possible. Results were obtained in an area where the pest was normally severe. These trials both depended partially or completely on the high-rate systems. Additional trials in the Imperial and Palo Verde Valleys were used to develop a better understanding of the SIT when combining the technologies (Staten et al. 1999; Walters et al. 1998, 1999, 2000).

3.3.3. Sterile Insect Release

Releases of sterile moths in this programme had two purposes: a suppression tactic in and of itself, and as a resistance prevention strategy (see discussion in Section 3.3.1.). The release of sterile PBW was started in 1968 in the San Joaquin Valley of California as part of a containment/exclusion strategy to prevent establishment of the pest (Staten et al. 1993, 1999). Releases were continuous in areas of detection from 1970 through 2011.

Over time three sterile moth rearing facilities were established to produce PBW for field release, namely a temporary facility in Harlingen, Texas (1968-1970) and two separate facilities in Phoenix, Arizona, from 1969-1995, and 1995 to the present. The current existing facility is unique in that it was designed to produce twice the known maximum needs of the then-existing San Joaquin Valley programme.

The purchase of a 66 000 square feet (6 131 m^2) building in Phoenix, its extensive renovation and most of its equipment were paid for by the CCPCB (through the California Department of Food and Agriculture (CDFA) and managed by Mr. Wally Shropshire as chairman from 1969 through 2010, and Mr. Ted Sheely, the current chairman).

The Phoenix mass-rearing facility has been managed by USDA with California funding and minimal federal (appropriated) funds until 2005. From 2005 onward it phased into a fully USDA federally funded operation, less a few expenses such as property taxes. The production and funding of sterile moths for release then became a USA government obligation. All other field costs remained with the grower community (Tables 1 and 2).

All moth handling, packaging, chilled shipment, handling pre-release, and release procedures were developed for the San Joaquin Valley containment/exclusion programme. This occurred long before eradication started in the fully infested areas. The reporting of the successful San Joaquin Valley programme is not complete, but segments and procedures are partially covered by Rudig and Keaveny (2008) and Staten et al. (1993). Many of the sub-areas of this valley have more favourable growing conditions for PBW population development than did areas which had moderate to heavy infestations. Examples include Safford, Arizona and the El Paso Valley of Texas. Wind-borne PBW movement into the San Joaquin Valley was documented and monitored by the CDFA programme. In addition, Stern and Sevacherian (1978) and Stern (1979) established its potential through monitoring of the desert between the San Joaquin Valley and with consideration of plant growth analysis and studies of the overwintering potential.

By 2005 the Phoenix mass-rearing facility had enough space, all major equipment needed, and the technology developed to produce an expected 20-28 million moths per day. Previously it had been producing an average of 5 million moths per day. Production for the years in which the "expanded" eradication programme was in effect is shown in Table 3. Range of production resulted from varied season lengths of the multiple treatment areas.

The sterile moths were directly collected after adult emergence from pupae with immediate chilling at $\pm 35^{o}$F (1.66^{o}C). They were maintained at as near that temperature as possible through irradiation and packaging. Shipment occurred in specialized pre-chilled shipping containers to the release destinations. Any holding time of the sterile moths at destination was in cold rooms or cold boxes. They were then loaded into specialized aerial release machines installed in small aircraft (Pierce et al. 1995).

The release aircraft must be capable of working speeds of 120 miles/h (193 km/h). A Cessna 206 aircraft served this purpose in this programme. Release height above the cotton fields was maintained at an average of 500 feet (152 m) above ground. Sterile moths were normally released within 24 hours post-collection. When insects were held an additional day, loss in quality was observed. Individual non-*Bt* fields were specifically targeted. Release grids were used for a lower release rate over *Bt*-fields.

Table 3. Daily sterile moths produced at the Phoenix Arizona mass-rearing facility (in millions) used for release in the bi-national PBW eradication programme areas

Year	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Production goal/day	5.0	10.0	22.0	22.0	22.0	26.0	20.0	20.0	14.5	5.0
Mean/day	5.4	12.5	22.9	25.1	24.1	27.2	22.2	22.9	16.3	5.8
Range low	6.8	1.3	16.2	19.6	10.2	19.6	20.2	20.3	14.7	4.1
Range high	15.5	6.7	30.1	33.2	30.7	32.9	27.2	32	22.8	7.3

3.3.4. Conventional Insecticides

Insecticides were those applied to kill the pest as a direct result of their application. This form of treatment was and is the only method available to manage a rapidly expanding population when larvae are first found in the cotton bolls. In many areas, before initiation of PBW eradication, this occurred early in the cotton fruiting cycle and lasted for 2-4 months.

In the case of the eradication programme, all other control activities started either at planting (*Bt*-cotton) or before a moth can be mated and lay its first fertile egg on a plant with a susceptible fruiting form. Optimum application of mating disruption was before the 6-leaf crop stage; sterile release started even before.

An indicator of success in the first few years of programme initiation was the reduction in insecticide sprays from those previously used by the producers individually. Choice of insecticide was made based on local recommendations and knowledge. The most commonly used insecticides were chlorpyrifos as Lockon or Lorsban (Dow Chemical), or a pyrethroid labelled for PBW control. As shown in the following outcome Section, very low percentage of the total area required any traditional insecticide treatment. That predominantly occurred in the first two years of programme operations. This approach was particularly applicable in Texas and New Mexico, before sterile moth release was available.

3.3.5. Cultural Control

Among the earliest research conducted on PBW involved cultural practices for its control (Noble 1969). As PBW exploded through Arizona, invading southern California in the 1960’s and 1970’s, cultural control became a major area of research (Naranjo et al. 2002). Over a 40-year period, each growing area within the generally infested cotton areas has developed a balance between profitable production and essential regulated cultural practices.

PBW eradication used all existing regulations as a standard. Management encouraged or was involved in any needed regulatory enforcement of these best practices. Of greatest importance were the programme actions that were needed for post-harvest crop destruction and host-free periods to minimize carry-over between cotton crops.

4. OUTCOMES

The successful eradication programme outcome is presented on a state by state basis in the general order that the seven states initiated their activities as part of the programme phases (Fig. 1).

Management credits herein provided for each state are for those managers who were responsible for day-to-day decisions, operation, all data acquisition and evaluation. Managers listed were accountable for day-to-day success as well as setbacks. These individuals are considered as the most important contact points in each state. The first listed are those in the operational offices where daily decisions were made.

The programme was feasible because only non-*Bt* cotton areas required centralised control cost and intensive day-to-day attention. We provide total cotton area planted and percent *Bt*-cotton to partially illustrate the magnitude and intensity of operations in each of the states. States and cotton areas within the states with the highest ratios of non-*Bt* required the most intense management per acre or ha. The data presented are in units of measurement found in the respective field records (acres or ha).

First programmatic treatments occurred in Texas in 2001. Overall, the last PBW detected as adult unmarked moths were captured in 2012. The last sterile release treatments occurred in 2013. Population collapse is illustrated by annual adult native moth capture.

An understanding of positive economic impact is found in the reduction and eventually elimination of any detectable larvae. Direct cost-benefit is best illustrated by the decreasing use of annual inputs in high-rate pheromone (PBW Rope), sprayable pheromone systems, and conventionally applied insecticides. Lessons learned from the first state to start were extremely beneficial as the subsequent states entered the programme.

4.1. Texas, USA (Phase I)

Edward Herrera, Supervisor, El Paso /Trans-Pecos District, 1999-2013
Osama El-Lissy, TBWF Programme Director, 1999- 2000
Charles Allen, TBWF Programme Director, 2001-2009
Larry Smith, TBWF Programme Director, 2009-present

The El Paso / Trans-Pecos growers of western Texas were the first group to initiate the PBW suppression and eradication efforts reported here. The Texas Boll Weevil Foundation (TBWF) was, in 1999, fully functional and successfully involved in its part of a USA cotton belt-wide eradication effort for boll weevil, *Anthonomus grandis*

Boheman (Allen 2008). Recently detected weevil populations had become a major concern in western Texas, and treatment was restricted to these populations in 1999 through 2005 in this area.

In 1999, the grower community of western Texas passed a referendum to join the TBWF for boll weevil eradication and to initiate PBW population evaluation. In 2001, the PBW area-wide suppression activities were added to eliminate economic loss from PBW for the growers in the area. Details and complete results for the first years are covered by Allen et al. (2005). Control activities began in 2001 without sterile releases and in the absence of isolation. The programme was modelled after the Arizona Parker project (Antilla et al. 1996).

The Texas PBW programme encompassed two distinct agronomic areas (Fig. 1). The El Paso "Valley" in the USA is separated only by the Rio Grande (Bravo) River from the Juárez "Valley" of Mexico. Mexico did not start programme activities the same year (2001) despite the fact that, in many cases, fields from the two countries were less than 200 meters apart. More than half of the Texas programme was in this valley.

Cotton in the El Paso work unit was more than 50% Pima *(G. barbadense*). This species of cotton is considered to be the most PBW susceptible commercial cotton grown as it was all non-*Bt*. Initial PBW populations were very high as depicted in trap counts in the year 2000 (Table 4). Due to lack of isolation, population suppression and economic loss prevention were the only achievable goals for the first two years of the Texas programme (2001-2002).

In contrast, the area east of El Paso comprised a distinctly different production system. This included the general vicinity of the Pecos River and the town of Fort Stockton, Texas. Land was characterized by a shorter season and high usage of *Bt*-cotton. In this distinctively different area, Pima production was minimal, being grown principally in one isolated organic cotton block. Fields in this zone were dispersed over a very large geographic area and occurred in isolated clusters. Separation of such cotton blocks was frequently more than 50 miles.

From 2001-2004 (Table 4), programme treatment options on non-*Bt* cotton were limited. Required treatments were heavily concentrated in the El Paso Valley area. Aerially applied NoMate fibre, with 0.000586 lb (0.265 g) pyrethroid per acre mixed in the adhesive as an "attract and kill" formulation, represented one important control approach. As the seasons progressed chlorpyrifos at 24 fluid ounce per acre (1.68 kg/ha) was used as an overspray.

Deployment of the high-rate PBW Rope during the first two years was limited to sensitive areas near schools, etc. Its use expanded over time reaching a peak in 2003 and then gradually declining to zero over time. The initiation in 2002 of eradication programme activities in part of New Mexico and the Juárez sector of Chihuahua state in Mexico was a major improvement.

Table 4. Summary data pink bollworm programme in Texas 2000 through 2004

Year	2000	2001	2002	2003	2004
Total acres cotton	48 281	48 222	39 538	36 100	40 826
% *Bt*-cotton		49	44	35	38
PBW Rope (acres)		5399	9056	23 551	19 815
Cumulative acres aerial low-rate pheromone		142 842	123 129	58 017	26 224
Acres pheromone + insecticide*		47 897	43 386	34 945	25 162
Pyrethroid (acres)		0	0	0	2039
Larvae/100 bolls		4.53	0.81	0.13	0.76
Native moths trapped (million)	1.40	0.75	0.27	0.18	0.09

* *Chlorpyriphos at full labelled rate applied at the same time as a low-rate pheromone Acres treated are also included in low-rate pheromone treated acres*

The PBW populations on Pima cotton in the El Paso zone were critical in this state's programme. They accounted for most of the non-*Bt* cotton in the Texas programme. In 2001 Pima made up 51% of the cotton in the El Paso valley. As a result of the suppression, total trap counts of native moths were reduced from 1.4 million in 2000 (pre-control) to 0.74 million in 2001.

By the end of the last year (2004) without state-wide sterile moth releases, the season-long capture of all moths totalled 0.09 million moths. These moths were captured in traps serviced weekly at one trap per 5-10 acres of cotton. This was the highest trap density used in the programme. The annual pre-control number of native moths per trap per service averaged 17.77 in 2000 vs. less than one in 2004 (0.94).

Larval populations in bolls further illustrate progress during this 4-year suppression period. Each year 60 randomly selected fields were sampled season-long. Larval counts decreased in a steady progressive fashion; details are found in Allen (2005). From an economic perspective it is important to note the general decrease in inputs needed for control including intensively managed aerial application of low-rate pheromones and conventional insecticide.

Limited sterile moth releases on isolated fields was initiated in the Trans-Pecos area in 2004. In 2005, funding for a targeted 10 million sterile moths for release per day was obtained for the core programme area of Texas, New Mexico, and northern Chihuahua. Sterile moths were released season-long in 2005 in Texas and New Mexico.

In Juárez, Mexico, the start of sterile releases was administratively delayed for some time. During this delay moths earmarked for Mexico were heavily released in Texas along the river border between the two countries. Sterile moth movement into Mexico and therefore coverage, was far better than expected. Eventually moth distribution in 2005 of the targeted 70 million per week were carried out over the entire three state area. At this time non-*Bt* fields were directly targeted by release aircraft. The ability to release these moths in the cotton fields on already suppressed populations before any mated female moth could deposit eggs on a susceptible plant was a game changer. As populations of native PBW declined, sterile moth release numbers were diverted to expanding treatment areas.

Table 5 provides the data for the period 2005-2012, when general sterile moth releases began, until after the last native or unmarked moth was trapped. In 2005, a total of 1336 million sterile moths were released in Texas. Over 1.4 million sterile moths were trapped together with 11 917 native moths. With the exception of the year 2009, this downward trend in native moth capture was very positive as shown in Table 5.

Table 5. Summary data pink bollworm eradication programme Texas programme 2005-2012

Year	2005	2006	2007	2008	2009	2010	2011
Total acres cotton	43 358	42 304	39 533	33 029	34 299	38 268	48 447
% *Bt*-cotton	35	22	30	48	40	49	55
PBW Rope (acres)	9226	9686	2843	3198	1682	8050	0
Acres aerial low-rate pheromones	6228	7597	4964	0	0	0	0
Chlorpyrifos (acres)	2923	3653	2804	0	0	0	0
Pyrethroid (acres)	0	0	3613	0	0	0	0
Sterile moths recovered (million)	1.45	0.86	3.19	2.23	2.93	1.05	2.09
Native moths trapped	11 917	3302	1363	14	3291	16	60

No native moths were trapped in or after 2012. The last pre-season randomly selected monitoring fields with larvae were found in 2005 and 2006. Season-long larvae/100 boll recovery levels in these fields were 0.01 and 0.20 respectively.

Thereafter no further larvae were found even when fields with positive traps were specifically targeted. The last year conventional insecticides and dual insecticide-pheromone treatments were applied was in 2007. This was the result of one hotspot population along the Texas-Mexican border. The two fields with the greatest difficulty were an upland non-*Bt* cotton field on the Mexican side of the border and a 7.9-acre Pima cotton field in Texas, separated by less than 150 m distance. Larvae had been found late in the season in the Mexican field in 2006. After extensive treatment in 2007 it was not possible to detect larvae. All of these fields were treated with PBW Rope in 2008. In both 2008 and 2009 the only non-sterile inputs were PBW Rope applications.

In 2009, two unexpected events occurred shortly before cotton was harvested. These events were widely separated geographically and with different, but logically, highly probable origins.

The first occurred in and around the Pecos River Valley, where field traps started capturing large numbers of native moths on September 29. Captures were distributed over 6 023 acres, with only 30 acres of non-*Bt* cotton. No native moths had been captured before this time in this area. Historically, wind-borne movement of PBW was common at this time of the year and has been documented frequently in monitoring traps in non-production areas along desert highway trap lines. A storm front movement from an isolated heavily infested organic farm near Midland, Texas, matched this hypothesis. Pierce et al. (2013) strongly verified this conclusion in a 3-year study.

The second unexpected event started in the El Paso Valley, October 19, in an area with intensive Pima cotton. On August 22, two irradiation canisters with $\pm$180 000 moths per container left the Phoenix facility without having proper radiation tags filed. They would have been transported in shipping boxes holding 2.1-2.2 million insects each. All moths captured were in an area documented with GPS flight recorders from this shipment. No native moth captured was more than half a mile (0.8 km) from a release swath from this flight. The vast majority of the 2626 non-marked moths in the El Paso area were directly within the expected swath of this aircraft. No larvae could be found, although exit holes and some characteristic damage was observed. The capture curves fitted with expected late-season life cycle length. The programme had examined a total of 67 246 blooms and bolls prior to the outbreak without any detection. Native moth capture had not occurred in the entire area before October 12. It was only logical to conclude that the unanticipated captures were due to a release of non-irradiated moths. To mitigate this situation, procedures for irradiation safeguarding were reviewed and significantly stiffened at the Phoenix PBW mass-rearing facility.

In 2010, this entire area (8050 acres) was treated with PBW Rope (Table 5) and received enhanced sterile moth releases. The native moths captured in 2010 were scattered throughout the El Paso zone, but not in the PBW Rope treated area. The last native moths captured were in 2011. The Texas PBW programme, as described, has not had a further detected moth or larvae.

4.2. Chihuahua, Mexico (Phase I)

Ing. Alfonso Soto Martinez, Gerente del Comité Estatal de Sanidad Vegetal, 2001-2008
Ing. Antonio Medina Arroyo, Gerente del Comité Estatal de Sanidad Vegetal, 2009
Ing. Juan Carlos Ramirez Sagahon, Gerente del Comité Estatal de Sanidad Vegetal, 2010-2011
Ing. Antonio Medina Arroyo, Gerente del Comité Estatal de Sanidad Vegetal 2011-2013
Ing. Jesús Escárcega Terán, Gerente del Comité Estatal de Sanidad Vegetal, 2013 to declaration.

Chihuahua has the largest cotton growing area of any state in Mexico. It is also a state in which production areas grew rapidly during this programme. Eradication of PBW and boll weevil were and are within the same management programme under the Comité Estatal de Sanidad Vegetal. Maps and details for 2002 through 2007 can be found in Staten and Ramirez-Sagahon (2008).

The state programme was ultimately organized in four work units, Ascensión, Meoqui, Ojinaga and Juárez, all in northern Chihuahua (Fig. 1). Trap placement in Chihuahua was standardized at 1 trap/4 ha and 1trap/20 ha in non-*Bt* and *Bt*-cotton respectively.

4.2.1. Ascensión, Chihuahua

This work unit included all cotton grown in the north-western portion of the state. This cotton, with only a few exceptions, was grown with a shorter season and colder winter. This is typical of all cotton found in the highland Chihuahua deserts, where centre pivot irrigation predominates. Historically boll damage was sporadic and usually occurred late in the season. With this in mind, the programme was designed to be heavily dependent on the planting of *Bt*-cotton and PBW Rope treatment. By 2004, the PBW population had been reduced to a level in which sterile insects would not be required.

During the critical initial three years of the programme (2002-2004), the percentage of *Bt*-cotton was at 67-69% (Table 6). During this time all non-*Bt* cotton was treated with PBW Rope. This cotton was well dispersed throughout the cotton production area. Native moth captures decreased by 3.6 and 3.7-fold each year. As the programme progressed, the application rate of PBW Rope was reduced in areas with moderate risk (based on previous year's moth trap capture) from 500 to 250 per ha.

In 2005 some fields did not receive pheromone treatment. Numbers of non-*Bt* fields treated also generally declined with the exception of the 2006 year. This was preceded by the only season to season escalation in native moth captures in traps. This occurred principally late in 2005, when minimum numbers of larvae were found in non-*Bt* monitoring field searches (less than 10).

By 2006 only 38% of the cotton was *Bt*-cotton (Table 6). 2006 had the only appreciable escalation of PBW Rope use. Conventional insecticides were used in only two years directly for PBW suppression. The last native moth capture in the Ascensión area of Chihuahua was in 2007 (12 specimen). Staten and Ramirez-Sagahon (2008) noted that this represented two native moths per every 10 000 traps serviced.

Table 6. Summary data pink bollworm eradication programme for the Ascensión area of the state of Chihuahua

Year	2002	2003	2004	2005	2006	2007	2008	2009
Total hectares (ha)	11 268	16 499	25 637	23 088	28 430	29 228	26 632	10 209
% *Bt*-cotton	69	68	67	47	38	29	30	31
PBW Rope (ha)	3507	5177	8566	8936	13 110	1118	461	0
Insecticides (ha)*	581	0	0	86.9	0	0	0	0
Native moths	20 256	5489	1467	4204	63	12	0	0

* *Conventional aerially applied insecticides expected locally to be the most effective*

4.2.2. Meoqui, Chihuahua

This unit is Chihuahua's southern-most cotton growing area. This area of Chihuahua is bordered by the states of Coahuila and Durango. It contained all the cotton cultivation found around the cities of Delicias and Jimenez. It is an older, diverse production unit mostly using impounded water from the Rio Conchos. It has enough overwintering habitat to make boll weevil its key pest.

Area-wide treatment for both PBW and boll weevil started in 2002. The weevil required ULV malathion with emphasis on pin square treatments and treatments in mid- and late-season based on weevil trap captures. This was followed by treatments targeting weevils going into diapause. A high percentage of *Bt*-cotton was present in all years except 2006 (Table 7). The PBW Rope was ideally suited for use in these circumstances. In the initial three years (2002-2004), all non-*Bt* cotton was treated with PBW Rope; application was targeted for 6 leaf cotton in the spring.

In 2005, PBW Rope was applied in the spring on non-*Bt* fields that had positive trap captures the previous year or at detection in early and mid-season. Only in the first year were 44.3 ha treated for PBW with a conventional insecticide and a sprayable pheromone, in addition to PBW Rope. Suppression in these fields was triggered where even a single larva was found by targeted scouting. No aerial treatments were applied thereafter.

Table 7. Summary data pink bollworm eradication programme for the Meoqui area of Chihuahua

Year	2002	2003	2004	2005	2006	2007	2008	2009	2010
Total hectares (ha)	930	5151	9332	6754	4332	933	1588	45	1704
Non-*Bt* (ha)	164	380	278	321	2195	114	266	2	254
% *Bt*-cotton	82	93	97	95	49	88	83	99	85
PBW Rope (ha)	164	380	278	180	139	25	12	0	0
Insecticides (ha)	44.3	0	0	0	0	0	0	0	0
Native moths trapped	N/A*	377	2970	1203	13 410	2632	953	265	0

* *N/A= not available; 5046 moths were captured in the combined Meoqui and Ojinaga work units*

Unfortunately, native moth capture summary data for 2002 are not available as they were combined for the areas of the Ojinaga and Meoqui work units. Throughout the programme in Chihuahua, trap grids were not completely established until July 2002. During this programme's 2002 establishment period, boll weevil treatment and trapping, as well as PBW Rope application, required availability of early-season resources.

Weekly PBW trap data as reported in Staten and Ramirez-Sagahon (2008) provide an understanding of important occurrences in the first two years, with graphics showing separate weekly trap captures for each area. There were appreciable trap captures in 2002. In 2003, captures were reduced season-long, however, in Meoqui during September 2004, trap capture of native PBW escalated. From September to the last service date in October 2914 moths were captured with no correlation to non-*Bt* fields. Migration was suspected but not verified.

In 2006 no moth captures occurred anywhere in Meoqui before the week of September 11. From that point on, there was a massive escalation in captures throughout the region with no correlation to non-*Bt* cotton. This included a new growing area with no PBW history and major captures in new *Bt*-fields with no cotton history. Highway trap lines were quickly established to the cotton growing area south of the state of Chihuahua in the La Laguna area, in the states of Coahuila and Durango, and to the Texas-Mexico border in Ojinaga. The conclusion, that a major weather-driven migration was responsible, was inescapable.

In 2007, many of the PBW positive fields were shifted to *Bt*-cotton or other crops. PBW Rope use continued but declined. By 2009 no treatment for PBW was needed. There have been no further PBW captures since 2009. By 2010, the La Laguna area was moving forward for a combined PBW and boll weevil programme.

4.2.3. Ojinaga, Chihuahua

This work unit, as stated above, was managed together with the Meoqui unit for three years. It had two distinct habitats for boll weevil and PBW. A well-established area was in a surface irrigated system at the confluence of the Rio Concho and the Rio Bravo (Grande, USA) rivers. This area is a local commerce centre at Presidio, Texas and Ojinaga, Chihuahua. Initially Texas had some cotton on its side of the border. On the Mexican side, removed from this river valley, where rapidly developing areas of centre pivot irrigation. The growing season in these areas is shorter than in the cotton areas in the river valley. Its rapid expansion is reflected in the increase in cotton areas (Table 8).

During the initial three years all the non-*Bt* cotton was treated with PBW Rope. Need and use of pheromone was reduced thereafter; the last treatment for PBW occurred in 2010 on 78 ha. No conventional insecticides were used for PBW in the Ojinaga unit. Boll weevil was treated with ULV malathion.

Table 8. Summary data pink bollworm eradication programme for the Ojinaga area of Chihuahua

Year	2002	2003	2004	2005	2006	2007	2008	2009	2010
Total hectares (ha)	1382	3987	9541	10 936	20 102	19 885	19 611	12 585	27 220
% *Bt*-cotton	42	52	82	85	81	60	42	69	86
Pheromone (ha)	803	1907	1715	130	245	769	248	315	78
Insecticides (ha)	0	0	0	0	0	0	0	0	0
Native moths trapped	N/A*	390	79	4	5415	96	225	89	0

* *N/A = not available, 5046 native moths were captured in the combined Meoqui and Ojinaga work units*

As noted in Staten and Ramirez-Sagahon (2008), native moth captures were common when traps were deployed in 2002. With area-wide suppression, numbers dropped drastically in the following years through 2005, when only four PBW moths were captured. However, as detailed in the discussion of the Meoqui unit, Ojinaga's 2006 trap captures exploded. A continued declining south to north gradient was apparent, with the largest portion of total captures occurring in the southern part of this area near Camargo, Chihuahua. The lowest captures occurred in the river valley on the Texas-Chihuahua border.

A season total of 5415 PBW moths were recovered in 2006. This escalation started during the reporting week of September 24, as was true of the Meoqui area. Native PBW had not been detected before this time in 2006. Similar patterns of capture occurred through 2009. Nevertheless, the last moth detections were in 2009 at 89 moths for that season. In 2010 no moths were detected with trap services exceeding 4000 traps per week.

4.2.4. Juárez, Chihuahua

This unit contained the state of Chihuahua's most destructive PBW populations. Its situation was inseparable from the El Paso, Texas valley unit as described in the Texas Section. The major differences were in a latter programme starting date (2002) and the absence of Pima cotton. Cotton was largely non-*Bt* in the Juárez unit and non-*Bt* cotton fields on the Mexican side of the border were all *G. hirsutum* varieties (upland cotton).

The first year's start was with a short time frame as described in the previous areas of Chihuahua. This unit started with only the cotton areas in the Rio Bravo (Rio Grande, USA) River Valley. As time progressed, new centre pivot irrigated cotton areas near Villa Ahumada, south of Juárez, were added to the unit. This centre pivot cotton did not create problems. *Bt*-cotton was primarily grown in the outskirts of the city of Juárez's urban interfaces. This scattered cotton production was found in the north-western end of the valley. *Bt*-cotton ranged from 33% in 2002 to 13% in 2008 (Table 9).

Table 9. Summary data pink bollworm eradication programme for the Juárez area of Chihuahua

Year	2002	2003	2004	2005	2006	2007	2008	2009
Total hectares (ha)	5251	7579	8689	7915	8898	7625	7371	5052
% *Bt*-cotton	33	24	29	29	23	20	13	18
PBW Rope (ha)	3528	5736	6177	1070	1063	1159	194	155
Insecticides (ha)	697	60	70	30	0	114	0	0
Sterile moths released (millions)	0	0	0	69.4	205.7	346.5	371.9	344.5
Native moths trapped	9886	5573	1247	1108	1957	447	4	0

Conventional insecticide was most heavily applied at the start of the year 2002; in total 697 aggregate ha were treated over that season. The second highest aggregate treatment occurred in 2007 at 114 ha. Use of aerially applied insecticides was limited to 30-70 ha in 2003-2005. All non-*Bt* fields were treated with PBW Rope during the initial three years of operations (2002-2004). As in other areas of Chihuahua, detection thresholds were used after 2004 and PBW Rope treatments declined after that year.

In 2002 native moth captures were the most intense of any area in Chihuahua. From August 25 to November 10, a total of 9486 native moths were captured in 11 586 total traps serviced. As in other areas of Chihuahua, native moth captures declined during subsequent years (Staten and Ramirez-Sagahon 2008).

By 2006 and 2007, the majority of all captures were recovered in one general area of the state of Chihuahua, which was contiguous with fields on the Texas side of the border. In 2008, the Chihuahua programme successfully encouraged producers to shift most of these fields to *Bt*-cotton. The last native PBW in the Juárez unit were trapped in 2008.

In 2010 and 2012, PBW Rope was applied to 129 and 153 ha respectively, in coordination with Texas treatments targeting finds in adjacent Texas fields still with native moth captures. These treatments were triggered based on late-season captures in 2009 and 2011 in Texas (Table 5). Sterile moth releases, started in 2005, therefore continued through 2012 at 180.2, 119.8, and 118.3 million per year. This was done in concert with Texas captures in adjacent fields. There is no ecological separation between the two areas.

4.3. New Mexico, USA (Phases I and II)

Joe Friesen, Executive Director PBW and BW Foundation, 1999-2013
Patrick Sullivan, Executive Director PBW and BW Foundation, 2013 to present

The New Mexico Phase I programme, managed by the New Mexico PBW and BW Foundation, covered all the cotton along the Rio Grande River in the Mesilla Valley and the Hatch Valley (Fig. 1). These two valleys follow the river from El Paso, Texas, north to the Caballo Lake Dam ($\underline{+}$100 miles), where cotton is no longer a dominant crop. This area, established before 1930, has a diversified agriculture with a high percentage of its area dedicated to pecans, peppers, vegetables, alfalfa, and grain. Urban interface is significant around its biggest city of Las Cruces. The majority of cotton acreage is in these two valleys, where it is irrigated with lake-stored water supplemented with groundwater in close proximity to the river.

A smaller area of cotton production is found further west near Deming, New Mexico. It is pump-irrigated and similar to the Ascensión area of Chihuahua, Mexico south of this part of New Mexico. Details for the PBW programme for the years 2000 through 2007 can be found in Friesen and Staten (2008). In four of the first five years of the programme, non-*Bt* exceeded *Bt*-cotton (Table 10). Non-*Bt* cotton was made up of Pima and upland cotton in order of importance. Of this cotton, 2-5% was certified organic.

From 2002 through 2004 all non-*Bt* cotton was treated with PBW Rope. As outlined in Friesen and Staten (2008) this was complicated by local cultivation practices applied for weed control. Cotton was planted in nearly flat low beds, but then cultivation raised the soil in the plant row while forming a deep furrow. As a result, PBW Rope applied at the 6th leaf node on a young plant was quickly covered in dirt. Pheromone emission was therefore blocked rendering the treatment ineffective.

To overcome this cultivation problem, in 2003 sprayable pheromones and dual insecticide-pheromone treatments were applied from ground spray rigs and by air at a targeted 10-day interval until the PBW Rope could be applied. This was logistically difficult and drastically increased programme input costs. It also contributed to the higher than expected usage of sprayable pheromones and insecticide shown in Table 10.

Table 10. Summary data pink bollworm eradication programme in New Mexico (Phase I)

Year	2002	2003	2004	2005	2006	2007	2008	2010
Total acres	17 061	21 061	21 701	21 722	21 627	16 957	14 664	13 246
% *Bt*-cotton	37	51	46	45	36	80	74	76
PBW Rope (acres)	10 690	9300	9493	1991	627	1325	0	0
Acres aerial pheromone	0	17 025	9843	3445	0	63	0	0
Acres pheromone + insecticide	0	13 115	4806	255	0	0	0	0
Mean traps serviced/week	1782	1906	2371	2231	1652	910	412	633
Sterile moths (millions)				N/A	322.7	365.9	307	
Sterile moths recovered					330 308	394 842	279 385	15 907
Larvae detected		227	2	0	0	0	0	0
Native moths trapped	51 764	126 033	18 126	2978	203	15	0	1

Treatments were concentrated in pockets. By 2005 this level of treatment could be reduced. Even so, with over 11 900 acres of non-*Bt* cotton, only a combined cumulative 3703 acres of sprayable pheromone and conventional insecticides were applied. PBW Rope applications in 2007 were based largely on native moth captures in 2006. The last year of pheromone treatment in the New Mexico programme was 2007.

Sterile moth releases started on a partial, "experimental" basis in Deming, Hatch, and north of Las Cruces in 2004. The entire area received its first full complement of sterile moths in 2005. Although *Bt*-fields were not specifically targeted, sterile moths were present in all fields. In 2007, all non-*Bt* fields received direct sterile moth releases. Less than 50% of these non-*Bt* fields required any additional pheromone treatment.

Native moth captures were again used to document the decline of detectable populations. In 2003, total counts were much higher than in the first year (2002). Nevertheless, in 2001 pre-programme captures of moths per trap per week still peaked at ±7 times greater when compared to similar data for 2003. Post-2003 captures of native moths declined each year through 2007, when only 15 native moths were trapped for the entire year.

In 2010 one moth was captured in a field just north of El Paso, Texas, which abutted a Texas field with concurrent late-season captures. These fields were literally separated by a line on a map representing no more than 20 feet in distance. This small area was included in the Texas sterile moth release operations for the remainder of the 2010 season and in 2011.

By 2006, cotton in Chavis, Eddy, and Lee counties of New Mexico was managed by contract as part of the Texas boll weevil eradication programme. During this time the area was under extensive ULV malathion treatment for boll weevil. The cotton produced was predominantly *Bt*-cotton. Pierce et. al. (2013) discusses the last 9 moth captures in 2009. All evidence indicates these captures were remnants of the same population movement that affected the Pecos valley area of Texas in that same time frame. In that study no New Mexico captures occurred in 2010 and 2011. APHIS records show negative surveys in 2011 to 2015.

In Hidalgo county, a small area of cotton production on the Gila river at the Arizona-New Mexico border was managed in Phase II as part of Arizona's Safford district (Section 4.4.1.) without separation of data.

4.4. Arizona, USA (Phases II, IIIa and IIIb)

Larry Antilla, ACRPC Director, 1991-2011
Leighton R. Liesner, ACRPC Director, 2011 to present

The Arizona Cotton Research and Protection Council (ACRPC) was established in 1984 to deal with an outbreak of boll weevil in Arizona. The outbreak was rapidly expanding in scope. The resulting programme culminated in declared boll weevil eradication in 1991 (Neal and Antilla 2001). The organization remained intact for continued monitoring of boll weevil and PBW populations. Its numerous projects included the successful Parker area-wide mating disruption trial, as noted previously.

It became a model for other areas of what could be accomplished. It was the key example used to convince Texas growers to make a first commitment to eradication (Allen et al. 2005).

The organization became deeply involved in a coordinated programme to address area-wide resistance management of PBW in *Bt*-cotton with the University of Arizona (Tabashnik et al. 2000; Antilla et al. 2001; Dennehy et al. 2004). The understanding of the presence of resistance and its risk potential was intense. As an issue and threat, it was completely controlled as exemplified by declines of resistance expression (Tabashnik et al. 2010, 2013).

When sterile moths became available, the organization was in place for an area-wide eradication programme. It had extensive pre-programme population monitoring data and knowledge of the relevant areas. The ACRPC and the University of Arizona were critical in clearing and implementing a special state 24C label allowing the utilization of sterile insects in lieu of structured non-*Bt* cotton refugia for PBW resistance management. A 2005 revised EPA primary *Bt*-cotton label issued for 2006 could have ended eradication efforts as they would have rendered some cotton untreatable (see transgenic cotton Section 3.3.1. of this chapter).

4.4.1. Eastern Three Fourths of Southern Arizona (Phase II)

With the passage of a grower-approved referendum on PBW eradication funding in 2005, Arizona's ACRPC started its first year with all tools at their disposal in 2006. It started in approximately 85% of cotton state-wide. It constituted all cotton in surface and groundwater irrigated areas within the eastern three fourths of southern Arizona (Fig. 1, Phase II).

As shown in Table 11, of the 165 683 acres, 93.1% were *Bt*-cotton in 2006. The Safford Valley exceeded this overall average, providing the majority of the Phase II area's non-*Bt* cotton. During this time, much of the cotton planted was Pima (*G. barbadense*).

An unpublished cooperative effort with ACRPC, USDA-CPHST, and Pacific BioControl led the development of the application technology of PBW Rope pre-wound on a bamboo stick in lieu of the ropes being directly "tied" on small cotton plants. This allowed ACRPC to mechanize treatments for many of their non-*Bt* fields. In all non-*Bt* fields, treatment could thus be initiated earlier. Arizona was successful in applying PBW Rope on all non-*Bt* cotton in 2006-2009. Late 2011 native moth captures led Arizona to again treat all non-*Bt* cotton with PBW Rope in 2012. As shown in Table 11, aerially applied pheromone treatments declined from 2006 to 2010 (from 6409 to 0).

In 2006, Arizona treated a cumulative 6409 acres with conventional insecticides predominantly on a small cluster of fields near Eloy. This event did not reoccur in subsequent years. All conventional insecticide use was eliminated in 2009. Arizona conducted boll surveys in late-season as part of an ongoing *Bt* resistance monitoring programme. Larval PBW populations ceased to be detectable by 2009 in this portion of the state. Adult PBW capture decline from 2006, the first year of treatment, through 2011, was equally impressive.

Table 11. Summary data pink bollworm eradication programme in Arizona (Phase II - Arizona Zone 1)

Year	2006	2007	2008	2009	2010	2011	2012	2013
Total acres	165 683	145 947	118 435	124 191	168 255	213 413	159 244	136 244
Non-*Bt* acres (%)	11 465 (6.9)	6389 (4.4)	2029 (1.7)	2144 (1.7)	7387 (4.4)	11 946 (5.6)	6607 (4.1)	4427 (3.2)
PBW Rope (acres)	11 465	6389	2029	2144	0	0	6607	0
Acres aerial pheromone	6409	1458	31	64	0	0	0	0
Insecticides (acres)	2907	1197	62	0	0	0	0	0
Sterile moth releases (millions)*	1682	1443	1410	1990	1123	1593	1300	382
Sterile moths recovered (millions)	1.447	0.918	0.551	0.307	1.137	1.047	0.489	0.012
Larvae detected (%)	1126 (2.0)	31 (0.08)	2 (0.02)	0	0	0	0	
Native moths trapped	657 752	199 726	2306	866	453	566	0	0

* *In 2006, California Phase IIIa's release numbers are included in this table*

4.4.2. North of Yuma Arizona along the Colorado River (Phase IIIa)

Eradication treatments of cotton areas north of the Yuma, Arizona area along the Colorado River (Fig. 1) began in 2007. It included the Parker and Mojave valleys along the Colorado River. Southern California started its eradication treatments at the same time. The area had high ratios of *Bt*- to non-*Bt* cotton (Table 12). Prior to 2007 many of the growers in this area used the un-treated non-*Bt* cotton option (5%) for resistance management. At least some of this was not harvested (sacrificed) due to PBW damage.

With the initiation of sterile moth releases for resistance management, all non-*Bt* cotton could be and was treated at or just before 6 leaf with PBW Rope on a bamboo stake. This occurred from 2007 through 2010, and in 2012. The decision to treat in 2012 was state-wide after unexpected increases in late 2011 (see above). With a preponderance of *Bt*-cotton, PBW Rope treatment, and sterile moth releases, the need for sprayable pheromone and conventional insecticides ended quickly (Table 12).

Throughout the pheromone-treatment years, the majority of all native moth captures were actually in *Bt*-cotton, either near a non-*Bt* cotton field or in fields which were rotated out of a non-*Bt* refuge field the previous year. Captures were reduced by 97.9% between 2007 and 2008. Larval populations were no longer detectable after 2007.

Table 12. Summary data pink bollworm eradication programme in Arizona (Phase IIIa - Arizona Zone 2)

Year	2006*	2007	2008	2009	2010	2011	2012	2013
Total acres	N/A	16 546	12 835	12 108	18 654	28 940	24 204	19 485
Non-*Bt* acres (%)	N/A	509 (3.1)	993 (7.7)	1812 (15)	1116 (6)	1278 (4.4)	299 (1.2)	285 (1.5)
PBW Rope (acres)	N/A	509	993	1 812	1 116	0	299	0
Areal pheromone (acres)	N/A	290	0	111.5	0	0	0	0
Conventional (acres)	N/A	117	0	0	0	0	0	0
Sterile moths recovered	N/A	56 689	47 432	32 546	18 125	17 150	21 904	10
Larvae detected	784	31	0	0	0	0	0	0
Native moths trapped	N/A	155 104	3259	1183	36	17	0	0

* *Pre-programme treatment*

4.4.3. Yuma and Lower Colorado Basin (Phase IIIb)

The profile of the Yuma area, Phase IIIb was similar to Phase IIIa and the rest of the Lower Colorado Basin (Fig. 1). In all these areas, when non-*Bt* cotton is produced full season, the PBW population development potential is extensive, as most of the winter is frost-free. Fortunately, in Yuma most of the cotton is rotated with high value winter vegetables. That portion of the area’s cotton crop is terminated in August and September. Consequently, the PBW population growth potential from September through November is severely curtailed in these fields. This, and high percentages of *Bt*-cotton, was extremely important in potential PBW population reduction (Table 13).

All non-*Bt* fields were treated with PBW Rope for the first five years of programme operations. A portion of the non-*Bt* fields were treated with aerially applied pheromone in 2008 and 2009 after PBW Rope efficacy ended. Conventional insecticides were only needed in 2008 and were minimal. Larval populations were not detected after 2009. After 2007, pre-programme survey moth captures declined sharply with no native moth captures in 2011 and one in 2012.The one capture in 2012 was in early April before hostable fruit set. It was the last native moth detection in the programme in the USA.

Table 13. Summary data pink bollworm eradication programme in Arizona (Phase IIIb - Arizona Zone 3)

Year	2007*	2008	2009	2010	2011	2012	2013
Total acres	N/A	10 358	12 957	15 571	24 676	19 116	11 502
Non-*Bt* acres (%)	N/A	236 (2.3)	717 (5.5)	344 (2.2)	2010 (8.1)	458 (2.4)	178 (1.5)
PBW Rope	N/A	236	714	344	2010	458	0
Acres aerial pheromone	N/A	248	930	0	0	0	0
Conventional (acres)	N/A	386	0	0	0	0	0
Sterile moths recovered	N/A	11 861	75 027	39 718	75 822	75 961	130 978
Larvae detected	44	7	2	0	0	0	0
Native moths trapped	61 166	21 032	4175	85	0	1	0

* *Pre-programme operations*

4.5. Southern California, USA (Containment Programme, Phases IIIa and IIIb)

Jodie Brigman District, Supervisor CDFA, 2001-2017
Jim Rudig, Programme Manager CDFA, 2006-2011
Victoria Hornbaker, Programme Manager CDFA, 2011, interrupted, current

The California organization (California Cotton Pest Control Board, CCPCB) was in place and operating in southern California before 2007, with all needed CDFA staff working on PBW activities, including the long-standing San Joaquin Valley containment/exclusion programme. They were deeply involved in population monitoring particularly in long standing monitoring of seasonal PBW movement throughout the area. Important efforts included monitoring for *Bt* resistance management in cooperation with the other states, led by the University of Arizona (Dennehy et al. 2004).

When PBW invaded southern California in 1963-64, the Imperial Valley, the Coachella Valley, and the Blythe-Palo Verde Valley had an extensive and prosperous cotton industry, frequently comprising more than 100 000 acres. Before PBW establishment, southern California produced very high yields with limited, targeted insecticide. The tenets of integrated pest management pioneered here, as described by Stern et al. (1959), had become a world standard.

In this southern California area, cotton was produced using a February-March planting window and harvested well into December. As part of the Lower Colorado River Basin (Fig. 1), with its neighbours of Yuma in Arizona, San Luis Río Colorado in Sonora, and Mexicali in Baja California, cotton's long-season growth regimen allowed the introduction and establishment of PBW to produce more generations per season than anywhere else in its North American range. Results of the invasion were significant yield losses and extensive insecticide use. Secondary pests, exemplified by whitefly, became common.

By the time southern California entered the PBW eradication programme in 2007, neither the Coachella Valley, nor the Imperial Valley had any commercial cotton. Only the Blythe area, the Palo Verde Valley, Bard/Winterhaven area (adjacent to Yuma), and Needles north of Parker, still produced some cotton in southern California. Most importantly, southern California had the highest ratio of *Bt*- to non-*Bt* cotton of any area in the programme (Table 14).

Southern California was unique in that it also had a small but important okra production. Okra is a weak host for PBW in the south-western USA and PBW has a decided preference for cotton. In addition, okra pods are harvested before larvae can develop, serving as a mechanical control. In the above agronomic environment, no conventional insecticides or sprayable pheromone systems were used. All non-*Bt* cotton was treated with PBW Rope from 2007-2011. Okra in the Imperial Valley was treated with PBW Rope for the initial three years. PBW Rope was also applied in the Coachella valley in 2008.

The small amount of non-*Bt* cotton was targeted at the onset of the programme at the standard mean of 250 sterile moths/acre/day (618/ha/day). All okra was targeted at a mean of 200 sterile moths/acre/day. In their most important role, sterile moth releases were used for PBW resistance prevention and management. *Bt*-cotton was targeted at a standard of 10 sterile moths/acre/day (24.7/ha/day). Adult moth monitoring showed a consistently decreasing number of native PBW moths captured each year that the programme progressed (Table 14).

The last native moth was captured in southern California in 2011. It was captured in a highway trap line, not in a field trap.

Table 14. Summary data pink bollworm eradication programme in southern California

Year	2007	2008	2009	2010	2011	2012	2013
Total acres cotton	16 555	9 635	6 132	10 445	19 175	18 293	16 171
Non-*Bt* acres (%)	130 (0.8)	30 (0.3)	71 (1.1)	0 (0.0)	85 (0.4)	105 (0.6)	30 (0.2)
Okra (acres)	205	460	597	560	513	555	515
PBW Rope cotton (acres)	130	30	71	0	85	0	0
PBW Rope okra (acres)	205	460	327	0	0	0	0
Sterile moths released (millions)	76.46	72.15	36.71	124.74	5.27	1.25	0
Native moths trapped	447 067	16 395	6142	147	1	0	0

4.6. Northern Sonora, Mexico (Phase IIIa)

Ing. Javier Valenzuela Lagarda, Gerente de Comité de Sanidad Vegetal, 2006 to declaration.

The cotton growing area of northern Sonora is found predominantly along the lower Colorado River in San Luis Río Colorado. In addition, it includes cotton in Sonoyta on the Arizona/Sonora border more than 200 km to the east. The Sonoyta data include scattered fields near the city of Caborca. In the latter, like much of Sonora's southern coastal production areas, agriculture has shifted to vegetable production. At this time no cotton production remains in Caborca. The only other cotton in Sonora during this programme was in the state's southern coastal areas. The Sonoyta area was limited and of a shorter season than San Luis. The San Luis growing area is separated from the Yuma, Arizona growing area by the small twin cities of San Luis Río Colorado and contiguous San Luis, Arizona. It is separated from the Mexicali Valley of Baja California only by the normally dry Colorado River and riparian area. The cotton is planted in early March through mid-April. In San Luis irrigation for cotton production is limited after August. This limits reproduction in later generations of PBW.

During 2007 and 2008, only non-*Bt*-cotton was produced in the Sonoyta / Caborca area. By 2013, this trend was completely reversed in favour of *Bt*-cotton in Sonoyta, while after 2010 the scattered fields around Caborca were no longer in cotton. The increased ratio of *Bt*- to non-*Bt* cotton was more pronounced in San Luis proper as well. By 2013, 99% of all cotton was *Bt* as illustrated in summary Table 15.

Table 15. Northern Sonora, Mexico pink bollworm eradication programme summary data (Phase IIIb, Figure 1)

Year	2007	2008	2009	2010	2011	2012	2013
Total hectares (ha)	3126	3885	2974	3657	6881	6751	3515
% *Bt*-cotton	54	72	75	87	91	92	99
Pheromones (ha)	1425	1093	751	478	550	237	10
Insecticide + (ha)	454	197	55	33	0	0	0
Sterile moth releases (millions)	0	0	152.5	198.4	208.8	210.3	117.2
Larvae detected	22 fields	9 fields	7 fields	1 field			
Native moths trapped	1 139 586	159 421	35 771	1139	163	7	0

In 2007 and 2008, sterile insect release was not available until 2009. The programme objectives were to drive populations down below conventional pest management field treatment thresholds. Northern Sonora's objectives were to treat all non-*Bt* cotton with PBW Rope on bamboo stakes. Limited conventional insecticide plus sprayable pheromone was required, but progressively reduced through 2010 (Table 15). Programme management treated fields with the highest risk with a second application of PBW Rope at 50-65 days, when triggered by native moth captures. Insecticide plus sprayable pheromone was used only when a larva was detected in boll samples.

In the first year (2007), with a full trap grid (1 trap per 4 ha non-*Bt* and 1 trap per 20 ha *Bt*-cotton), captures totalled 1 139 586 native moths season-long. The San Luis Rio Colorado area contributed most of these captures. It was influenced by its adjacent neighbour, the Mexicali valley, which was not yet in the eradication programme. In 2008 captures declined significantly to 159 421 (Table 15).

Perspective pre-programme data on file show season-long mean native moths per trap per week from 2006 through 2008 to be 106, 46, and 16. This downward progression continued to zero native moths captured in 2013. Solid evidence of reproduction is found when any larvae can be detected in a field. During the first year, 22 fields were positive, all in San Luis Río Colorado. By 2010, even with extensive "directed" sampling of fields with native moth captures, only in one field a larva was detected.

No PBW has been detected in this programmatic area after May of 2012. The last seven native moth captures coincide in time and space with the last adults captured in neighbouring Mexicali and Arizona.

4.7. Mexicali, Baja California, Mexico (Phase IIIb)

Ing. Enrique Montano, Gerente del Comité Estatal de Sanidad Vegetal, 2006-2007
Ing. Roberto Roche Uribe, Gerente del Comité Estatal de Sanidad Vegetal, 2008 to declaration.

The Mexicali Valley, during the course of this eradication programme, contained all the cotton in Baja California (Fig. 1). It was established as a production area in 1912. Its water source, like San Luis Río Colorado, is the Colorado River, its eastern border. The northern limit of this valley is the California, USA border with Mexico. As with San Luis Rio Colorado, if cotton were to be grown for its longest potential season, it would generate extremely high populations of PBW. Irrigation for cotton has long been terminated in late August. Yield potential is still high, with planting and harvest windows consistent with its Sonoran neighbour. This area has no PBW population separation from northern Sonora.

When PBW entered this system, as in San Luis Río Colorado, it drastically affected production practices. Insecticide use escalated for secondary pests as well as PBW. Shorter growing cycles became the reality. As in all areas, the introduction of *Bt*-cotton was profound. Its use escalated even as growers ceased to find PBW resulting from eradication activities in non-*Bt* cotton (Table 16).

Pre-programme monitoring data for 2007 has been provided, indicating extremely high native moth captures. Programmatic control activities in non-*Bt* cotton started in 2008 with pheromone treatment of all fields. Two different high-rate systems were used in 2008. The PBW Rope was used on 73% of non-*Bt* fields. A second high-rate system was used on the remaining non-*Bt* fields.

In 2009-2012 all non-*Bt* fields were treated with the PBW Rope, targeting a pre-6 node cotton development window. After 2010, all PBW Rope applications at or before 6 leaf were applied on the bamboo stake. Dual insecticide-pheromone applications were used on fields in which trap captures exceeded one moth per trap per night, or in which larvae could be found. This occurred in 2008, from the week of 4 August through 15 September. Field re-treatment varied depending on trap captures. By 2011, only one field required treatment (36 ha, Table 16). Sterile moth releases started in 2009 with the majority directed over non-*Bt* fields. This continued through 2013 in areas where native moths were captured in 2012.

In population assessment, no larvae were detected after 2009. These data were from a programme evaluation survey of randomly selected non-*Bt* fields. Fields were selected when mapping of cotton fields was complete early in the season. Field selection occurred before boll set.

Table 16. Mexicali valley, Baja California, Mexico pink bollworm eradication programme summary data

Year	2007	2008	2009	2010	2011	2012	2013
Total (ha)	20 643	19 984	17 385	20 153	33 671	32 829	22 814
% *Bt*-cotton	62	68	78	84	96	96	96
Pheromone (ha)	0	6505	3771	3170	1455	1511	0
Insecticide+ (ha)	0	2750	1652	264	36	0	0
Sterile moth releases (millions)	0	0	822.6	766.3	825.5	755.4	258
Larvae detected (%)	1450 (29)	181 (3.2)	4 (0.007)	0	0	0	0
Native moths trapped	2 705 400	709 203	162 226	15 258	401	18	0

All native moth capture data in Table 16 were totals from the programmatic standard season-long trap grids. Pre-programme valley monitoring in 2007 produced a season-long moths/trap/week average of 103.9 native moths. Many of these delta traps were past trap capacity and no longer capturing all moths which entered the trap. At the end of the first year of programmatic treatments, season-long average capture/trap/week dropped to 9.2.

As was true in Sonora and Arizona, 2012 was the last year in which native adult moths were captured. In 2012, a total of 18 native moths were all captured on or before the week of 26 May. These were the last native moths captured and the last detection of any life form of PBW anywhere in this bi-national programme.

5. CONCLUSIONS

On November 22, 2012 ten municipalities in north-western Chihuahua were declared free of PBW (as officially eradicated). This area was the Ascensión work area which had not had a detected population for 5 years. Subsequently, on December 8, 2014, eradication was declared for the remainder of the state of Chihuahua. On February 3, 2016, PBW was declared eradicated from Sonora and Baja California (SENASICA 2018).

Though not covered in this report, PBW has in the meantime also recently been eradicated from the Mexican states of Coahuila and Durango (Diario Oficial 2018). This latter cotton area (La Laguna) centres around the city of Torreón in the states of Coahuila and Durango, which was the first reported area infested with PBW in continental North America (Noble 1969). The state of Tamaulipas, which is contiguous to Texas and has likewise been involved in PBW eradication activities, also had no PBW captures in 2018, but has not yet been declared PBW-free (SADER 2018).

In the USA, eradication could only be declared after *Bt*-cotton labelling issues for refugia (grower variety selection) were resolved. This occurred after 6 years of continuous negative surveys. The United States Secretary of Agriculture signed the eradication proclamation for all USA cotton production areas on October 19, 2018 (USDA 2018). Eradication has been successfully achieved over a very diverse geographic and ecological range because many years of research and development had provided multiple surveillance and control tools (Noble 1969; Naranjo et al. 2002). These were tools which could be used synergistically. The area-wide integration of tools was then successfully tailored to varied habitats over the pest's broad range.

Resistance to *Bt*-cotton is, in the view of the first author, the most important entomological issue concerning cotton worldwide. Movement of a multi-gene resistant PBW population back into this bi-national programme area would be of the gravest concern.

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THE SUPPRESSION OF THE FALSE CODLING MOTH IN SOUTH AFRICA USING AN AW-IPM APPROACH WITH A SIT COMPONENT

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SUMMARY

The false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), is native to sub-Saharan Africa, where it infests various commercial, and wild, fruit-bearing plants. This major pest is not present in the Americas, Europe, and Asia, and therefore has phytosanitary implications, which impose severe limitations on potential South African exports. Consequently, this pest represents a severe threat to the fruit industry of South Africa, in terms of socio-economic impacts on both fruit production and job security. Although the pest can be managed to some extent with insecticides, mating disruption, and orchard sanitation, a long-term environment-friendly solution was needed. This became more evident as *T. leucotreta* developed resistance to available insecticides, while stricter quarantine measures were enforced by importers of African citrus. In 2002, research commenced on an area-wide integrated pest management (AW-IPM) programme in conjunction with the development of the Sterile Insect Technique (SIT) for the false codling moth. Commercial sterile insect releases started in the 2007-2008 season over 1500 ha of citrus orchards in Citrusdal, Western Cape Province, but by 2017-2018 had gradually expanded to almost 19 000 ha in three different citrus producing regions of South Africa. The programme is currently owned by the Citrus Growers Association (CGA) that have contributed to the steady growth of the SIT programme in the citrus industry. Over the past ten years the status of *T. leucotreta* as a pest threat was systematically reduced in areas where the SIT was practiced on an area-wide basis, compared to non-release areas.

Key Words: Citrus, navel orange, area-wide, Sterile Insect Technique, pest management, *Thaumatotibia leucotreta,* Tortricidae, resistance, quarantine, South African exports, Western Cape

1. THE PROBLEM

The false codling moth *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) is a polyphagous indigenous pest of both cultivated crops and wild plants in sub-Saharan Africa. False codling moth was first noted in the Paarl region of the

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Western Cape Province (South Africa) around 1969 (Hofmeyr et al. 2015). Although it attacks many different deciduous, subtropical, and tropical plants, it prefers citrus as one of its primary hosts.

By the mid-1970s *T. leucotreta* was detected at a holiday resort, 170 km north of the Paarl region near Citrusdal, an important citrus exporting region in the Western Cape Province (Hofmeyr et al. 2015). By the end of the 1970s it had spread through some parts of the valley, with heavy infestations in navel orange orchards (Hofmeyr et al. 2015).

The presence of this insect represents a high phytosanitary risk for South African fruit exports to the USA, Asia, and Europe. The economic threats imposed by the pest to the fruit growers and the industry of South Africa may also have severe socio-economic consequences for food and job security. The situation was exacerbated after *T. leucotreta* developed resistance against available registered insecticides and stricter regulations were imposed on exporters (Hofmeyr and Pringle 1998). This included a zero tolerance for *T. leucotreta* and the requirement of a post-harvest cold treatment (Hofmeyr et al. 2016a, 2016b).

2. PRE-OPERATIONAL ACTIVITIES

Although the pest has been managed to some extent, by integrating control tactics such as insecticides, mating disruption, and orchard sanitation, a longer-term solution was needed. In 2002, research was conducted to develop an area-wide integrated pest management system (AW-IPM programme) with an SIT component (Hendrichs et al. 2007; Klassen and Vreysen 2021). Citrus Research International (CRI) (Pty) Ltd, the Citrus Growers Association of South Africa (CGA), the Joint Food and Agriculture Organization of the United Nations/International Atomic Energy Agency Division (FAO/IAEA), the United States Department of Agriculture (USDA) through its Agricultural Research Service (ARS) and Centre for Plant Health Science and Technology (CPHST), joined resources and efforts to develop and test the efficacy of a SIT programme for *T. leucotreta.*

During the first phase of research, the radiation biology and inherited sterility of *T. leucotreta* (Bloem et al. 2003) was investigated, which was followed by field cage trials to evaluate mating compatibility and competitiveness (Hofmeyr et al. 2005). The results of these biological studies on the effect of gender and irradiation dose on *T. leucotreta* are shown in Fig. 1. As expected, fertility of both male and female moths declined with increasing irradiation dose (Bloem et al. 2003). This dose effect was greater for crosses involving irradiated female moths, which were almost completely sterile when treated with a dose of 200 Gy, while irradiated males still had a residual fertility of 5.2% when treated with a dose of 350 Gy (Fig. 1) (Bloem et al. 2003). Similar to other Lepidoptera, *T. leucotreta* exhibited inherited sterility when partially sterile male moths copulated with wild female counterparts (Carpenter et al. 2004). The resulting F_1 progeny was shown to be fully sterile, mostly male, and took longer to develop (Bloem et al. 2003). Furthermore, the F_1 generation would either fail to hatch or would develop into sterile, but fully competitive F_1 adults, that would provide additional pest population suppression in the subsequent generation.

The promising results led to the next research phase in the Olifants River Valley, which involved a SIT pilot study in citrus orchards during the 2005-2006 season (Hofmeyr et al. 2015). This involved the release of sterile *T. leucotreta* adults in a 35·ha navel orange orchard, surrounded by natural vegetation, while another navel orchard was used as a control.

Releases were performed over a 29-week period, with a total of 2000 sterile moths released per week. Thirteen delta traps evenly spaced over the orchards, equipped with synthetic pheromone (Cardiff Chemicals, Cardiff, UK) in Lorelei dispensers, were used. The goal was to create an overflooding ratio within the orchards and this was maintained at no less than 1 wild: 10 sterile moths per week (Hofmeyr et al. 2005; Hofmeyr et al. 2015). The encouraging results from this pilot study indicated a 77% decrease of wild *T. leucotreta* trap catches and an approximate 95% reduction of *T. leucotreta* related fruit infestations, compared to the non-SIT area.

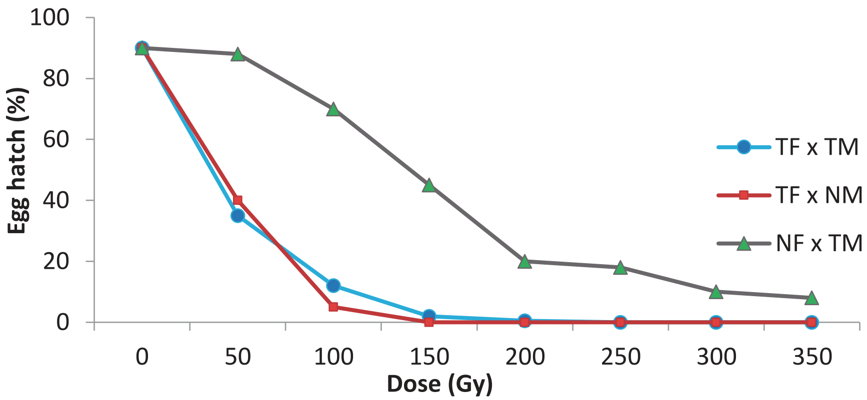

Figure 1. Effect of irradiation dose administered to T. leucotreta *adults on the mean egg hatch (%) per mated female. Males and females were treated (T) with 50, 100, 150, 200, 250, 300, and 350 Gy and inbred (TF x TM) or out-crossed (TF x NM, NF x TM) to untreated adults (N) (adapted from Bloem et al. 2003).*

The results of this 2005-2006 season pilot project in the Citrusdal region are shown in both Figs. 2 and 3. From the results obtained in this initial trial, the South African citrus industry was convinced to fast-track the commercial introduction of the SIT programme for *T. leucotreta*.

As a result, in 2006, the private company *Xsit* (Pty) Ltd. was established to manage the production, sterilisation, and the release of sterile *T. leucotreta* in the Citrusdal region. New equipment was designed to upscale moth production and to replace the insufficient infrastructure being used for the small-scale rearing of *T. leucotreta* (Hofmeyr et al. 2015). The new mass-rearing facility became operational in early 2007, and the release of irradiated moths commenced in November of that year.

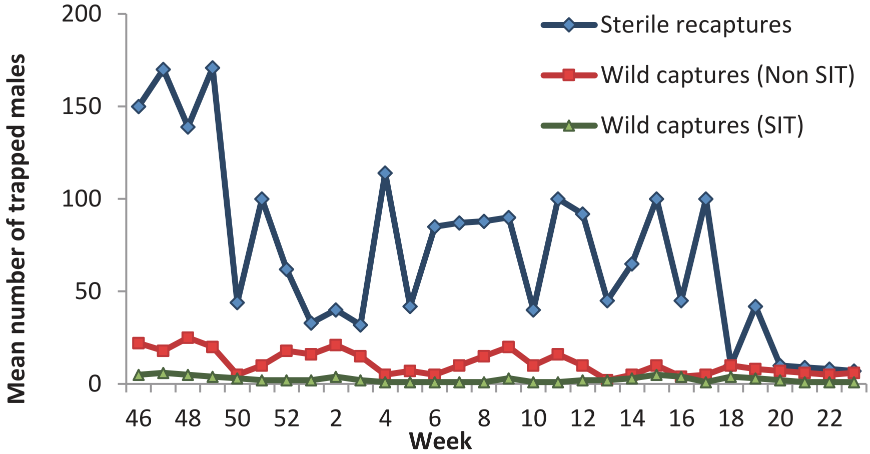

Figure 2. Capture of released (irradiated and topically marked) and wild T. leucotreta *males in SIT-treated and non-SIT-treated navel orange orchards as part of a SIT pilot project carried out in the Citrusdal region during the 2005-2006 season. A minimum ratio of 1:10 wild:sterile moths were maintained throughout the pilot trial in the SIT-treated orchard (adapted from Hofmeyr et al. 2015).*

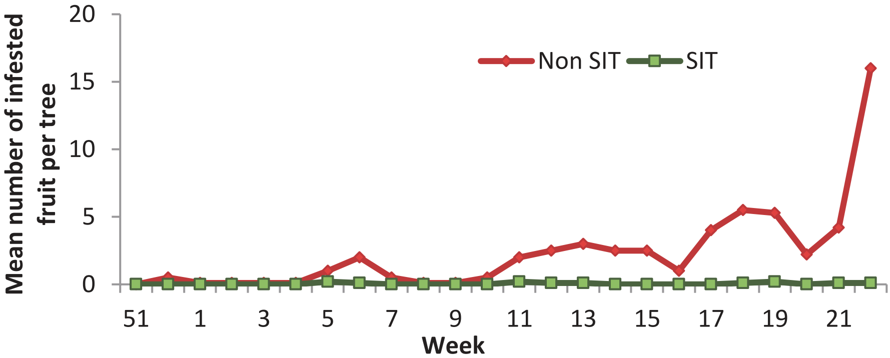

Figure 3. Fruit drop due to T. leucotreta *infestation in non-SIT and SIT-treated citrus orchards (35 ha) as part of a SIT pilot project carried out in the Citrusdal region, Western Cape Province, during the 2005-2006 season.*

3. THE FIRST DAYS OF THE SIT PROGRAMME, EXPANSIONS AND IMPROVEMENTS

Since the commercialization of the AW-IPM programme with a SIT component in Citrusdal in 2007, many new systems and equipment have been designed, developed, and manufactured in a relatively short time. Production monitoring systems for traceability, cold chain management, and quality management of reared moths were developed and constantly improved.

From 2007 to 2018 the initial 1500 ha SIT-treated area in the Citrusdal region was gradually increased to 4800 ha by incorporating the rest of the Olifants River Valley. Sterile insect releases are also being carried out over 6500 and 2200 ha of the Sundays River and Gamtoos River valleys in the Eastern Cape Province, respectively. During the 2016-2017 season, the release of sterile *T. leucotreta* was also expanded to the lower Orange River area in the Northern Cape Province and the Hex River Valley of the Western Cape Province, the latter, an important table grape export region, treating 1500 and 4000 ha respectively. At the time of writing (2018), this privately-owned programme was providing sterile insects on a weekly basis to cover more than 18 000 ha.

There was a progressive seasonal improvement in wild *T. leucotreta* suppression following routine releases of sterile moths in all treated areas. The results showed a reduction in crop losses and fewer rejections of fruit consignments destined for exports due to *T. leucotreta* presence. While this rapid growth of the programme was very exciting, it was accompanied by many challenges and hardships that sometimes threatened its existence.

3.1. Rearing Equipment

During the initial days of programme implementation, some of the old equipment and processes developed for the pilot trial were utilized in the commercial rearing programme. However, with the expansion of the programme, it became apparent that most of these systems were inadequate, and only suitable for small-scale rearing of *T. leucotreta* (Hofmeyr et al. 2015).

3.1.1. Larval Diet Preparation Equipment

A new artificial diet (Moore 2002; Moore et al. 2014) was introduced for rearing purposes in 2007. The diet was prepared using large-scale equipment from the baking industry, and then pulsed into 500 ml glass jars fitted with breathable replaceable paper membranes, allowing for gas exchange, in the metallic screw-lids (Hofmeyr et al. 2015). The individual jars were placed by hand into stainless steel baskets, containing 25 rearing jars each. Baskets were then stacked on a steel trolley, holding up to 16 baskets before they were pulled into an oven. Although the oven was an innovative piece of equipment, it was not ideally suited, as evidenced by the uneven cooking of the diet, and lack of sterilisation. After baking two trolleys per oven cycle with 400 glass jars each, they were placed in a room for cooling. Each jar of diet was inoculated with approximately one thousand 24-h old eggs via placement of an egg sheet, sterilised by an 8% formaldehyde solution, on top of the diet (Hofmeyr et al. 2015).

As the programme expanded and diet preparation increased from 6000 to 20 000 bottles a day, the handling and diet preparation processes had to be re-considered. Disadvantages of the jars included relatively high costs, susceptibility to breakage, and requirement for individual handling and cleaning after larvae emerged. In 2013, new technologies to prepare the diet were investigated. These included radiowave, microwave, infrared, steam, and extrusion. After completing the initial trials, only radiowaves, microwaves, and extrusion seemed potentially viable.

Different cooking times and temperatures were tested to develop a cost-effective method for delivering a diet producing high larval yields without a negative impact on larval development. Although several parameters were tested, the following criteria were critical in validating the optimum cooking/sterilisation process:

1. Proportion of large 5th instar larvae (0.04 g ± 1 and >10 mm) produced.
2. Feed conversion ratio, denoted by the amount of diet required to produce one large 5th instar larva.
3. Absence of viral and bacterial infected larvae - denoted in the amount (g) of diet required to produce one healthy larva.

To validate the correct process and cooking protocol (time vs. temperature), the number of large larvae (5th instar) produced was counted. It was evident that both the microwave and the extrusion processes resulted in the highest number of large larvae with the least feed (best feed conversion). The original Xsit diet required 0.35 g to rear one large larva, while both the microwave and the extrusion required only between 0.21 g and 0.22 g, respectively to rear one large larva. It was clear that the best results were obtained with the laboratory microwave and the extruder, where approximately 90% of the reared larvae reached 5th instar on day 12, while only 40% of the control diet reached 5th instar at the same time (Fig. 4).

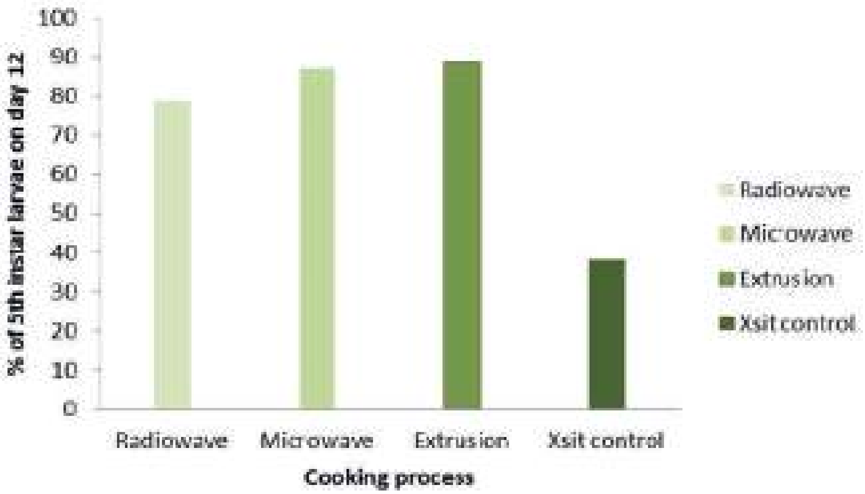

Figure 4. Percentage of large larvae per diet preparation treatment. Results of the preliminary laboratory studies were confirmed in this laboratory trial. Microwave and extrusion samples resulted in the highest amount of 5th instar larvae on day 12.

The next step was to test both processes in a commercial trial. In 2015, several experiments were conducted in a commercial microwave. These trials were repeated three times under different settings, but the positive results obtained with the laboratory microwave (1000 W) could not be replicated in the commercial microwave. After further investigation in obtaining a commercial microwave, the lack of support of the industry was evident, and it was decided to continue with the extrusion process. Commercial trials with different extruder processes and settings were tested until the end of 2016, when the correct settings and consistency of the diet were obtained, with proven results of growth and yield of larvae replicated on a semi-commercial scale; nearly 90% of larvae had grown on the extruded diet, and reached 5^{th} instar on day 12, compared to only 40% on the Xsit diet.

Xsit purchased its own extruder plant in July 2017. However, after the commissioning of the extruder, it was clear that the larval diet was not similar or even comparable to the product developed over the past four years. The extruded larval diet was sticky and could hardly be packed or handled, while larval growth was retarded, and yields were low. The larval diet produced was therefore, not fit for use. For six months, extensive trials were carried out, testing all variables, including raw product variability and extruder conditions. It was concluded that the main cause of the problem was (a) the dextrinisation and gelatinisation of the starch in the diet, and (b) the inconsistent granule size of the maize meal, which comprises 80% of the diet. During the extrusion process the starch granules swell when pressurised under high temperatures during cooking and drying, and then shrink as soon as the product cools down. In the event, when the diet is cooked at a too high temperature, the starch molecules lose the ability to swell, leading them to shear and burst. This will lead to the loss of the semi-crystalline structure of the starch, while the smaller amylose molecules leach from the granule to form new chemical structures which cannot be digested by the larvae. During cooling, the semi-crystalline structure recovers and, provided that the granules did not burst, will re-align to a similar position or structure prior to cooking. This phenomenon is known as retrogradation (Oates 1997; Wang et al. 2015). As the initial research was conducted in different stages, the diet was cooled prior to drying, while at the new commercial plant the extruded diet was immediately transferred to the oven for drying, leading to dextrinisation and gelatinisation of the starch. This assumption was confirmed during trials where the diet was allowed to cool before drying. As a final outcome, excellent results were obtained which were similar to those obtained in the research done over the previous four years. An additional cooling unit was introduced after extrusion, before drying.

3.1.2. Rearing Containers

During the original rearing of the larvae in glass jars, the screw lids and membranes were removed when larvae reached 5^{th} instar. The jars were placed on their sides to assist larval exit. An integrated aperture below each basket held the pupation substrate, which is a square of polycarbonate honeycomb material, 570 mm x 530 mm x 11 mm with 6 mm diameter aperture, placed on a 570 mm x 530 mm x 3 mm fibre sheet.

In 2012, the whole false codling moth colony suffered from a severe bacterial infection in the facility. After an intense investigation it appeared that miniscule holes between the honeycomb cells became breeding sites for *Bacillus cereus*, Gram-negative opportunistic bacteria that undermine the immune system of the larvae, killing them in a matter of days. In response, the polycarbonate sheets were replaced with disposable, pre-manufactured sheets of corrugated, single-face cardboard.

In parallel with the development of the new diet preparation system described above, a replacement of the glass jars was pursued. Several types of containers were investigated, including paper bags, starch bags, polyethylene cups, and disposable polyethylene bags. The most significant challenge was to find a similar membrane to the one used in the jars. The new extruded diet was more prone to drying out, but one had to keep in mind that the regulation and exchange of gasses were critical. A material with these specific properties, while preserving moisture, had to be found.

In 2016, disposable polyethylene bags (280 mm x 160 mm), with a breathable polyethylene-based microporous membrane, were introduced. These were automatically filled by a diet dispensing machine. A volume of water equal to 47% of the total volume of the dry mix was added to form a fluffy diet, while 250 g of larval diet was required to produce at least 550 larvae per unit. The bags were consequently phased in to replace the glass jars. Egg sheets containing 800 ± 100 *T. leucotreta* eggs, dipped in an 8% formaldehyde solution to prevent contamination by any bacteria and/or virus, were then placed on the diet and sealed. The bags were then assembled on a rearing cart containing 480 bags. When ready for pupation, usually on day 12 of the rearing cycle, the larvae chew their way out of the bags and descend on silk threads to the cardboard pupation substrate, placed 30 mm below the bags.

After the implementation of this new system, significant production losses were experienced due to larvae dying in the bags before reaching the 5th instar. Upon investigation it was determined that the HVAC system was not capable of handling the large volume of CO_2 generated by the large number of larvae reared per m^2. Consequently, a new HVAC system with increased capacity and higher air change rate was designed and installed by the end of the 2016-2017 season to ensure sustainable production of sterile insects on a continuous basis.

3.1.3. Moth Emergence Cabinets

The pupation boards were placed into custom designed steel emergence cabinets to permit moth emergence and collection. Each cabinet, 1550 mm x 630 mm x 940 mm, was welded on a 900 mm supporting framework (Fig. 5). The cabinets were divided longitudinally with a perforated stainless-steel sheet separating two compartments: a back compartment, 740 mm deep with an access door, containing 50 horizontally placed pupation sheets, and a front compartment, 200 mm deep with a glass door to the outside, allowing moths to move phototactically from the back into the front compartment (Boersma and Carpenter 2016). The front compartment was lightly dusted with talcum powder and was fitted with a collection cone at the bottom, attached to a plenum-based air-braking moth collection system.

Establishing the correct speed of the airstream that transferred the moths from the moth cabinet to the collection room was challenging, as too high airflow resulted in damaged moths, while too low airflow caused clogging of moths. This was resolved by adjusting the airspeed to 12 m/s for transferring moths to the collection pans, while reducing the airstream to 3 m/s as they enter the collection room, allowing for a soft landing (Hofmeyr and Pretorius 2010).

Scaling up the release area from 3300 ha to more than 18 000 ha induced a lot of pressure on these emergence cabinets, resulting in problems with temperature consistency, clogging of moths due to overcrowding, and production losses due to moths escaping from these old cabinets and equipment. In 2015 cabinets were re-designed, allowing for better airflow by making the following improvements:

1) the steel sheets of the cabinets were folded rather than welded for better durability;

2) extra space was provided at the back to allow increased airflow between pupation boards, resulting in fewer temperature spikes;

3) use of rubber sealed Perspex doors for better sealing.

Figure 5. Moth emergence cabinets.

In the moth collection pans adult moths need to be kept immobile. This is a critical procedure to prevent mating in the collection pans as well as the prevention of damage to adult moth's wings. Mated adults or those with damaged wings may have a negative effect on their field performance. However, no set or established temperature range were used during the moth collection, handling, and transport. This led to moths being exposed to temperatures below their critical thermal limits (below 6ºC), resulting in poor field recaptures in the warmer months of the season (Boersma and Carpenter 2016; Boersma et al. 2017).

In 2015, new cooling and handling protocols were introduced with a cold chain with a set temperature range from the moth cabinets to the orchard to ensure moths were kept between 6-10ºC, which resulted in better quality and recapture of sterile moths in the field.

3.2. Sterilisation Dose

As soon as the moths reached a required temperature of 10ºC in the collection pans, they were placed into cardboard boxes (140 mm x 140 mm x 50 mm) and irradiated with a dose of 150 Gy (Bloem et al. 2003) in a 20 kCi ^{60}Co source panoramic irradiator. During the 2017-2018 season, the irradiation dose was increased to 200 Gy to compensate for an apparent reduced sterilisation effect of the 150 Gy dose with reduced dose-rate of the cobalt source. Although the strength of the cobalt source has weakened, and moth exposure time adjusted accordingly, the reason for this decrease in radiation impact is not known and is currently being investigated.

3.3. Release Methods and Devices

The irradiated moths are stored in a holding room between 6-8°C for approximately 12-24 h, and then transported to orchards or an airfield in a refrigerated vehicle at the same, regulated temperature range.

From 2007 to 2010, sterile moths were released with all-terrain vehicles (ATVs) or "quad bikes", manned by a driver and an assistant responsible for releasing the moths by hand into the trees (Hofmeyr et al. 2015). Later the ATVs were equipped with a release box with a release auger. Although this release method was relatively inexpensive, it had a few disadvantages:

1. Human factor: releasing an accurate and constant number of moths in orchards was not possible.
2. The terrain where some of the orchards are located is rough, making driving while releasing an equal number of sterile moths difficult, leading to inadequate moth distribution.
3. Access to farms was sometimes difficult.
4. Logistical constraints: covering releases twice a week in a valley which stretches more than 100 km in its length and 60 km in its width became a logistical constraint; this led to an increasing cost of maintaining the ATV's and preventing breakdowns with a constant challenge of completing the releases in time, versus maintaining the quality of the product.

Since 2010, releases of the moths have progressed from ground releases with ATVs to aerial releases using gyrocopters, and later to fixed-wing aircrafts. Moth releases with fixed-wing Piper Pawnee aircrafts commenced at the end of 2015 but were gradually replaced by helicopters in 2017.

In 2010, Xsit outsourced the releases to a company that used gyrocopters. The release system and holding boxes (hopper) of the sterile moths were slightly modified, and fitted to the gyrocopter, making aerial releases possible. The results obtained were excellent. The recapture rate of the sterile moths increased, while the wild false codling moth population decreased to the lowest levels since the start of the Xsit programme. Unfortunately, after the tragic loss of two pilots, in two separate incidents, the gyrocopters were grounded by the South African Civil Aviation Authority, and releases had to be continued using fixed-wing aircraft. Although the results obtained from the fixed-wing aircraft were comparable to the gyrocopter in certain areas, it had a few disadvantages:

1. The minimum speed the aircraft flew were significantly higher than the gyrocopters (160 km/h vs. 100 km/h), resulting in poorer recaptures.

2. The minimum height the aircraft flew were higher than the gyrocopter (160 feet vs. 100 feet), leading to poorer recaptures.

3. Flying in small valleys and mountainous areas were impossible, adding to logistical constrains by filling gaps with ground releases.

4. Quality degradation due to the prop wash of the aircraft (the force of wind generated behind a propeller) causing moths to be blown into a swirl.

Starting the 2017-2018 season, fixed-wing releases were gradually phased out and replaced with small helicopters (R22) to simulate the conditions of gyrocopter releases. This increased efficiency and resulted in sustainable results, contributing to an even greater suppression of wild false codling moth over the past two seasons. Currently the possibility of releasing the sterile insects by unmanned aerial vehicles is being investigated, with the first experimental releases occurring in early 2018. Current aviation legislation in South Africa, in conjunction with costs, still make this venture impossible on a commercial scale.

4. THE RESULTS AND IMPACT

The SIT programme for *T. leucotreta* is governed by phytosanitary requirements, demanding a zero-tolerance level of pest incidence in fruit. The SIT programme is one component of an area-wide approach integrating multiple tactics to mitigate the threat posed by the *T. leucotreta*. This includes obligatory orchard sanitation by growers, with various alternative control measures.

The frequency of sterile male releases varies for each insect species and depends mainly on the survival of the sterile insects in the target area. The ability of sterile insects to survive and remain sexually active as long as possible in the field is essential, and if their longevity declines, the frequency of releases needs to be increased to ensure optimal overflooding ratios at all times (Dowell et al. 2021). The success of the sterile *T. leucotreta* release programme is very much determined by the ability to ensure the release of pre-determined numbers of sterile moths into the orchards that will guarantee a minimum sterile:wild male overflooding ratio of 10:1 (as assessed by trap catches).

In the warmer months, sterile *T. leucotreta* has a shortened life span, therefore requiring two releases of 1000 sterile adults per week. These double releases take place between November to April, while only one release of 2000 sterile adults per week takes place in the cooler months (September to October, and May to June), when the longevity of the moths increases. If the desired overflooding ratio is not achieved, supplementary releases of sterile moths are conducted. Maintaining a continuous optimal overflooding ratio maximises the probability that a wild moth will mate with a sterile moth in the field, thereby resulting in no viable or fertile offspring and an eventual population decline (Carpenter et al. 2004; Hofmeyr et al. 2015).

Once the wild population has been reduced to such a level that no wild moths are captured on a consistent basis, the release of sterile moths can become the main or sole control method, as is currently the case in numerous citrus orchards in various valleys. Furthermore, the pre- and post-harvest absence of *T. leucotreta* infested fruit, are additional indicators of programme success.

In recent years, the success of the SIT programme is evident by a marked reduction of the wild *T. leucotreta* populations, in some areas below economic thresholds. The released and wild populations are monitored at weekly intervals, using delta traps baited with the female sex pheromone to attract male moths. Sterile males are differentiated from their wild counterparts by their pink intestines caused by a food dye that is mixed with the larval diet (Hofmeyr et al. 2015).

Commercial results for the three main growing areas currently serviced by the programme, one in the Western Cape, and two in the Eastern Cape, are encouraging. Despite many challenges, the SIT has proven to be a sustainable approach to reduce the occurrence of wild *T. leucotreta* to well below economic thresholds (results from the recently incorporated Hex River Valley in the Western Cape, and the lower Orange River Valley in the Northern Cape will not be reported here).

4.1. Olifants River Valley, Western Cape

The positive achievements of the area-wide programme were evidenced by the progressive increase in the numbers of sterile male *T. leucotreta* moths trapped from 2007 to 2010 (Hofmeyr et al. 2015). In addition, trap catches of wild male adults declined from 13.0 moths per trap per week prior to the sterile moth releases in 2006, to 2.0, 0.4, and 0.1 moths per trap per week in 2012, 2013, and 2014, respectively. In addition, infestation of *T. leucotreta* in citrus fruit was reduced from 2.6% in the 2010-2011 season to 0.1% in 2013 (Barnes et al. 2015; Hofmeyr et al. 2015).

During the 2006-2007 season, i.e. before the start of the area-wide SIT programme, each tree had on average 30 fruit damaged by larvae of the false codling moth. As the SIT programme advanced, and the ratio of sterile to wild adult *T. leucotreta* increased, the average infestation rate declined to only 0.2 damaged citrus fruits per tree per season. The Perishable Products Export Control Board of South Africa reported a substantial reduction of pre-harvest crop losses (Hofmeyr et al. 2015).

Despite this initial success, the number of trapped wild *T. leucotreta* males increased during the 2010-2011 season, as did crop damage, to an average of 1.56 infested fruits per tree (Fig. 6). However, this was still 95% lower than the damage level before the release programme started. Higher than normal ambient temperatures experienced during the spring and summer of 2010-2011 were attributed as the cause of the increased wild male captures. This resulted in at least one additional generation, leading to more pressure on the SIT programme resulting in the increased fruit damage.

Despite this temporary upsurge in wild male catches, the suppression of the wild *T. leucotreta* population in the SIT-treated areas was restored and progressively improved from 1.5 moths per trap per week in the 2010-2011 season to 0.1 moths per trap per week in the 2015-2016 season (Fig. 6). The gyrocopter accidents coupled with production issues during the 2016-17 season resulted in a slight increase of average wild male catches from 0.3 and 0.4 males per trap per week. This, however, did not result in an increase in fruit infestation. This could be explained by the increased rearing efficiency, resulting in the release of better-quality moths, and the fact that the wild population of *T. leucotreta* was so low that sterile released adults were more effective, outcompeting the low numbers of the wild population (Hofmeyr et al. 2015).

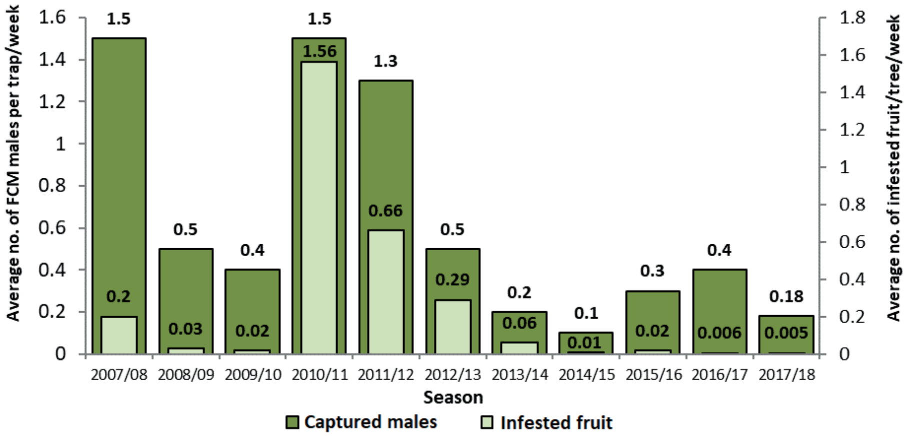

Figure 6. Reduction in numbers of wild T. leucotreta *males and infested fruit in sterile insect release areas in the Olifants River Valley, Western Cape Province from 2007-2008 to 2017-2018 seasons (data obtained from Xsit).*

4.2. Sundays River Valley, Eastern Cape

Sterile moth releases were initiated in the Sundays River Valley in 2011-2012, and since then the density of the wild *T. leucotreta* population has progressively declined with successive seasons, resulting in less infested fruit (Fig. 7). There was, however, an increase in the average number of trapped wild males in 2013-2014, but this was due to areas with historically high population densities being added to the SIT programme.

A similar trend of higher wild trap catches was seen in the Sundays River Valley in 2016-2017 due to challenges experienced in the mass-rearing facility. During the 2017-2018 season wild false codling moth catches decreased to only 1 wild male per trap and 0.02 infested fruit per tree respectively, the lowest since the start of the SIT programme.

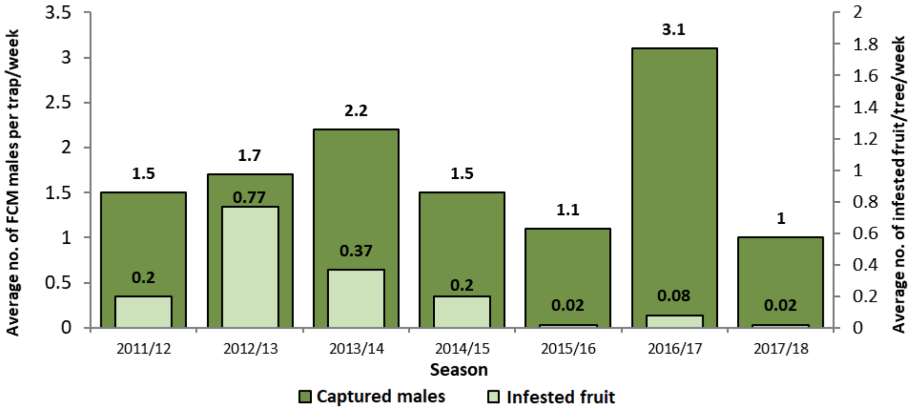

Figure 7. Reduction in numbers of wild T. leucotreta *males and infested fruit in the Sundays River Valley, Eastern Cape Province from 2011-2012 to 2017-2018, as a result of sterile insect releases (data obtained from Xsit).*

4.3. Gamtoos River Valley, Eastern Cape

The natural population of *T. leucotreta* was much lower in the Gamtoos Valley as compared to the other areas, causing much less crop damage. This was evidenced by much lower trap catches of wild males during the first season of sterile moth releases in comparison to the other areas. As a result, the SIT programme was able to reduce the wild moth population density and the number of infested fruits within the first release season (2014-2015).

In the following season, wild males were suppressed to such a low level that basically no infested fruit were recorded for the entire season, while a slight increase was recorded in the 2016-2017 season for the same reasons mentioned above (challenges at the rearing facility) (Fig. 8).

5. THE REASONS FOR SUCCESS

This AW-IPM programme with a sterile male release component against the *T. leucotreta* has had a significant impact on the citrus industry in South Africa; securing exports to the rest of the world in a sustainable manner. The success of the programme can be attributed to the following factors:

1. *Single crop industry*: The citrus trade, unlike other fruit sectors such as the deciduous fruit industry, is a single crop industry led by the Citrus Growers Association. This results in easier decision-making, management, and funding, since all stakeholders have the same vision. This played a significant role in the success of the programme as it was an industry-driven project to secure sustainable citrus exports for the growers.

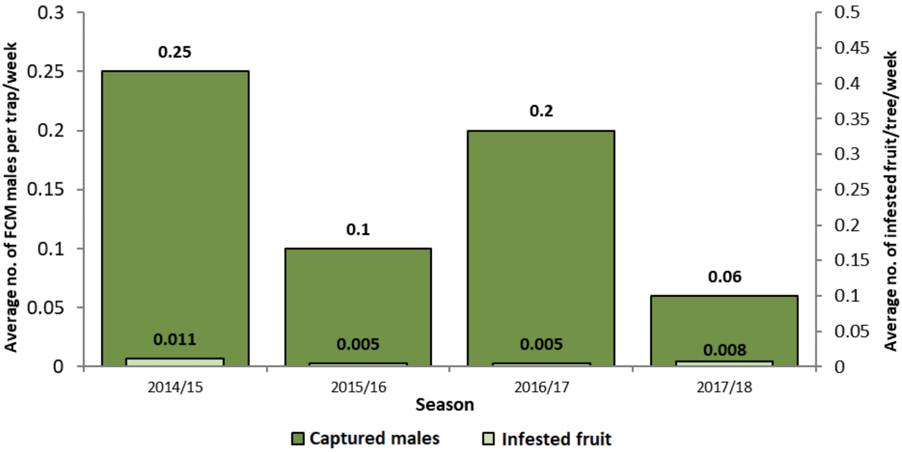

Figure 8. Reduction in numbers of wild T. leucotreta *males and infested fruit in the Gamtoos River Valley, Eastern Cape Province from 2014-2015 to 2017-2018 seasons, as a result of sterile insect releases (data obtained from Xsit).*

2. *Area-wide integration of suppression methods:* The SIT programme was managed as part of an area-wide programme. Xsit did not only take responsibility of both the monitoring of wild *T. leucotreta* and infestation, but also played a significant role in the monitoring of sanitation practises and the treatment of hot spots (an area with a high wild *T. leucotreta* population), in conjunction with other integrated pest control practises.

3. *Management to ensure sustainable sterile moth production*: Well-experienced and capable management was in place which ensured sustainable production of sterile insects, while the shareholders of the programme were also industry-related individuals, which ensured that the interests of the programme were always well-managed.

4. *Support of farmers and industry*: Most farmers were in favour of the programme, understanding the advantages of the AW-IPM approach. However, education and training were provided throughout the programme to ensure farmers were kept informed about industry-related matters.

5. *Phytosanitary regulations*: Since *T. leucotreta* is a regulated quarantine pest, governed by phytosanitary regulations in line with a systems approach (FAO 2017) for the controlling of the pest for export purposes, a zero-tolerance policy is enforced. Although there are several choices of control measures for *T. leucotreta* under the systems approach, it encouraged farmers to take part in the SIT programme if they wanted to export their fruit.

6. *In-house research*: Xsit employed its own researchers, constantly exploring better means of rearing, processing, and releasing insects, staying informed of the newest technology.

7. *Set protocols and procedures*: The use of standard protocols and procedures combined with continuous training of employees are essential for the efficient rearing of insects. The correct handling and distribution of insects are also essential to ensure good quality of insects in the field. Maintaining a cold chain proved essential to prevent damage to the sterile insects during transport, while the proper handling temperature has to be selected as this affects the competitiveness of the adults in the field (Boersma and Carpenter 2016).

6. FUTURE PLANS

The need for AW-IPM programmes with a SIT component to manage *T. leucotreta* in other countries where this pest is present, has become more apparent. Export crops such as avocados in Angola, and chilies in Kenya may also require the use of a SIT-based AW-IPM in the future to deal with this polyphagous pest.

Meanwhile, many export crops in South Africa, such as table grapes, stone fruits, and citrus grown in other regions are anticipating the introduction of the SIT. Xsit currently services 14 000 ha of the 70 000 ha of citrus in South Africa, while 4000 ha of table grapes are already enrolled in the programme. This indicates that there is scope for integration and expansion of this valuable technology to production areas not yet under this area-wide pest control method.

The potential of *T. leucotreta* becoming a major invasive pest in different countries globally is a reality, representing a threat to agriculture and food security. With the SIT now developed for this pest, if invasive false codling moth outbreaks are detected early, efficient integration of the SIT on an area-wide basis will allow eliminating them in an effective and environment-friendly way.

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PUTTING THE STERILE INSECT TECHNIQUE INTO THE MODERN INTEGRATED PEST MANAGEMENT TOOLBOX TO CONTROL THE CODLING MOTH IN CANADA

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SUMMARY

The Okanagan-Kootenay Sterile Insect Release (OKSIR) programme, in southern British Columbia, Canada, has been successfully applying the Sterile Insect Technique (SIT) as part of a sustainable area-wide integrated pest management (AW-IPM) programme to control the codling moth *Cydia pomonella* L. in pome fruits in the region for over 20 years. Chemical, cultural and biological techniques that complement the SIT are also integrated into orchard and regional pest management plans by the programme and/or individual growers. The AW-IPM programme is supported by close monitoring of codling moth populations in orchards and adjacent urban properties; enforcing suppression of codling moth infestations in orchards and urban areas; removing derelict orchards, wild host trees and poorly managed host trees; and increasing public awareness and education. Successful collaboration between the OKSIR programme, the pome fruit industry, area residents and various government organizations has reduced codling moth populations by 94%, relative to pre-programme levels, and codling moth damage to less than 0.2% of fruit, in more than 90% of the orchards in the programme area. Local pesticide sales indicate a 96% reduction in the amount of active ingredient used against the codling moth since 1991. Implementing the SIT through an innovative social approach to local community-centred area-wide pest management has posed many challenges and created many learning opportunities. The codling moth mass-rearing facility in Osoyoos, British Columbia, has the capacity to produce 780 million sterile codling moths annually, but only a portion of that is used seasonally to treat 3400 hectares (ha) of pome fruit made up of small orchards intermixed with residential areas in the Okanagan Valley. As a result of climate change and increasing global trade, destructive insect pests are migrating to new habitats throughout the world. These new threats must be managed in ways that protect both the agrifood industries and the natural environments in which the industries operate. The OKSIR programme is an effective and easily transferred model to meet these challenges, especially as a supplement to other biological control methods. The programme is also a

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compelling model of success that can encourage other regions to use the SIT in their pest management toolbox to combat codling moth infestations across multiple local community jurisdictions using environment-friendly, cost-effective methods based on proven technology. The OKSIR programme is exploring the sale of surplus sterile moths, egg sheets or possible virus production as an opportunity to offset costs of incorporating additional area-wide approaches to combat other invasive pests.

Key Words: beneficial insects, biological control, virus, pheromone-mediated, mating disruption, pesticide resistance, area-wide integrated pest management, IPM, SIT, *Cydia pomonella,* British Columbia

1. INTRODUCTION

The codling moth *Cydia pomonella* L., the proverbial “worm in the apple”, damages pome fruit directly, and is a key pest of this crop in most of the areas where it is cultivated (Beers et al. 2003). If left uncontrolled, the codling moth can damage 50 to 90% of an apple crop (Ontario Ministry of Agriculture, Food and Rural Affairs 2011). Though the codling moth originated in Asia Minor, it arrived in the Okanagan region in the early 1900s, making it a pest for nearly as long as apples have been produced commercially in the area (Bloem et al. 2007). The Okanagan region in southern British Columbia, the western-most province of Canada (Fig. 1), is unique in Canada because of its dry, sunny climate (hot, dry summers and mild winters). Tree fruit production has been a hallmark of the region for over 100 years.

In this region, and most other areas where pome fruits are produced, broad-spectrum insecticides were previously used heavily to control codling moth populations (Madsen and Morgan 1970). Codling moth populations in many areas had started developing resistance to some classes of insecticides, and concerns over development of cross-resistance were mounting (Dunley and Welter 2000). The use of broad-spectrum insecticides also had indirect costs related to the loss of natural enemies and pollinators; it was a source of environmental contamination, and consumers were concerned over insecticide residues on food (Vreysen et al. 2010). For these reasons, negative public attitudes towards the use of pesticides stimulated support for codling moth control strategies that did not rely on synthetic pesticides (Madsen and Morgan 1970).

Twenty years of research and planning culminated in the early 1990s with the implementation of the Okanagan-Kootenay Sterile Insect Release (OKSIR) programme (Dyck et al. 1993). Though the programme was initially more expensive than a conventional insecticide programme, a number of factors contributed to its adoption in the region. Restrictions on pesticide use, particularly those most effective at controlling the codling moth, were increasing. Concern for the impacts of insecticides on beneficial insects, the environment, and surrounding communities was mounting. Finally, reducing pesticides and codling moth populations created marketing advantages for local pome-fruit growers. In weighing these costs and benefits, and taking a long-term view of their implications, the programme was deemed to have a net benefit to the industry and community (Holm 1985, 1986; Jeck and Hansen 1987) and was therefore initiated in 1991.

2. THE STERILE INSECT TECHNIQUE

The concept of the Sterile Insect Technique (SIT) for insect control was conceived by E. F. Knipling in the 1930s, and first developed and applied in the 1950s to successfully control the New World screwworm *Cochliomyia hominivorax* Coquerel (Klassen et al. 2021). The SIT is a biological insect control method in which insects are mass-reared, irradiated to make them sterile, and then released into the environment at regular intervals to mate with wild insects. Wild female insects that mate with the sterilized male insects produce no offspring, thereby reducing the number of insects in the next generation. Continued use of the SIT at appropriate overflooding ratios thus leads to successively smaller generations, and can, if applied correctly on an area-wide basis, result in an area of low pest prevalence or even eradication of the population in that area (Dyck et al. 2021).

Since the 1950s, the SIT has been successfully used around the world to suppress, prevent, contain or eradicate many dipteran insect populations such as the New World screwworm, several tsetse flies *Glossina* spp., and fruit flies such as the melon fly *Zeugodacus cucurbitae* Coquillet, Mediterranean fruit fly *Ceratitis capitata* Wiedemann and Mexican fruit fly *Anastrepha ludens* Loew (Dyck et al. 2021).

The SIT has also been applied with success against various lepidopteran pests such as the cactus moth *Cactoblastis cactorum* Berg, the painted apple moth *Orgyia anartoides* Walker, the false codling moth *Thaumatotibia leucotreta* Meyrick, and the pink bollworm *Pectinophora gossypiella* Saunders (Carpenter et al. 2007; Suckling et al. 2007; Dyck et al. 2021; Bello et al., this volume; Boersma, this volume; Staten and Walters, this volume).

3. OKANAGAN-KOOTENAY STERILE INSECT RELEASE PROGRAMME

At the inception of the OKSIR programme, other non–insecticide-based insect control methods, such as pheromone-mediated mating disruption, were considered in order to reduce codling moth populations in southern British Columbia (Judd et al. 1996). Due to the heterogeneous landscape of orchard and residential areas in most of the region (creating a non-contiguous orchard area (Cardé 2007; Witzgall et al. 2008)), the SIT was considered a more suitable solution to manage the codling moth. The OKSIR programme was initially conceived in 1991 as an eradication programme, and was based on research conducted by Proverbs and colleagues in the 1970s and 1980s (Proverbs et al. 1978, 1982).

The mandate and objectives changed in 1997 when the goal became suppression of the codling moth below economic levels through delivery of an efficient, effective and sustainable AW-IPM programme using the SIT as the main control tool. It was concluded that permanent area-wide suppression rather than eradication was a more realistic goal because of the large programme area, the limited human and financial resources available, and because an expensive quarantine programme that would be necessary during and after eradication for which the Federal Government of Canada, which regulates quarantines, had no plans (Bloem et al. 2007).

A community-based, AW-IPM approach was essential to reduce costs and increase the effectiveness of the programme. Area-wide measures applied over a geographically- or politically-defined area enable control of an entire pest population, a prerequisite to sustainable pest management. Insects do not respect property boundaries, and a field-by-field approach that focusses narrowly on the value loss to individual crops cannot successfully control pest populations because unmanaged cultivated and wild host trees on neighbouring public, private or abandoned land are recurrent sources of reinfestation (Hendrichs et al. 2007).

An AW approach requires a strong partnership among all stakeholders to succeed. For OKSIR, a partnership was therefore developed between the region's local governments and the tree-fruit industry. The Province of British Columbia enacted legislation establishing a mandatory community-based, AW-IPM programme (Municipalities Enabling and Validating Act [RSBC 1960] 1989) that required all property owners, including both residential and commercial host-tree owners, to control codling moth infestations and participate in local funding of the programme.

Researchers at Agriculture Canada, local governments and growers selected the SIT to reduce the use of insecticides because of concerns about excessive pesticides in the environment, an attempt to delay insecticide resistance, and to support agritourism opportunities in the area. This collective action, i.e. action taken jointly by the stakeholders in pursuit of their perceived shared interests, made it possible to deliver results in a much larger geographic scale than could be provided or protected by a single farmer (Lefebvre et al. 2015b).

3.1. Programme Area

The programme area covers approximately 600 km^2, and at its onset serviced approximately 8 900 ha (22 000 acres) of pome fruit; the surface of pome fruit within this area was gradually reduced to 3 440 ha (at the time of writing). The large area to be serviced, and the need for pre-release sanitation (discussed below), required the programme to be implemented sequentially across three zones (Fig. 1). Pre-release sanitation and construction of the rearing facility began in zone 1 in 1992 followed by moth release in 1994. Pre-release sanitation started in 1998 and 2000 in zones 2 and 3, respectively, with moth release occurring after two years of sanitation efforts.

3.2. Rearing Facility

At the heart of the OKSIR programme is a state-of-the art insect mass-rearing facility. Construction of the facility located in Osoyoos, British Columbia was completed in 1993. The facility is capable of producing more than 2 million sterilized moths per day or 780 million sterile codling moths annually. Maintenance and operational costs, as well as capital upgrades, are funded by local property tax requisitions.

3.3. Programme Services

The OKSIR programme services include pre-release sanitation, mandatory SIT application, surveillance, enforcement and education:

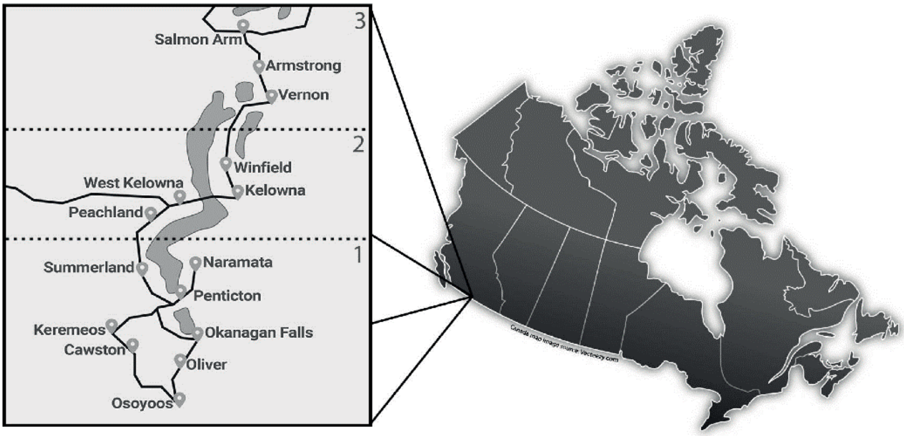

Figure 1. Location of the OKSIR programme. The map of Canada (right) indicates where the OKSIR Programme is located in British Columbia, and the inset (left) illustrates how the programme area, covering a linear distance of ca. 175 km, was divided into three zones.

3.3.1. Pre-Release Sanitation

The first phase of the programme was pre-release sanitation. This entailed the removal of thousands of unmanaged/abandoned host trees to reduce refugia for the codling moth. The programme also coordinated and supported the suppression of codling moth populations in orchards through the use of conventional insecticides, cultural practices and pheromone-mediated mating disruption. Wild codling moth populations had to be reduced as much as possible throughout all communities to increase the efficiency of subsequent SIT application. The programme continues to remove unmanaged/abandoned host trees and derelict orchards as needed.

3.3.2. Mandatory SIT Application

The programme delivers a mandatory area-wide control application of sterile codling moths to every orchard property, i.e. 2000 sterile codling moths of mixed sex (1:1)/ha/week for approximately 20 weeks per season. As necessary and practical, additional releases are made to address high pest pressure "hot spots."

3.3.3. Surveillance

Every orchard property is monitored with pheromone-baited traps (1 trap/ha) that are checked once a week. These spatially explicit trapping data are disseminated in real-time through the programme's website to allow growers to respond rapidly with supplementary controls if needed (OKSIR 2017).

Other monitoring techniques include: in-season fruit inspections, end-of-season assessment of fruit damage, and banding of host trees (corrugated cardboard strips wrapped around trees to trap mature larvae; later the strips are removed and destroyed). Codling moth host trees are also monitored on non-orchard properties (properties with less than 20 host trees) by visual fruit inspections and banding. It is important to recognize that the monitoring of non-orchard properties is focussed on a 200-m buffer zone around commercial orchards, though other properties are visited as needed.

3.3.4. Enforcement

Codling moth control is enforced throughout the programme area. The programme has the legal authority to enter orchards and residential properties to inspect for codling moth infestation and issue control orders for fruit stripping and tree removal. Dedicated enforcement ensures that all host-tree owners do their part to prevent outbreaks and ensure proper management of the codling moth.

3.3.5. Education

Education is essential to reduce enforcement actions as much as possible. Extensive education and outreach about the programme and codling moth control is done each season with growers and residential tree owners in the region. This is critical, especially during the first stages of programme implementation. Outreach occurs via media advertising, publications, newsletter articles, the programme website, field visits and public meetings.

An information technology specialist maintains the website that provides real-time trapping and phenological data to growers. The growers themselves become sources of positive promotion once they are convinced of the benefits that the OKSIR programme is bringing to their economies and the environment (Bloem et al. 2007).

3.4. Governance, Funding and Budget

The OKSIR programme is governed by a Board of Directors, comprising five elected community representatives from each of the four regional municipal governments within the programme's service area, and three grower representatives nominated by the pome fruit industry.

The programme has a central administration and is funded through local taxation. All properties in the region pay a tax based on assessed land value. Currently, the average residential property pays ca. CAD 11/year (USD 9/year), and growers pay a parcel tax of ca. CAD 340/ha. Overall, the programme obtains 60% of its funding from local property owners in the community and 40% from commercial growers. The percentage share of the funding allocation has been arbitrarily determined based on political will. The annual programme budget is ca. CAD 3.2 million. In 2016, CAD 1.7 million was collected from general taxpayers via land value tax and CAD 1.16 million from growers (approximately 3440 ha) via parcel tax (cost/ha). The programme received an additional ca. CAD 350 000 from the sale of excess products, interest income and grants.

Of the total operating budget in 2016, CAD 1.1 million was used for field operations, CAD 1.1 million to operate the mass-rearing facility, and CAD 681 000 to cover administrative costs. The programme services are delivered by 16 full-time staff and up to 75 seasonal staff working in the field and mass-rearing facility. From 2010 to 2017, the programme operated without increase in either land value or parcel taxes.

4. SUCCESS OF THE OKSIR PROGRAMME OVER 20 YEARS

Over the more than 20 years that the OKSIR programme has been operating, it has achieved sustained codling moth suppression (Fig. 2). Overall, there was a 94% reduction in codling moth population, relative to pre-programme levels in the OKSIR programme area as measured by pheromone traps (Fig. 2).

It must be noted that in zones 2 and 3, on recommendation of an operational advisory committee, the OKSIR programme piloted zone-wide use of pheromone-mediated mating disruption in commercial orchards as an alternative to the SIT from 2011-2014. Pheromone trap captures during these years are not directly comparable to other years or zone 1 during these years because a different trapping system was used. For economic and other reasons, in 2015 the OKSIR programme returned to using the SIT in commercial orchards across the entire programme area (Cartier 2014). Since 2000, and in all zones, mean codling moth trap captures remain well below the recommended treatment threshold of two codling moths per trap per week for two consecutive weeks (Vakenti 1972) (Fig. 2).

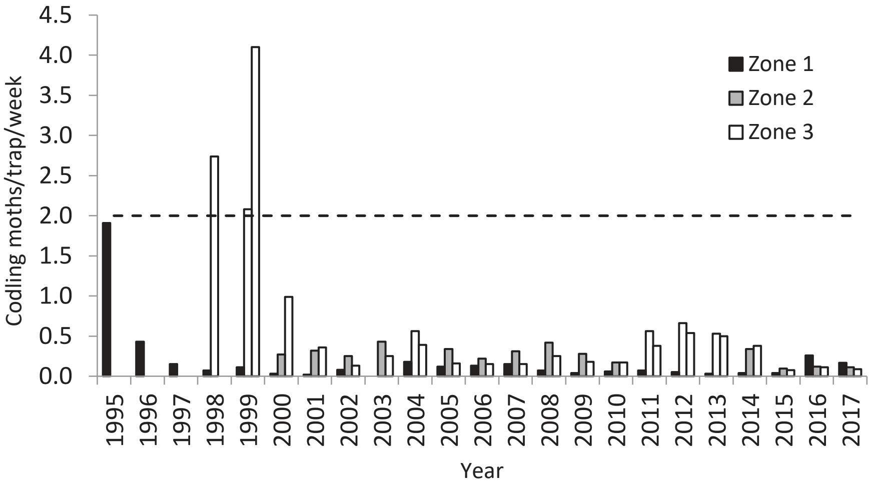

Figure 2. Mean wild codling moth captures per trap per week from 1995 to 2017 for each zone managed by the OKSIR programme in zone 1 (from 1995), in zone 2 (from 1998), and in zone 3 (from 1999), averaged over each fruit-growing season. The dashed line indicates the recommended threshold (two codling moths per trap/week for two consecutive weeks) at which insecticide controls supplementary to the SIT would be required.

In zone 1, codling moth fruit damage was effectively suppressed after approximately five years, and has remained that way in more than 90% of orchards (Fig. 3).

Codling moth suppression followed similar trends in zones 2 and 3, except that population suppression occurred at a slower rate (Figs. 2 and 3). It is difficult to pinpoint exactly why this was the case, and no single factor alone was likely responsible for this result. Reasons for slower population decline include:

- Larger urban centres in zones 2 and 3 with more infested backyard trees acting as refugia for codling moths.
- Pheromone-mediated mating disruption that was used extensively in zones 2 and 3 during the pre-release sanitation phase, may have been less effective than the extensive initial application of organophosphate insecticides for suppression in zone 1.
- The increased service area created greater demand on the programme's limited expert knowledge and human resources.
- The organic fruit producers reside predominantly in zone 1, meaning there may have been less grower buy-in or more scepticism in zones 2 and 3.

Ultimately, by 2015 more than 90% of orchards had less than 0.2% fruit damage due to codling moth (Fig. 3) in all zones.

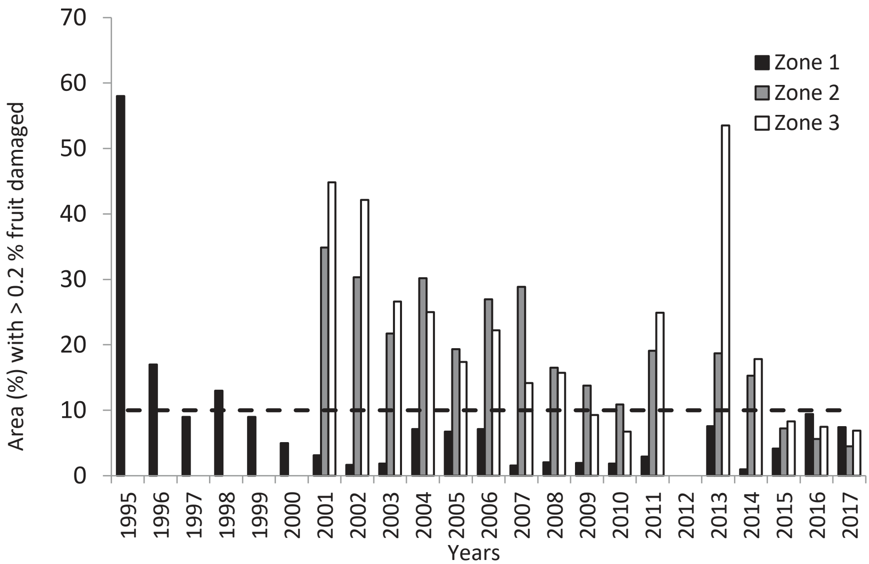

Figure 3. Percent of programme area with >0.2% of fruit damaged by the codling moth. Bars show data from 1995 (zone 1) and from 2001 (zones 2 and 3) to 2017 for each zone managed by the SIR programme. The dashed line indicates 10% of the programme area, an economic target set by the Programme's Board; 2012 data not available.

On average, 85% of the planted area was sampled every year for fruit infestation just prior to harvest (though this varied significantly across years due to limited human resources) ranging from 15 to 100%. Orchards that were not sampled were those with no evidence of codling moth populations, as evidenced by trap captures, early-season inspections, and previous sample history, and thus were placed in the ≤0.2% damage category.

The programme has contributed to a dramatic decrease in the amount of insecticides applied per ha of pome fruit. From 1991 to 2016, there was an estimated 96% reduction in insecticides used against the codling moth (Fig. 4). Other factors, such as changes in spray application rates in spindle versus traditional planting systems, new product formulations, etc., contributed in part to this reduction. Personal testimonies from growers indicated that many have not needed to spray insecticides to control the codling moth in more than 15 years.

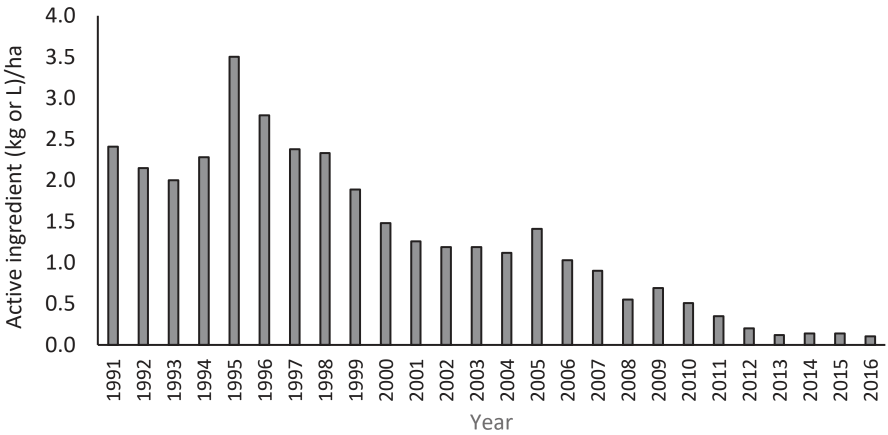

Figure 4. Estimated pesticide active ingredient (kg or L) applied per ha per year for all zones managed by the SIR programme from 1991 to 2016 based on the estimated proportion of sales for the 15 products registered for use against the codling moth (note: a number of these insecticides are also applied for other pests and/or crops). The estimates of active ingredients are divided by the area (ha) of planted pome fruit in the programme area to account for changes in sales due to amount of pome fruit under cultivation.

Anecdotal evidence collected during an external review of the OKSIR programme also suggests that there are collateral pest management benefits occurring (Carpenter et al. 2014). Leafrollers *Spilonata ocellana* Denis and Schiffermüller, *Choristoneura rosaceana* Harris and *Archips* spp., were reported to be on the decline similar to what organic growers have experienced, i.e. when insecticide use is reduced the natural enemy populations are allowed to increase and reduce some pest populations (Leach and Mumford 2008).