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TITLE: Investigation on Utilization of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) for Biological Control of Sugarcane Moth Borer Complex in Thailand

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THESIS

INVESTIGATION ON UTILIZATION OF *Cotesia flavipes* (CAMERON) (HYMANOPTERA: BRACONIDAE) FOR BIOLOGICAL CONTROL OF SUGARCANE MOTH BORER COMPLEX IN THAILAND

TEWEE MANEERAT

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Entomology)
Graduate School, Kasetsart University

2014

Tewee Maneerat 2014: Investigation on Utilization of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) for Biological Control of Sugarcane Moth Borer Complex in Thailand. Doctor of Philosophy (Entomology), Major Field: Entomology, Department of Entomology. Thesis Advisor: Associate Professor Wiwat Suasa-ard, Ph.D. 74 pages.

Sugarcane moth borers, as a complex, *Chilo infuscatellus* Snellen, *Chilo sacchariphagus* (Bojer), *Chilo tumidicostalis* (Hampson), *Scirpophaga excerptalis* (Walker), and *Sesamia inferens* (Walker) are destructive insect pests attacking sugarcane crop in central Thailand; however they are controlled by an effective larval endoparasitic wasp, *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae). Three objectives of this thesis are, firstly to explore the population trends of the sugarcane moth borers and their larval parasitoid, *C. flavipes*; secondly to investigate field augmentative biological control of the borers; and finally to access the yields of sugarcane gained from the augmentative release of *C. flavipes*. Experiments were conducted at four districts of Thailand in three successive years (2009-2011). In the population trends study, the incidence of the sugarcane moth borers was synchronized with their parasitoids throughout the end of the seasons at all planting locations. In the second experiment (augmentation), percentage of the borers infestation was significantly lower ($P<0.05$) in parasitoid release plots than in non release plots. Percentage of parasitization of the release plots was significantly higher ($P<0.05$) than in non release plots. Finally, average yields of sugarcane in release plots were significantly higher ($P<0.05$) than those in non release plots.

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ACKNOWLEDGEMENTS

I would like to express the deepest appreciation to my thesis advisor, Associate Professor Wiwat Suasa-ard, Ph.D., who has the attitude and the substance of a genius: he continually and convincingly conveyed a spirit of adventure in regard to research and an excitement in regard to teaching. Without his guidance and persistent help this dissertation would not have been possible.

I would like to offer my sincere thanks to examination chairperson Assistant Professor Nantasak Pinkaew, Ph.D. for his valuable suggestions for thesis manuscript.

I am grateful to Associate Professor Kosol Charernsom, Departmant of Entomology, Kasetsart University for identification and confirmation in specific of the sugarcane insects studied. I would like to thank National Research Council of Thailand for funding source, National Biological Control Research Center (NBCRC), Central Regional Center and Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom for providing all the possible facilities and necessary during this work.

Finally, I must express my profound gratitude to my parents for providing me with unfailing support and continuous encouragement throughout my years of study and though the process of researching and writing this thesis. This accomplishment would not have been possible without them.

Tewee Maneerat

November 2014

TABLE OF CONTENTS

LIST OF TABLES

.

LIST OF FIGURES

Figure **Page**

LIST OF FIGURES (Continued)

Figure **Page**

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

Figure **Page**

Appendix Figure

.

LIST OF ABBREVIATIONS

^{o}C	=	degree Celsius
cm	=	centimeter
ha	=	hectare
hrs	=	hours
kg	=	kilogram
mm	=	melimeter
n.d.	=	no date
RH	=	relative humidity
t	=	ton
s	=	second

INVESTIGATION ON UTILIZATION OF *Cotesia flavipes* (CAMERON) (HYMENOPTERA: BRACONIDAE) FOR BIOLOGICAL CONTROL OF SUGARCANE MOTH BORER COMPLEX IN THAILAND

INTRODUCTION

Global interest in sugarcane *Saccharum officinarum* L. has increased significantly in recent years due to its economic impact on sustainable energy production. It is one of the economically important cash crops widely cultivated in the tropics to subtropics; and it annually provides around 60 to 70 % of the world's sugar (Shah *et al*., 2009). As a result of its sweetness, it is used as raw material for the production of sugar according to an estimate 80 % of sugar production that is based upon it. Likewise sugarcane has been an important crop grown in Thailand for human consumption. Its industry has continually flourished the country, which has become one of the world's largest sugar exporters, after only Brazil since last decade. Last century sugarcane production was produced for sugar and sweetener only. Recently, new trends in biotechnology, the search for new materials preference for renewable energy production is in progress.

Due to the increasing consumption and rising of oil price, therefore, Thailand, is destined to import energy in more quantity and value. One alternative to be independent from oil import and foreign exchange loss is to find other sources of energy from the country's abundance of agricultural crops, which can be used for production of renewable energy. Definitely, there are many end uses to which the byproducts of the sugarcane industry can be put e.g. bagasse, syrup, filtered cake and trashes from the harvest. Bagasse and sugarcane leaves can be used as fuels for the electricity generator; and sugarcane syrup as well as molasses which is feedstock for ethanol production. Furthermore, sheaths and leaves are used for fertilizing and for producing ethanol.

Sugarcane planting technology is applied to increase its yields. However, the Thai sugarcane farmers have been facing problems in cropping practices. One of these is the insect pest damage which is the barrier that causing yields loss. In Thailand, during 2007-2010 sugarcane planting seasons, the sugarcane moth borer complex generated considerable economic damage to Thai sugarcane industries (Suasa-ard *et al.*, 2010). The various insect pest attacking sugarcane crops were more than 120 species which included sap feeding, leaf feeding and stem borers (Suasa-ard and Allsopp, 2000). Among these, the sugarcane moth borer complex are considered to be economically important; there are five species, four are in the family Crambidae: *Chilo infuscatellus* Snellen, *Chilo sacchariphagus* (Bojer), *Chilo tumidicostalis* (Hampson), *Scirpophaga excerptalis* (Walker); and one is in the family Noctuidae: *Sesamia inferens* (Walker) (Lewanich, 1975; Suasa-ard, 1982; Suasa-ard, 2000; Suasa-ard, 2010). Economic impact of the moth borers larvae could severely reduce quantitative crop yield and qualitative sugar content by boring either into the shoots or stalks of sugarcane plants (Sallam *et al*., 2010; Goebel *et al*., 2011). To lessen the damage, control strategies are applied for the insect pests.

Insecticide is commonly practiced in any pest control strategy also widely used in sugarcane plantations. However, residues effect may occur if it is misapplied on the crop. Insecticide treatments are generally ineffective, expensive. In order to save environment from chemical pollution, application of biological control approach has been given utmost attention; it combines environmental preservation and biodiversity conservation (Goebel *et al.*, 2010). Natural enemies, parasitoids, predators and entomopathogenic microorganisms have been utilized to control the sugarcane insect pests in various countries e.g. Pakistan (Hussnain *et al*., 2007), Brazil (Bueno, 2011), eastern and southern Africa (Jiang *et al*., 2004) and Thailand. (Mohyuddin, 1991; Katrina *et al.*, 2000; Sétamou *et al.*,.2002a; Suasa-ard *et al*., 2008; Nadeem and Hamed, 2011).

Highly effective and culturable natural enemies that are acceptable for the suppression population of sugarcane insect pests, particularly the sugarcane moth borer complex, is the koinobion larval parasitoid *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae). This species is widespread in Asia and has been successfully released in biological control program (Alam *et al.*, 1971; Mohyuddin, 1991) on a number of sugarcane borers e.g. *Chilo partellus* (Lepidoptera: Crambidae) (Jiang *et al*., 2004), *Diatraea saccharalis* Fabricius (Lepidoptera: Crambidae) (Gifford and Mann, 1967; Fuchs *et al*., 1979; Mahmoud *et al*., 2011), *Diatraea grandiosella* Dyarand, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) (Alleyne and Wiedenmann, 2001).

OBJECTIVES

Overall objectives

The overall goal of the thesis was to study field augmentative release of the larval parasitoid, *Cotesia flavipes,* as a biological control agent of the sugarcane moth borer complex.

Specific objectives

1. To explore the population trends of the sugarcane moth borer complex and their larval parasite, *C. flavipes*.

2. To investigate field augmentative release of *C. flavipes* for sustainable biological control of the sugarcane moth borer complex.

3. To access the yields of sugarcane gain from the augmentative release of *C. flavipes* by comparing between the release and non release plots.

LITERATURE REVIEW

Sugarcane

Today, sugarcane (*Saccharum officinarum* L.) is one of the world's most important crops. It has widespread implications for the earnings and well being of farm communities, as well as for consumers of this important food source (Chang, n.d.). Moreover, it is considered as a multi-usage crop serving a variety of sectors from food and pharmaceuticals to energy production. Recent advances in industrial biotechnology are providing new opportunities to capture additional revenue streams from bioproducts (e.g. bioplastics) using sugarcane stalks and residues ('bagasse') as energy feedstock. Almost 80 % of the world sugar is produced from sugarcane, mainly in developing and emerging countries (Licht, 2009).

In 2010, FAO estimates it was cultivated on about 23.8 million ha, in more than 90 countries, with a worldwide harvest of 1.69 billion tons. Brazil was the largest producer of sugarcane in the world. The next five major producers, in decreasing amounts of production, were India, China, Thailand, Pakistan and Mexico (Anonymous, 2013).

Genetically, sugarcane originates from New Guinea. This plant belongs to the Gramineae family (Naturland, 2000). It is a perennial cash crop which is of prominence to countries in the tropical and subtropical regions, between 22 ^{0}N and 22 ^{0}S, and some up to 33^{0}S (Figure 1). A sugarcane crop is sensitive to climate, soil type, irrigation, fertilizers, varieties, insects, diseases, and the harvest period. Both sufficient sunshine and water supply, either from natural rainfall or through irrigation, with a continuous period of more than six to seven months, can increase cane production (Anonymous, 2013).

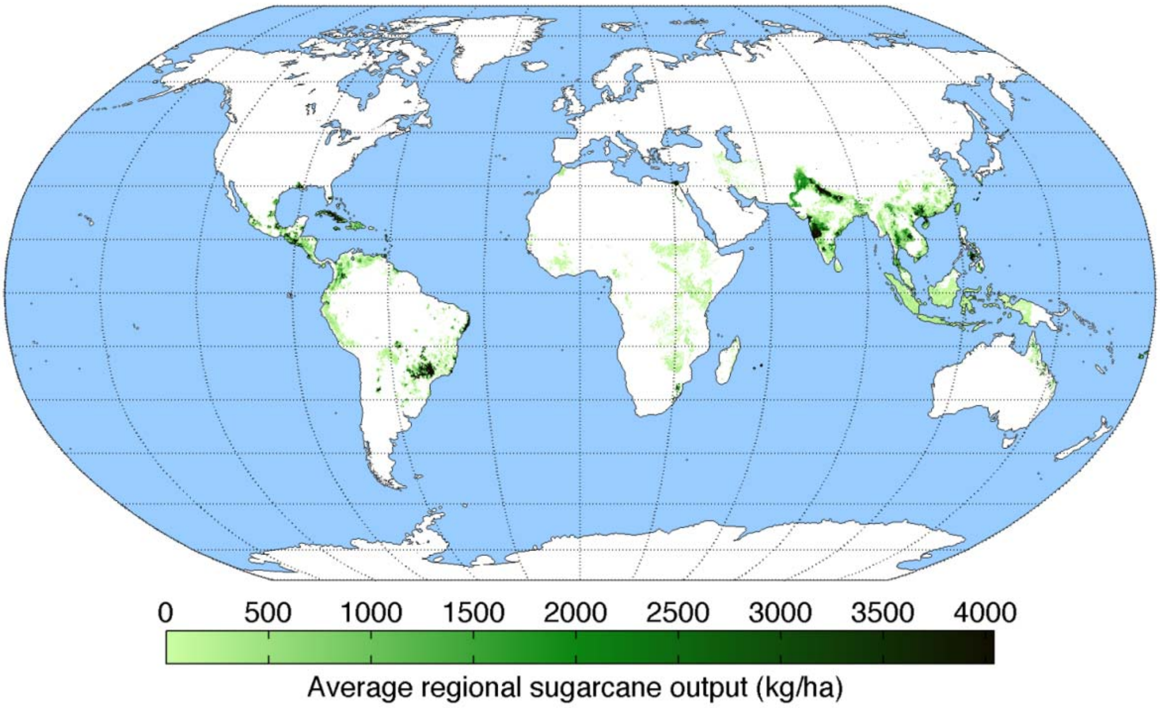
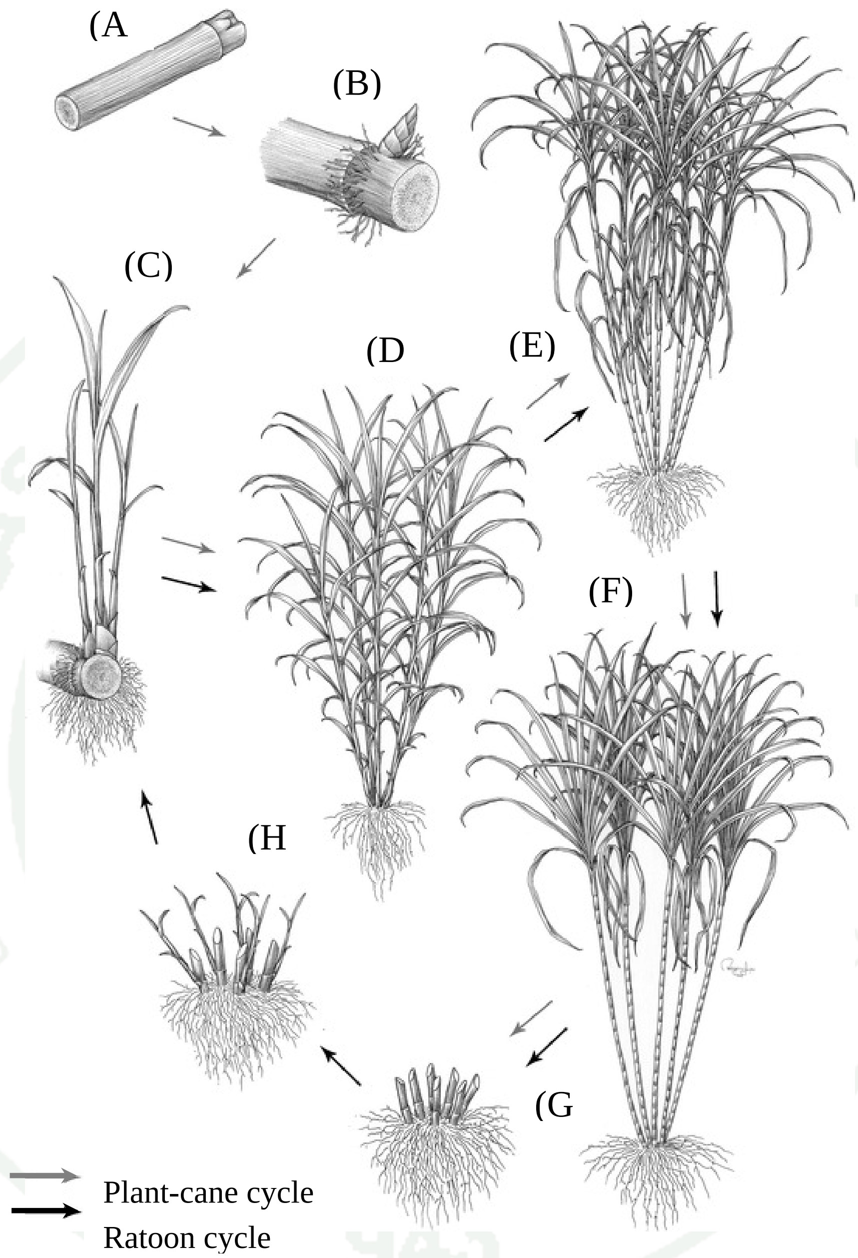


Figure 1 World map of sugarcane planting countries.

Source: Hishler (2010)

The sugarcane plant forms lateral shoots at the base to produce multiple stems (tillers). Later the tillers grow into cane stalks, typically three to four meters high and five cm in diameter, which when mature constitutes approximately 75 % of the entire plant (Anonymous, 2013). Number of plant tillers, generally 4-12 stems, depends on the variety and site conditions (Naturland, 2000).

Since sugarcane is perennial crop, it is harvested annually for up to five years before requiring replanting or six harvests when grown under rainfed conditions (Matsuoka *et al.*, 1999). In sugarcane plant phenology, there are seven phases of growth development; stalk pieces used in planting; beginning of bud sprouting and rooting, tillering initialtion, intense tillering beginning of maturation, manufacturable stalks in optimal sucrose concentration, harvesting and ratoon sprouting (Figure 2) (Cheavegatti-Gianotto *et al.*, 2011). Generally, two basic sugarcane production cycles exit. The plant-cane cycle starts with planting and ends after the first harvest. The ratoon, or ratoon-cane, cycle starts after the harvest of the plant cane and continues with successive ratoon crops until field renewal. The complete cycle of a sugarcane field lasts either four or five seasons, after which time the crop is renewed (Cheavegatti-Gianotto *et al.*, 2011).



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Source: Cheavegatti-Gianotto *et al*. (2011)

A mature stalk typically composes of 11-16 % fiber, 12-16 % soluble sugars, 2-3 % non-sugars, and 63-67 % water. The average yield of cane stalk is 60-70 % t/ha/year, however this figure can vary between 30 to 180 t/ha depending on knowledge and crop management approach used in sugarcane cultivation (Anonymous, 2013). The sugar content (saccharose) fluctuates between 11 % to 16% (Naturland, 2000).

Thailand’s sugarcane and sugar industry has been grown continually and the country now has become one of the world top ten of producer and exporter sugarcane. Especially, the white sugar product of that was exported onto world trade markets less than only Brazil (Euteneuer, 2011). The industry is one of the country’s key industrial sectors, which play an important role in the country’s economic and social development. This industry can generate revenue to local economy for more than 50,000 million baht annually from exports and domestic sales of sugar. It also, creates employment and income for more than 1,000,000 sugarcane farmers and people in related sectors. Sugarcane planting areas of Thailand have increased gradually since last decade. Most of the planting areas are in the north, followed by central, northeast and west, respectively (Figure 3) (Office of Agricultural Economics 2004; 2005; 2006; 2007; 2008; 2009; 2010; 2011; 2012; 2013).

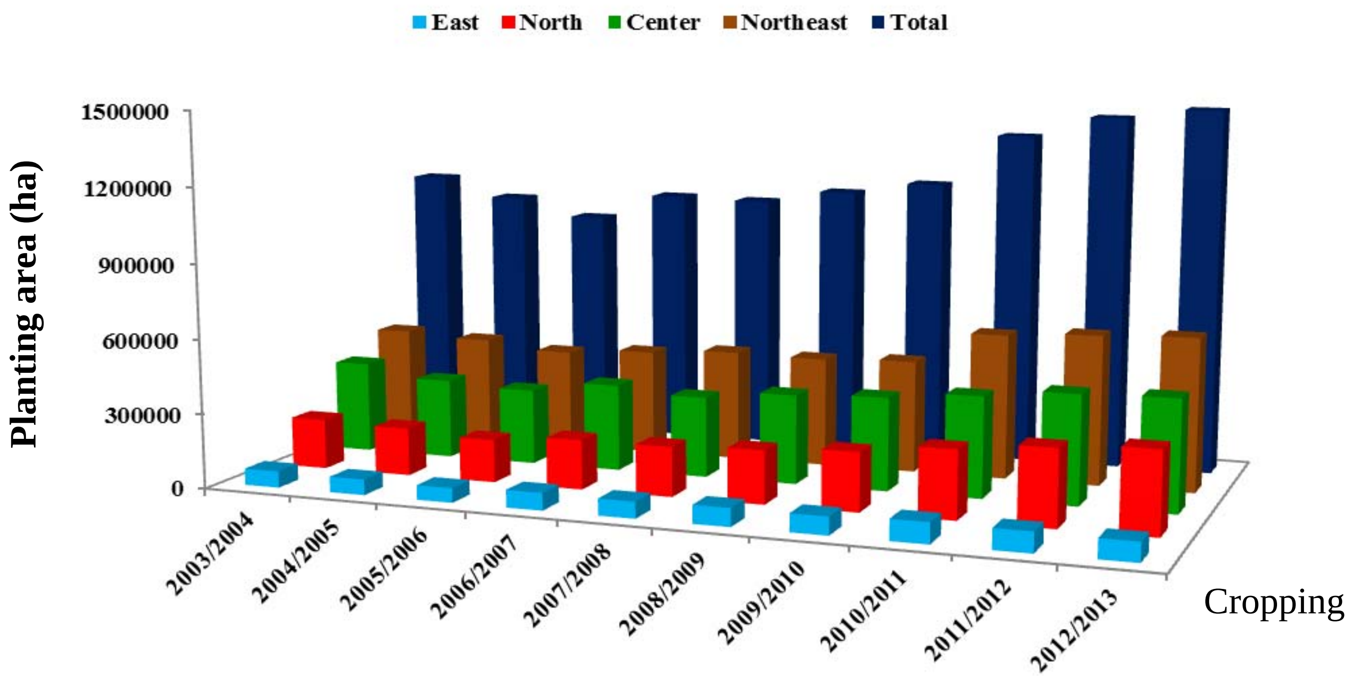

Figure 3 Thailand regional sugarcane planting areas in ten cropping seasons (during 2003/2004 to 2012/2013 cropping seasons).

Source: Modified from Office of Agricultural Economics (2004; 2005; 2006; 2007; 2008; 2009; 2010; 2011; 2012; 2013)

Inevitably whenever crops are grown, insect pests damaging the growing crops is one of the hindrance that farmers face. Sugarcane farmers are alike to experience this problem and quite severe. Since sugarcane is usually planted as monocrop, hence, the problems are more intense than other mixed cropping.

Sugarcane insect pests

Sugarcane crop is attached by a wide range of insects more than 1,500 species world wide (Box, 1953; Long and Hensley, 1972; Hussnain *et al*., 2007), particularly those economically important ones cause significant damage to all stages and parts of the crop (i.e. root, stalks and foliage) (Williams *et al.*, 1969; Hall, 1988). Practically they can be classified into four main groups. The first one is the leaf feeders which include armyworms (Lepidoptera: Noctuidae) and locusts (Orthoptera: Acrididae); the second one is sap feeders which are mostly hemipteran species, including aphids (Aphidoidea), scale insects (Coccoidea), whiteflies (Aleyrodidae), mealybugs (Pseudococcidae), planthoppers (Fulgoroidea) and froghoppers (Cercopoidea); the third one is the stalk feeders, around 50 species of moths attack sugarcane worldwide (Long and Hensley, 1972) and this group can be loosely classified, depending on the time of infestation and the feeding site, to be stem feeders and shoot feeders. They are by far the most damaging sugarcane pests in all cane growing countries (Kfir *et al.*, 2002); and the final one is the root feeders which are mainly the white grubs causing plant drying and increase risk of the canes collapse (Allsopp, 2010).

In Thailand sugarcane planting technology has been applied to increase the sugarcane yields. However, the Thai sugarcane farmers are still facing problems in cropping practices. One of these is the insect pest damage which is responsible for low yield of sugarcane. About 120 insect species are associated with sugarcane (Suasa-ard and Allsopp, 2000). However, only nine species of them are important including five species in the sugarcane shoot and stem borers: *Chilo infuscatellus* Snellen, *Chilo sacchariphagus* (Bojer), *Chilo tumidicostalis* (Hampson), *Sesamia inferens* (Walker) and *Scirpophaga excerptalis* (Walker). The others are the

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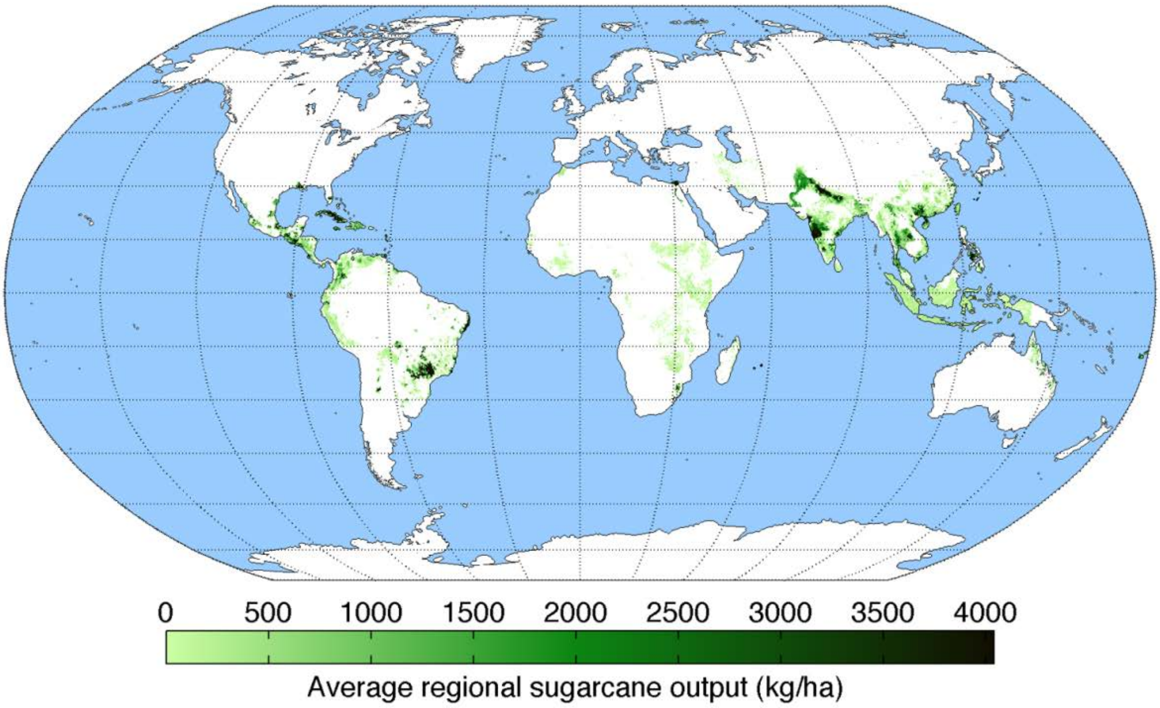

Figure 1 World map of sugarcane planting countries.

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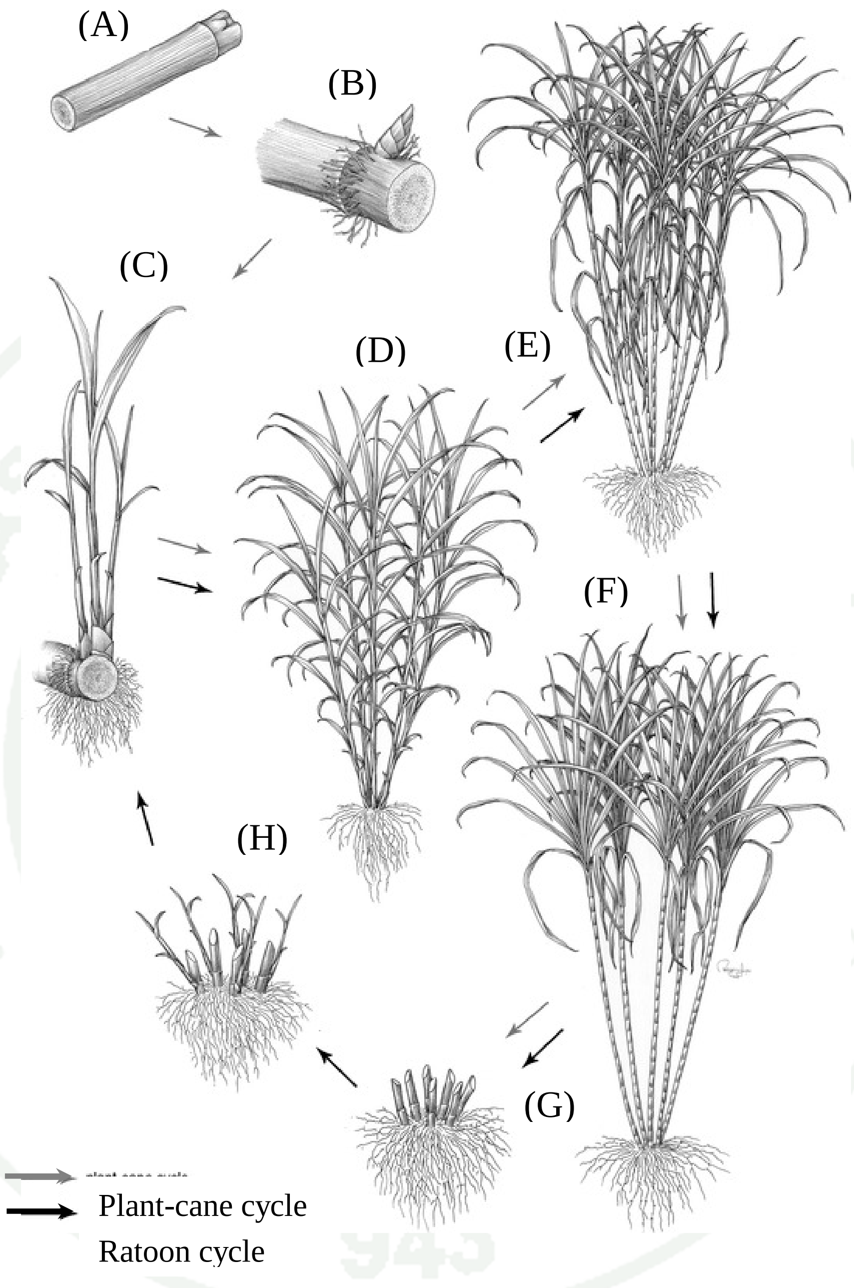

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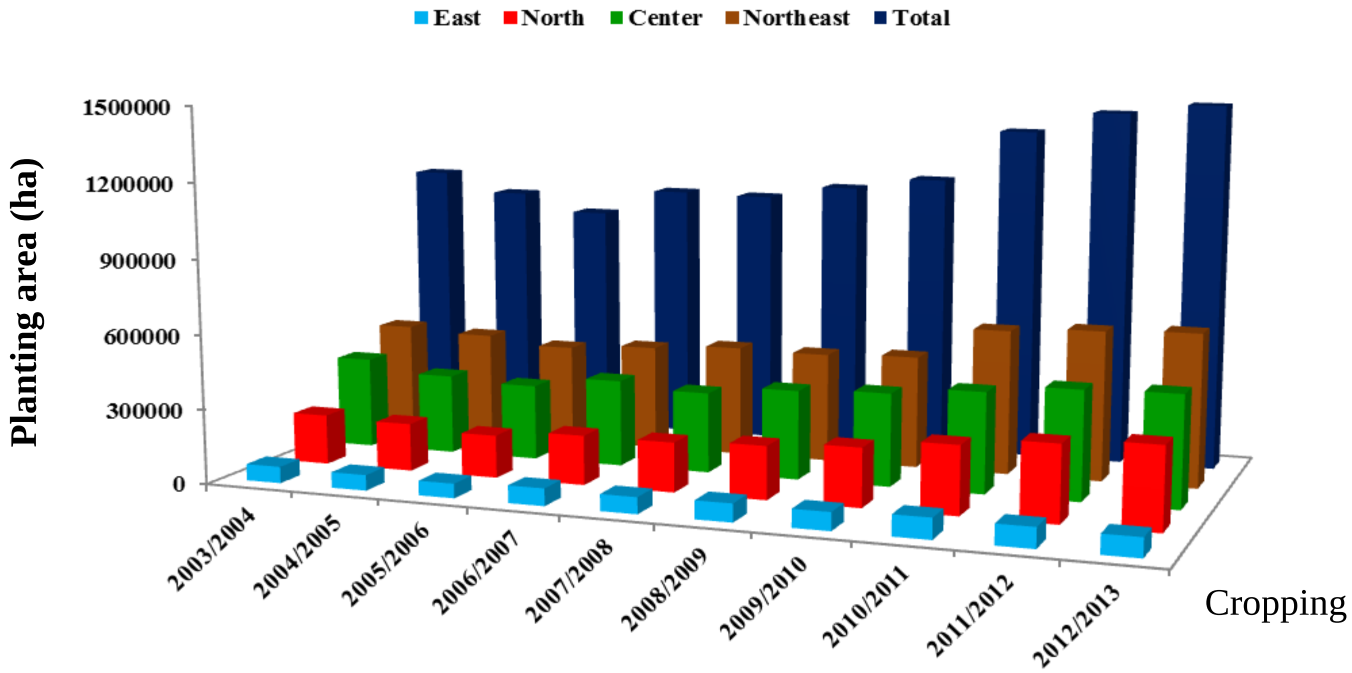

Figure 3 Thailand regional sugarcane planting areas in ten cropping seasons (during 2003/2004 to 2012/2013 cropping seasons).

Source: Modified from Office of Agricultural Economics (2004; 2005; 2006; 2007; 2008; 2009; 2010; 2011; 2012; 2013)

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In Thailand sugarcane planting technology has been applied to increase the sugarcane yields. However, the Thai sugarcane farmers are still facing problems in cropping practices. One of these is the insect pest damage which is responsible for low yield of sugarcane. About 120 insect species are associated with sugarcane (Suasa-ard and Allsopp, 2000). However, only nine species of them are important including five species in the sugarcane shoot and stem borers: *Chilo infuscatellus* Snellen, *Chilo sacchariphagus* (Bojer), *Chilo tumidicostalis* (Hampson), *Sesamia inferens* (Walker) and *Scirpophaga excerptalis* (Walker). The others are the sugarcane whitefly, *Aleurolobus barodensis* (Maskell)*;* the sugarcane longhorn stem borer, *Dorysthenes buqueti* (Guérin-Méneville)*;* the sugarcane scale, *Aulacaspis sacchari* and the sugarcane pink mealybug, *Saccharicoccus saccharii* (Cockerell). Among these, the sugarcane moth borer complex and the longhorn stem borer are the most serious ones.

Sugarcane moth borer complex

In Thailand, five species of sugarcane moth borer are grouped in an insect complex. These five species are stem borer, *C. infuscatellus,* stem borer, *C. sacchariphagus,* stem borer, *C. tumidicostalis,* top borer, *Sc excerptalis* and shoot borer, *S. inferens*. They are serious pests of sugarcane in Asia including Thailand. The distributions of these are showed in Figure 4. All five species caused “dead hearts” in sugarcane, with *Sc. excerptalis* being responsible for the majority of dead heart symptoms in both young and mature cane, *Chilo* spp. Cause dead hearts only in young cane and later tunnel inside cane stalks and damage the internodes, the later *S. inferens* is a shoot borer and causes dead heart in young cane plants. Significant damage results are the tunnels in the stalk made by the sugarcane borers. It can cause a loss of stalk weight and ultimaltly loss of sugarcane yield (Assefa *et al.*, 2010; Duna *et al.*, 2009; Ron and Nuessly, 2011; Singh, 2006; Suksen *et al.*, 2007).

Chemical treatments are generally ineffective, expensive and at the present none are registered for use, biological control represents a good approach that combines environmental preservation and biodiversity conservation strategies (Goebel *et al*., 2010). Interest has increased in using biological control, which has long been recognized as an important tool in suppressing insect pests. Beneficial insects have been successfully employed in a variety of augmentation and conservation strategies (Nordlund, 1984; Mohyuddin, 1991).

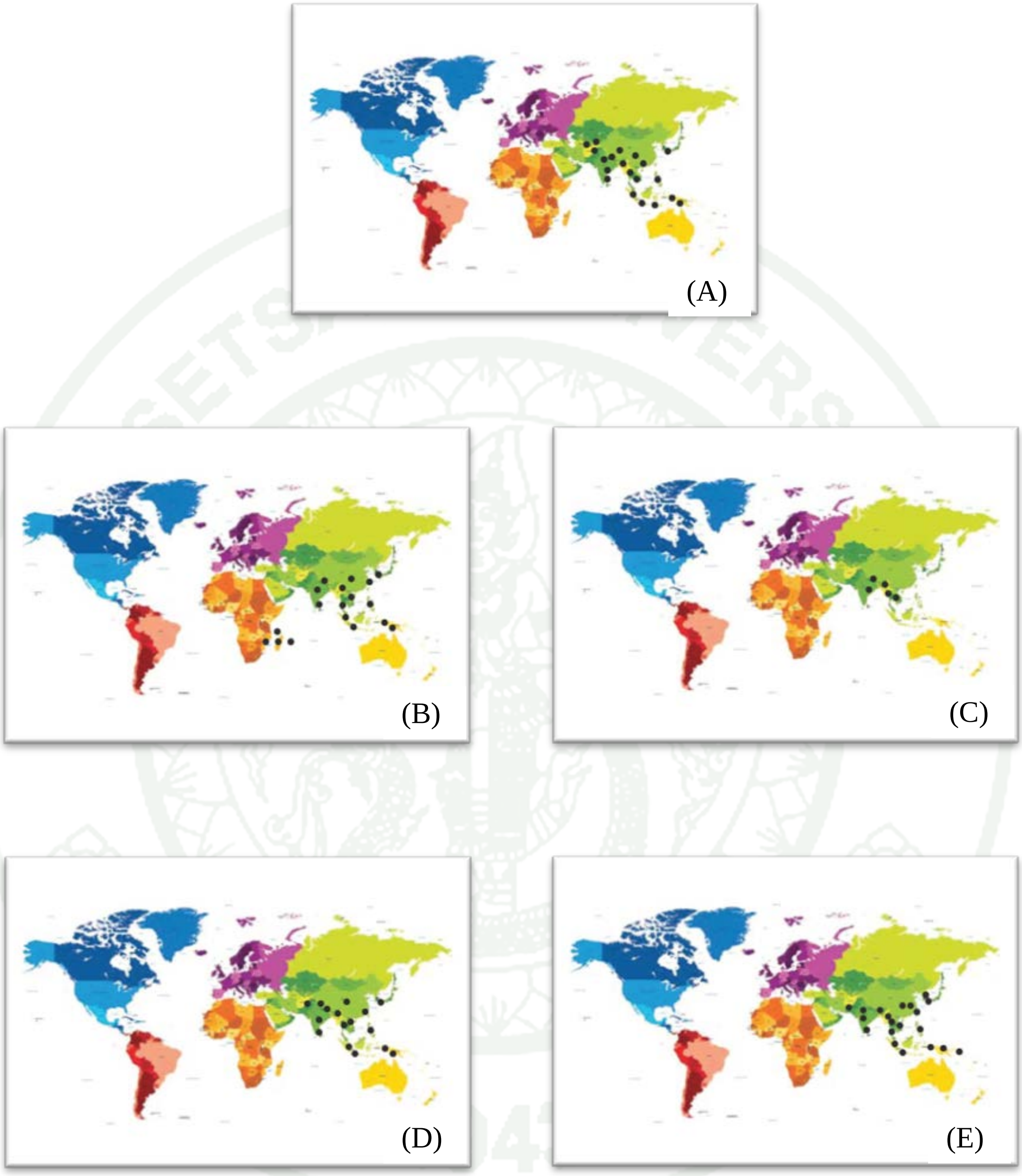


Figure 4 Distribution map of five species of the sugarcane moth borer complex
(A) *Chilo infuscatellus* Snellen (B) *Chilo sacchariphagus* (Bojer)
(C) *Chilo tumidicostalis* (Hampson) (D) *Scirpophaga excerptalis* (Walker)
(E) *Sesamia inferens* (Walker)

Source: Sallam (2012)

Cotesia flavipes **(Cameron) (Hymenoptera: Braconidae)**

Monoculture is the agricultural practice of producing or growing a single crop or plant species over a wide area and for a large number of consecutive years. Sugarcane is one of the monoculture crops that are widely grown in tropical and subtropical countries (Overholt *et al.*, 2003). This is an example of agroecosystems with low diversity of organisms both fauna and flora and it may be more susceptible to pest outbreaks (Theunissen, 1994; Altieri and Nicholls, 2004). Because of this increased susceptibility, management and external inputs are essential to support low diversity agroecosystems especially when outbreak of insect pests occurs. On the other hand, reliance on diverse plantings, a range of natural enemies that are supported by these plants, and associated crop management strategies can, in some cases, help maintain pest populations below economic thresholds (Altieri and Nicholls, 2004; Rodriguez-Saona *et al.*, 2012). In most cases, pest control becomes one essential alternative to suppress the pest population.

C. flavipes is a larval parasitoid used worldwide in biological control against tropical stem borers (Potting *et al.*, 1997a). It was selected as the preferred candidate of this study because it had a wide host range in diverse habitats (Potting *et al,*. 1997a), especially associated with sugarcane and cereal crops (Walker, 1994), and its history of success and importance in the control of stem borers in its aboriginal home in Asia (Overholt *et al*, 1994). It was successfully colonized in the New World, where it provided excellent control of the sugarcane borer in the Carribbean, (Baker *et al.*, 1992), Ethiopia (Assefa *et al.*, 2008), South America (Rossi and Fowler, 2004), Texas (Legaspi *et al.*, 1997; Meagher *et al.*, 1998; Sétamou *et al.*, 2002b) and Louisiana (White *et al.*, 2004) It was responsible for maintaining populations of sugarcane borer below economic levels (Sétamou *et al.*, 2002b) and reducing of yield loss (Lv *et al.*, 2011). It was an important natural enemies attacking larvae of pyralid, noctuid borers (Potting *et al.*, 1997b) and crambid borers (Polaszek and Walker, 1991). Some others insect pests that are parasitized by this parasitoid are *C. infuscatellus* (Fatima *et al.*, 2009; Hussnain *et al.*, 2007; Saikia and Nath, 2002; Suasa-ard and Charernsom, 1999; Tanwar and Varma 2002), *C. sacchariphagus* (Goebel *et al.*, 2010; Kfir *et al.*, 2002), *Sc. excerptalis* (Hussnain *et al.*, 2007) *C. tumidicostalis* and *S. inferens* (Suasa-ard and Charernsom, 1999; Suasa-ard *et al.*, 2010).

For the biology of *C. flavipes* (Figure 5), it has complete metamorphosis; the immature parasitoid develops in only one host, from which it obtains its nutritional requirements (DeBach and Rosen, 1991). Female of *C. flavipes* oviposits directly into the host haemocoel, and the number of eggs depend on the host age (Brewer and King, 1981). This parasitoid remains within the host during its whole embryonic and larval development, beings both dependent on the temperature conditions and development status of the host. The immature of parasitoids emerge from the body cavity of the host to pupate after producing a characteristic silk cocoon. Parasitized hosts usually die or become sterile after parasitoid emergence.

In spite of number of pesticide applications, cryptic habit of the damaging larvae protects them from the toxic effect of insecticides. Further understanding the host plant, insect host and parasitoids relationships is one of the key components for the augmentative biological control approach of sugarcane moth borer complex. Additionally inadequate work of the Thai literature has been reported on this subject; all are from other countries (Botelho *et al*., 1980; Van Hamburg and Hassell, 1984;Tanwar and Varma, 2002; Khan *et al*., 2013; Muhammad *et al*., 2014). Therefore population and augmentative release study of this parasitoid species is required for biological control of the sugarcane moth borer complex.

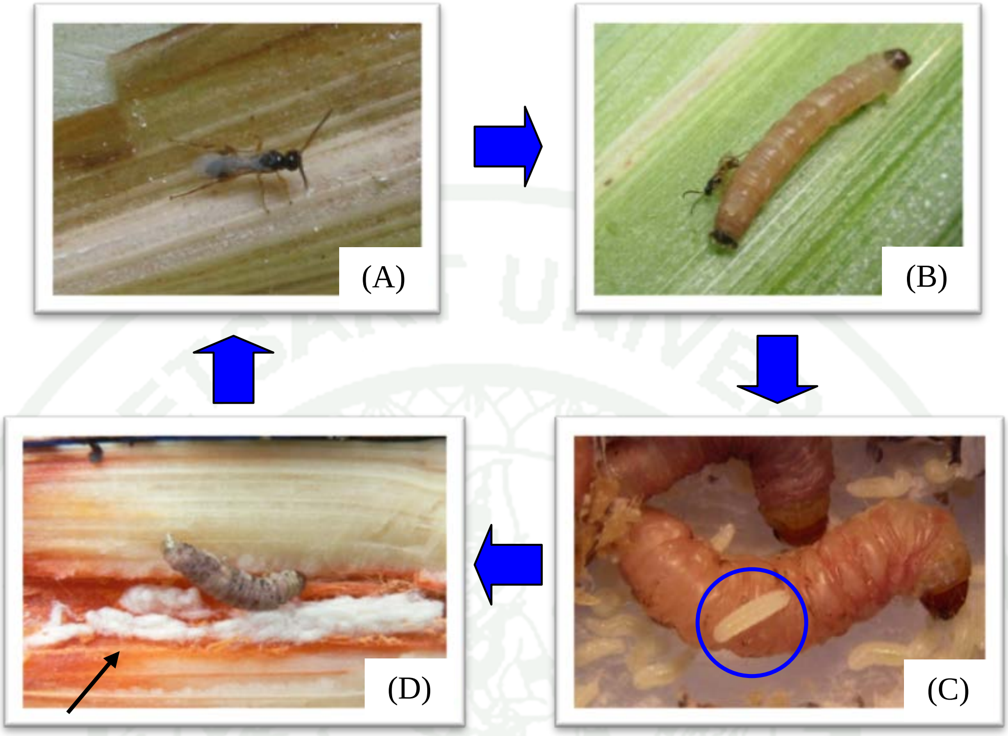


Figure 5 Biology of larval parasitoid, *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae), adult (A), egg laying on *Sesamia inferens* (Walker) (B), developing of late instar larval inside its host (C), and emergence of cocoons outside body of their host (black arrow) (D).

Augmentative release of natural enemies.

Augmentative biological control (or “augmentation”) is simply the release of additional numbers of a natural enemy when too few are present to control a pest effectively. Such releases might be made, for example, if existing natural enemy populations fail to colonize fields or orchards, or colonize too late in the season; it provides effective control of the pest (Obrycki *et al.*, 1997; Charlet, 2002).

Doutt and Hagen (1949) were the first researchers to take this approach over 65 years ago. They released green lacewings to control outbreaking of mealybug populations in pear orchards. Since Doutt and Hagen's pioneering study, augmentative biological control was applied experimentally in a large number of pest systems (Collier and Van Steenwyk , 2004).

However, Mahr (1994) proposed that there were two general approaches to augmentation: inundative releases and inoculative releases. Inundation involves releasing large numbers of natural enemies for immediate reduction of a damaging or near-damaging pest population. It is a corrective measure; the expected outcome is immediate pest control. Inoculation involves releasing small numbers of natural enemies at prescribed intervals throughout the pest period, starting when the pest population is very low. The natural enemies are expected to reproduce themselves to provide more long-term control. The expected outcome of inoculative releases is to keep the pest at low numbers, never allowing it to approach an economic injury level; therefore, it is more of a preventive measure. However, most successful augmentation relies on an ability to mass-produce large numbers of the natural enemy in a laboratory (Taiao, 2013).

One of the major stimuli for investigating biological control has been the drive to reduce a historic reliance on broad-spectrum pesticides for pest control. Hence, augmentation might be used as a substitute for pesticide applications if the pest is sufficiently suppressed by the released natural enemies (Collier and Van Steenwyk, 2004).

Utilization of *Cotesia flavipes*

C. flavipes caused the highest parasitism (82 %) on *C. partellus.* This result verified that *C. flavipes* was contributed to the reduction of *C. partellus* population in lowlands, regardless of the zone, and its rate of parasitism varied between crop stages, crop types, elevations, host and host stages. Findings of this study have particularly relevant information on the contribution of *C. flavipes* to the population reduction of stem borers, time or stage of its occurrence in relation to host stages and crop stages, and its distribution in relation to the availability of a suitable host across each zone. In conclusion, this larval parasitoid plays an important role in reducing stem borer populations and can be used as one component of integrated stem borer management in northeastern Ethiopia (Dejen *et al.*, 2013).

Moreover, the success of the introduced *C. flavipes* was demonstrated by its establishment and spread from the release points increasing parasitism, and decrease in stem borer density by 70 % (Jiang *et al.*, 2004).

MATERIALS AND METHODS

1. Study on the population trends of the sugarcane moth borer complex and the larval parasitoid, *Cotesia flavipes*

Population trend of the sugarcane moth borer complex and the larval parasitoid, *C. flavipes* were studied at 4 districts in 4 provinces in Central Region of Thailand (Figure 6 (A)); Kamphaeng Saen district, Nakhon Pathom province ($13^o59'2''N^o99^o59'38''E$) (Figure 6 (B)), U-Thong districts, Suphanburi province ($14^o22'32''N^o99^o53'32''E$) (Figure 6 (C)), Tha Muang district, Kanchanaburi rovince ($14^o0'12''N^o99^o33'0''E$) (Figure 6 (D)) and Takhli district, Nakhon Sawan province ($15^o42'48''N^o100^o08'07''E$) (Figure 6 (E)). Each study plot covered at least 1.6 ha. This study was done for a period of three years (2009-2011 i.e. three sugarcane planting seasons).

Sampling plan of the sugarcane moth borer larvae and *C. flavipes* was performed with systematic random sampling. Initially, sampling of populations of the sugarcane moth borer complex was done after bud sprouting and rooting (one-month old sugarcane seedlings). For the sugarcane moth borer complex larvae, sampling of each scheme was applied with 100 samples/plot (1.6 ha), five stools/row and 10 rows/plot (Figure 7). The sampling unit assigned was one stool/sample. Data of number of sugarcane moth borer larvae and cocoons of *C. flavipes* were recorded monthly till the sugarcane harvest, approximately 9 months.

Samples of field infested sugarcane stalk were assembled and placed in a synthetic container and later transferred to laboratory of National Biological Control Research Center, Central Regional Center (NBCRC, CRC). The sample stalks were cut off with a sharp knife. Number of sugarcane moth borer larvae were recorded and they were hold in a round shape box, 21.5 cm in diameter and 10.5 cm in height; with a lid which was cut in round shape (10 cm in diameter) and covered with polyester gauze for ventilation. The collected larvae were fed with pieces of young sugarcane stalks (Figure 8). Subsequently, after the parasitoid larvae which developed inside the sugarcane moth borer larvae, emerged and became cocoons, they were gathered in a plate, 5.5 cm in diameter and 1 cm in height. The old stalks, provided as food sources for those developing larvae, were replaced with the new ones every three days.

Identification of specimens

The specimen identification was carried out under the stereoscopic binocular microscope and the samples of the identified insects have been deposited in the Collection of the National Biological Control Research Center, Central Regional Center, Kamphaeng Saen district, Nakhon Pathom province, Thailand. The major characteristics applied to identify the larvae of sugarcane moth borers and the adults of *C. flavipes* were followed the descriptions given by Butani (1956) and Suasa-ard (1982); and Suasa-ard (1982), respectively. Later confirmation of the insect species was done by Kosol Charernsom (a taxonomic entomologist, Department of Entomology Kasetsart University).

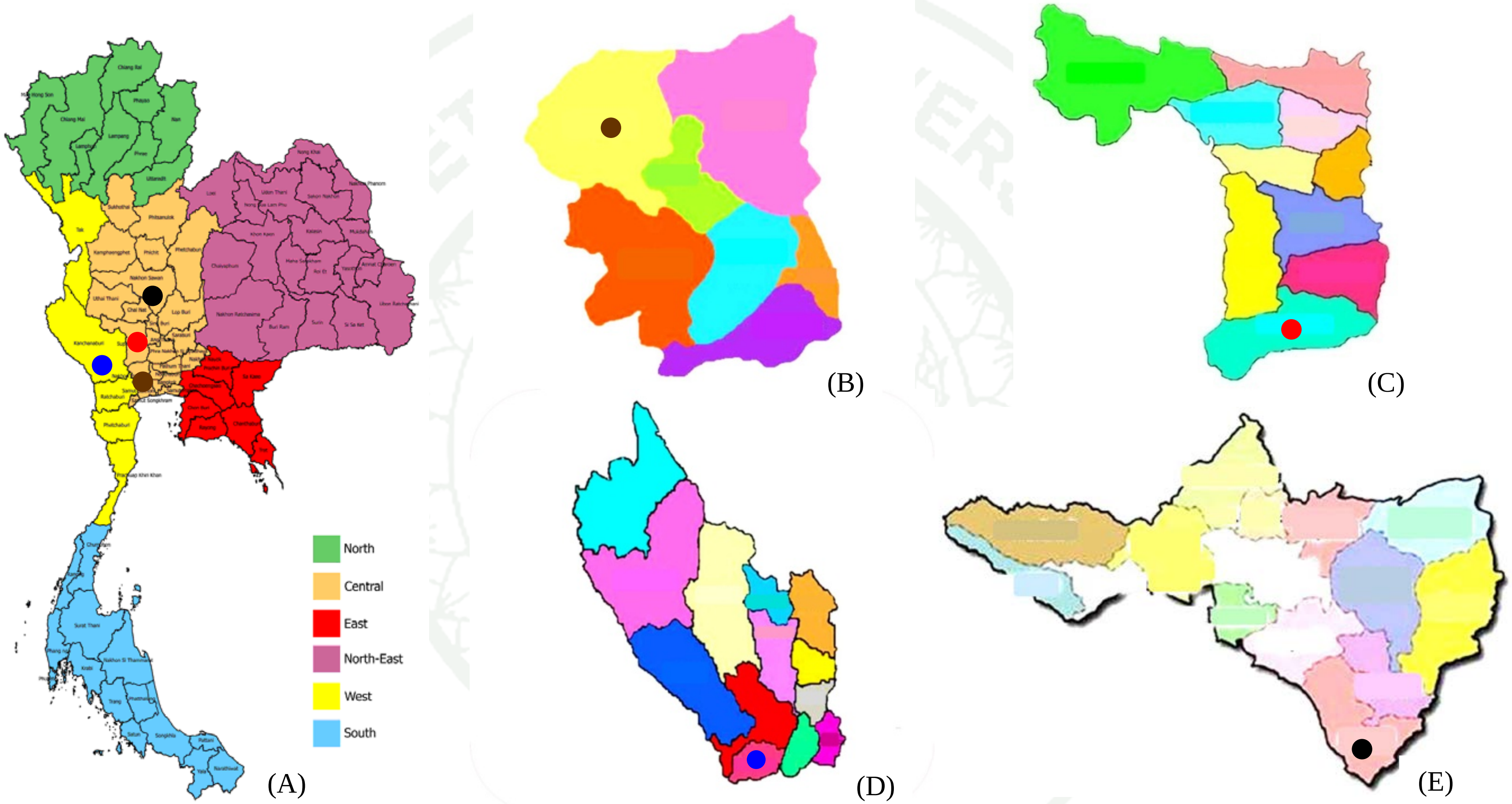


Figure 6 Sampling plots of population trends study in 4 provinces of Thailand (A), Kamphaeng Saen district, Nakhon Pathom province (B) (brown circle), U-Thong district, Suphanburi province (C) (red circle), Tha Muang district, Kanchanaburi province (D) (blue circle), and Takhli district, Nakhon Sawan province (E) (black circle).

Source: Modified from Anonymous (n.d.)

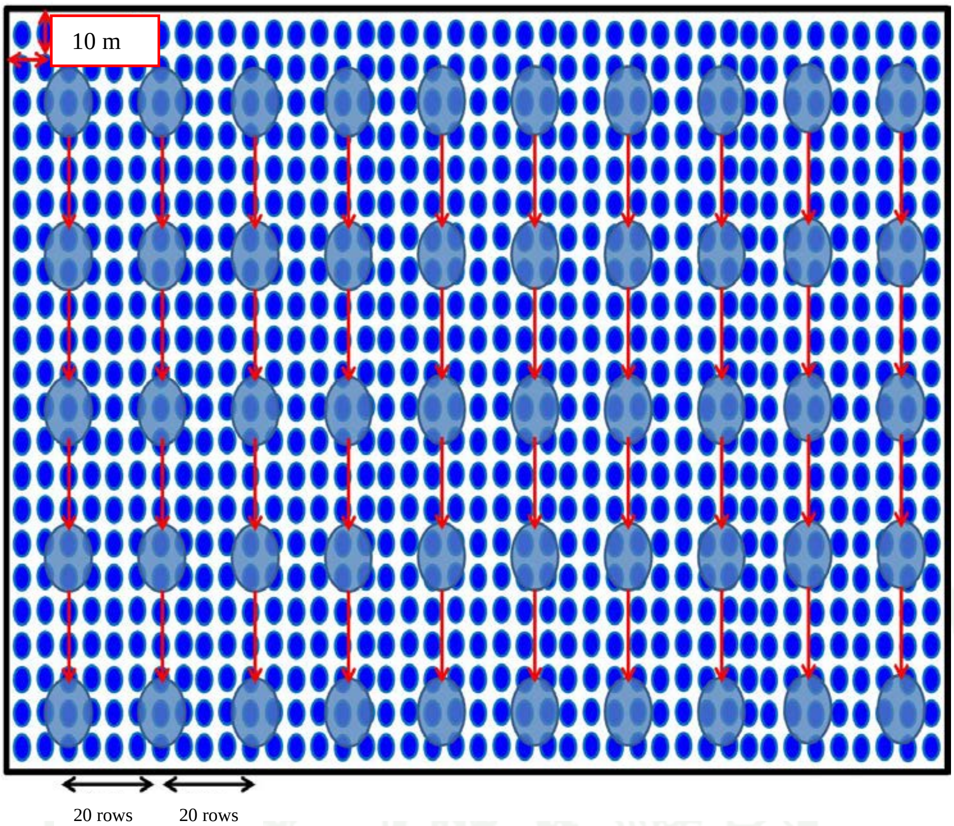


Figure 7 Layout of population sampling of the sugarcane moth borer complex and the larval parasitoids, *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) with systematic random sampling, after 1-month old of sugarcane, in area of 1.6 ha.

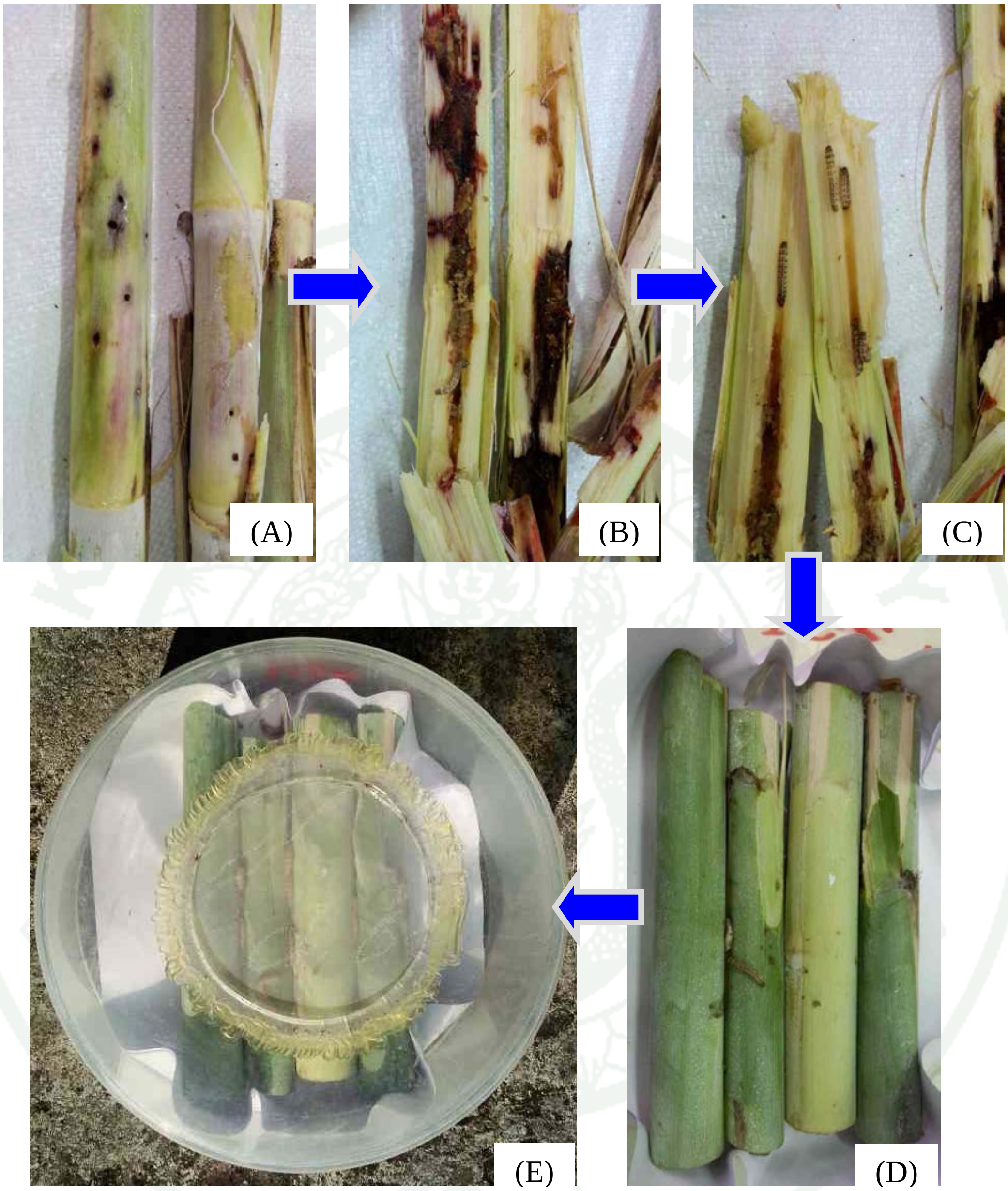


Figure 8 Collecting of the sugarcane moth borer larvae and the larval parasitoid, *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae). Stalks with the sugarcane moth borer larvae injury (A), symptoms of larval damages (B), the larvae inside the stalks (C), new young sugarcane stalks as food sources for the larvae (D), and the clear plastic tray contained the stalks and the collected larvae (E).

2. *Cotesia flavipes* release for sustainable control the sugarcane moth borer complex in the fields

There were two parts comprising in the mass rearing of the parasitoids for filed release i.e. stock cultures of the insect hosts and the parasitoids. Stock culture of *S. inferens*, the parasitoids' host, was the initial prerequisite. Subsequently the parasitoid was mass-reared on their insect hosts. The stock cultures were maintained under laboratory conditions at 27±2 ^{o}C and 75±5 % RH.

Stock Culture of *Sesamia inferens*

The stock cultures of the sugarcane moth borers method followed Suasa-ard *et al.* (2014). They were obtained by collecting mixed instar larvae of *S. inferens* from the sugarcane plots at Kamphaeng Saen district, Nakhon Pathom province. Then they were cultured in a round clear plastic box, 21.5 cm in diameter and 10 cm in height and fed with pieces of young sugarcane stalk (Figure 9). The larvae were removed to a new box and the old sugarcane stalks were replaced with the new ones every 3 days, until pupation. The pupae were kept in a new plastic box containing water-soaked filter paper for adequate moisture provision. Afterwards the newly emerged adults were relocated to another new plastic box. A piece of cotton soaked with 10% of honey was provided as food source for the adult moths. After oviposition occurred in the box, the eggs were collected with a small brush to a small round container, 5.5 cm in diameter and 1 cm in height. By this procedure on a continuous basis, it was possible to maintain a laboratory stock culture of *S. inferens*, at room conditions. The described practice was briefly showed in the flow diagram in Figure 10.

Figure 9 Stock culture of *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae) was maintained in a round shape clear plastic box 21.5 cm in diameters and 10 cm in height with pieces of young sugarcane stalks.

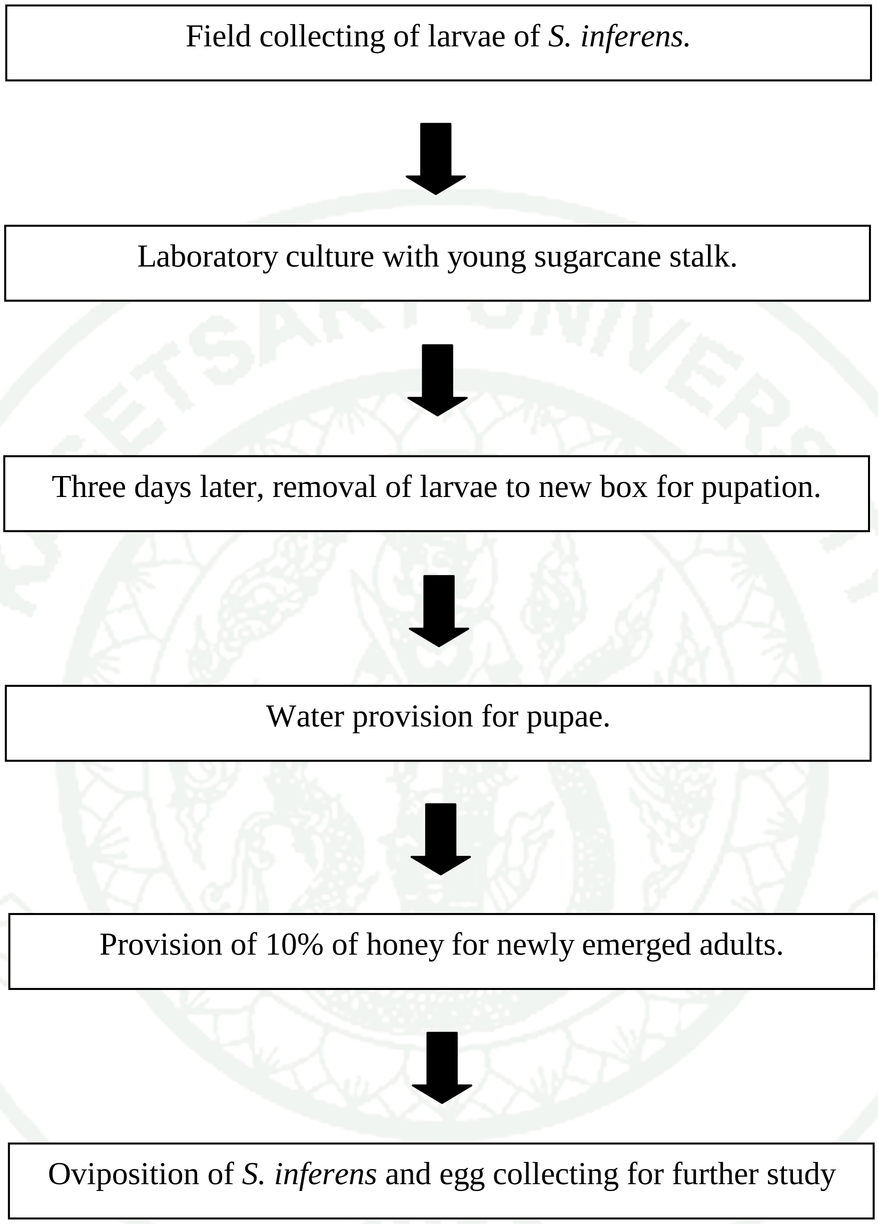


Figure 10 Flow diagram of maintaining laboratory stock culture of *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae), under room conditions (27±2 ^{o}C and 75±5 % RH) (After Suasa-ard *et al.*, 2014).

Stock culture of the larval parasitoid, *Cotesia flavipes*

Stock cultures of the larval parasitoid, *C. flavipes* method was after Suasa-ard *et al*. (2014). They were maintained by collecting its cocoons from the sugarcane moth borer larvae in the sugarcane plots at Kamphaeng Saen district, Nakhon Pathom province. The cocoons were placed in a cylindrical clear plastic box, 11 cm in diameters and 15 cm in height. The middle of the bottom of the box which was cut into a square hole, 0.2x1 cm, 100 % of honey was spread with a small brush on a piece of wax paper which was insert in the hole for adult food provision (Figure 11). A few clusters of cocoons were kept in the box until adult emergence. Adults of *C. flavipes* were reared in a clear plastic box, 11 cm in diameter and 15 cm in height and fed them with 100 % of honey for 24 hr. The fourth instar larvae were exposed to 24-h-old mated females of *C. flavipes* for parasitism. Individual larvae were held carefully with forceps and introduced in the parasitoid container through the hole located on one side. Individual larvae were exposed to *C. flavipes* females until being parasitized, which usually required 1-2 s. Only one oviposition of *C. flavipes* was allowed per larva.

After parasitization occurred, the moth larva was reared in a round clear plastic box, 21.5 cm in diameter and 10 cm in height with pieces of sugarcane stalk and they were changed every 3 days. Daily observation was done for cocoon collection. Through the wasp is an endoparasitioid, its cocoon is visually collected from the body of the parasitized moth larva. The cocoons were eventually transferred to a new plastic box for adult emergence. Following this step by step for the culturing method, the stock cultures of *C. flavipes* were laboratory propagated at 27±2 ^{o}C and 75±5 % RH. Brief flow diagram was showed in Figure 12.

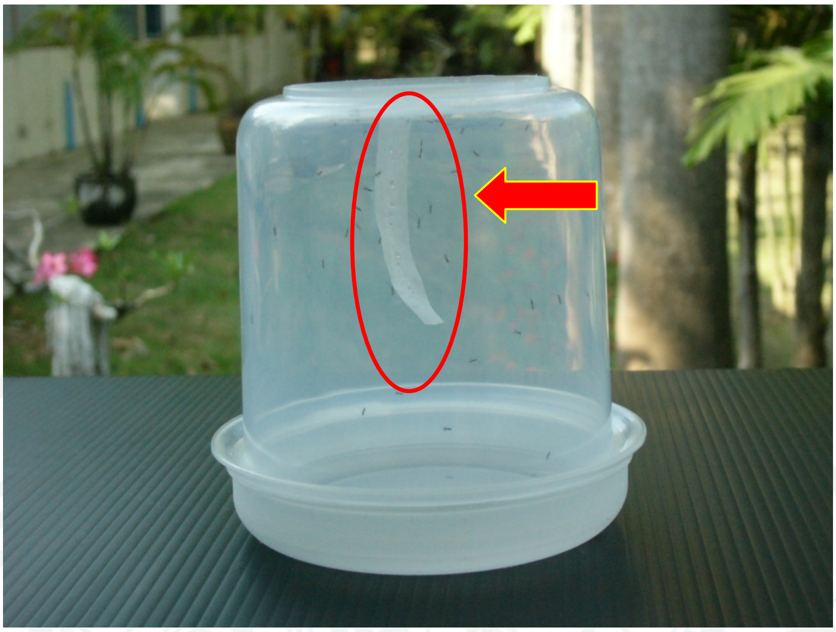


Figure 11 A cylindrical clear plastic box, 11 cm in diameter and 15 cm in height, for stock culture of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) adults. The insects were provided with a few drops of 100 % of honey on wax paper (arrow).

Field collecting of the sugarcane moth borer larvae
for cocoons of *C. flavipes*.

Provision of 100% of honey for *C. flavipes* adults.

Exposure of the 4th instar larvae of *S. inferens*
for mated females of *C. flavipes* parasitization.

Provision of sugarcane stalks for the parasitized *S. inferens*.

Daily collection of *C. flavipes* cocoons
from the parasitized *S. inferens*.

Emergence of *C. flavipes* adults.

Figure 12 Flow diagram of maintaining laboratory stock culture of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae), under room condition (27±2 ^{o}C and 75±5 % RH) (After Suasa-ard *et al.*, 2014).

Implementation plan

The variety of sugarcane planted in the experimental trials was LK 92-11. Sugarcane grows from a stem, into a tall upright plant. Unlike most stems that are planted upright, a sugarcane stem has to be furrowed and placed on its side in order for it to grow. Ordinary sugarcane planting of farmer practices can be described as follows. Firstly the mature and healthy sugarcane stalks are selected and their leaves and flowers are removed with a sharp knife or sickle. Upper leaves of the sugarcane stalks are chop off and the older stalks of the sugarcane are split into long pieces (each pieces with 2 or 3 sugarcane nodes). The soil is dug into a trench about 15-29 cm deep. The pieces of sugarcane stalk are put in horizontally, applied the 21-0-0 fertilizer (N-P-K), at the rate 625 kg/ha, covered with the soil and flew water for the newly planted sugarcane. The four-month old sugarcane plants are fertilized with 15-15-15 fertilizer (N-P-K) at the rate 625 kg/ha.

The implementation plan was conducted in three successive years, 2009-2011, and was arranged into the following action schemes: coordination with the farmers for field release, mass reared of natural enemies and field release.

Augmentative release of the larval parasitoid, *Cotesia flavipes,* for the control of the sugarcane moth borer complex in sugarcane plots

The experimental plots were located at the same locations as those described in population trend study (Figure 6). They consisted of eight plots, each covering at least 1.6 ha. Four were released with *C. flavipes* (release plots) with 1,250 adults/ha and the other four were conventional farmer practice (cypermetrin, insecticide was released one time (3-month old of sugarcane) to control the sugarcane moth borer complex) by the farmers, i.e. without the natural enemy release (non release plots). Initially, a survey of pest population was done after the emergence of sugarcane in the plots. For the moth borers sampling, systematic random sampling was applied with 50 samples/plot (0.8 ha), five stools/row and 10 rows/plot (Figure 7). The sampling unit was 1 stool/sample. The number of borer larvae found was recorded monthly till the sugarcane harvest. The release schedule of the natural enemies are shown in Figure 13.

Data collected were number of the sugarcane moth borer complex larvae infested sugarcane and parasitization of larval parasitoid, *C. flavipes* on the sugarcane moth borer complex larvae. Dependent-samples *t* test was applied for statistical analysis.

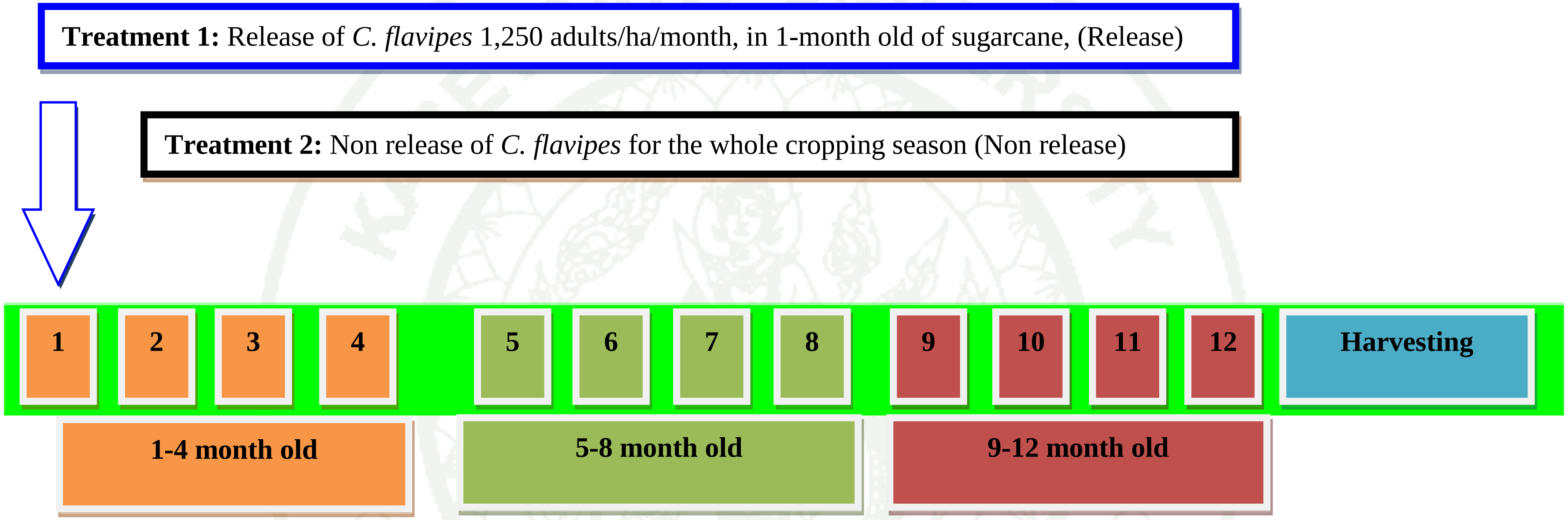


Figure 13 The release schedule of the natural enemy, *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) for augmentative biological control of the sugarcane moth borer complex in experimental plots.

3. Comparisons of yield of sugarcane in release and non release plots with *Cotesia flavipes*

The mature sugarcane stalks in release (release of *C. flavipes*) and non release (non release of *C. flavipes*) experimental plots were weighted after harvesting to evaluate the effect of damage caused by the sugarcane moth borer complex, on sugarcane yields, in three cropping seasons (2009/2010, 2010/2011 and 2011/2012). Afterwards the yield data (t/ha) were statistical analysis with Dependent-samples *t* test.

RESULTS AND DICUSSION

1. Population trends of the sugarcane moth borer complex and the larval parasitoid, *Cotesia flavipes*

According to the wide range of the collected population data of the sugarcane moth borer larvae and the cocoons of the larval parasitoid, *C. flavipes*, data transformation with log (N+1) had to be done (Fletcher *et al.*, 2005) before the implementation of graphical work to figure out the population trends.

Population trends data in the first crop (new stalk crop, 2009) and the second crop (1stratoon crop, 2010) were recorded during February to September. However, the third crop (2nd ratoon crop, 2011) the data were initially recorded in March 2011, rather than in February 2009 and 2010, because of the delay harvest. It was the primary cause of the mega flood occurred during November 2011 to January 2012 in central plain of Thailand including the experimental areas, resulting this circumstance.

In 2009 cropping season, at sampling site Kampaeng Sean district, Nakhon Pathom province (Figure 6), after the emergence of sprout sugarcane, the population of the sugarcane borers up surged abruptly in March (tillering initiation stage), because of the availability of the oviposition and food sources for the hatching larvae. It was possible that the adults moths started to lay their eggs as soon as the emergence of the bud sprouting and rooting of sugarcane plant. Generally the eggs of the moths take 7-9 days to hatch. This simply synchronized with the phenology of the plant. Afterwards, the population gave slight oscillation throughout the cropping season. Similarly population of *C. flavipes,* at the beginning of the cropping season also increased considerably parallel with its host populations. Nevertheless, the peaks of the population of the parasitoids were bimodal; the first peak was in April-May and the second peak was in July-August.

Analyses of the data of both the borers and the parasitoids in 2010 and 2011 cropping seasons at Kampaeng Sean district, Nakhon Pathom province sampling site indicated similar results of 2009 cropping season.

Interestingly, the rests of the study cropping seasons (2009-2011) and locations (U-Thong district, Suphanburi province (Figure 6 (A)); and Tha Muang district, Kanchanaburi province (Figure 6 (B)); Takhli district, Nakhon Sawan province (Figure 6 (C)) sampling sites exhibited similar outcomes. Additionally average populations of both the borers and the larval parasitoid of four sampling locations in the period of three cropping seasons also gave comparable figures (Figure 6 (D)) with 2009-2011cropping seasons, Kampaeng Sean district, Nakhon Pathom province sampling sites (Figure 14-16).

The effects of host plant phenology would have the impact of the development and dissemination of insect species (Bale *et al.*, 2002; Karuppaiah and Sujayanad, 2012). Finding of this study revealed that the fluctuation of the population density of these borers was dependent upon the phenology of sugarcane which is similar to those reported on *C. flavipes* parasitized *C. infuscatellus* (Khan *et al.*, 2013; Muhammad *et al*., 2014), and *D. saccharis* (Rossi and Fowler, 2002). Meanwhile, the population of *C. flavipes* relied on the population of the sugarcane moth borer complex in the fields as a numerical response (Price, 1975). In some extent, the larval parasitoid, *C. flavipes,* were able to regulate steadily the moth borer populations.

Since the up-down cyclic of population of the insects host (the sugarcane moth borer complex) was reliant upon the phenology of the host plant (sugarcane) being damaged and the insects host influenced the number of the parasitoid (*C. flavipes),* it unmasked the tritrophic level, plant-insect-parasitoid interactions (Leutourneau, 1988).

A number of literature have addressed the attractions of the parasitoid to its insects host in tritrophic system (e.g. Büchel *et al*., 2011; Cappuccino, 2008; Colazz *et* *al*., 2004; De Moraes *et al*., 1998; Dicke, 1994; Erb *et al*., 2010; Gross *et al.,* 2005; Prütz *et al*., 2004). One of the main factors is the volatile compounds that arouse the parasitoids. Examining plant-insect host-parasitoid in tritrophic interactions has been a great deal of interest over the past 25 years (Dicke, 1994; De Moraes *et al*., 1998). The presence of the larval parasitoid, *C. flavipes* in this study might be volatile compounds released by sugarcane plant (after the emergence of the talk in bud sprouting and rooting stage and it being damaged by the moth borers) incited the oviposition of the larval parasitoid, *C. flavipes*.

Some experiments were conducted to scrutinize these relationships. The egg parasitoid, *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) responded to volatiles emitted by leguminous plants induced by feeding and oviposition activity of the green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae) (Colazz *et al*., 2004). Erb *et al*. (2010) pointed out that herbivore-infested plants emit volatile compounds that highly attract to the parasitoids; also in their finding, the parasitoid *Cotesia margniventris* (Cresson) (Hymenoptera: Braconidae) strongly preferred volatiles of plant infested with its host *Spodoptera littoralis* (Boisdual). Further study of Büchel *et al*. (2011) indicated that not only the feeding injury on elm made by the insect herbivore, the elm leaf beetle *Xanthogaleruca luteola* (Müller) (Coleoptera: Chrysomelidae), but also the leaf beetle egg deposition which also made wound, were able to release volatile compounds that attract the eulophid parasitoid, *Oomyzus gallerucae* (Fonscolombe) (Hymenoptera: Eulophidae). Hence, it is possible that after the sugarcane borers laid their eggs on the sugarcane which also made some wounds, would emit some volatile compounds to intrigue the larval parasitoid, *C flavipes* to lay eggs on the larvae of its host as soon as the emergence of the sugarcane plants.

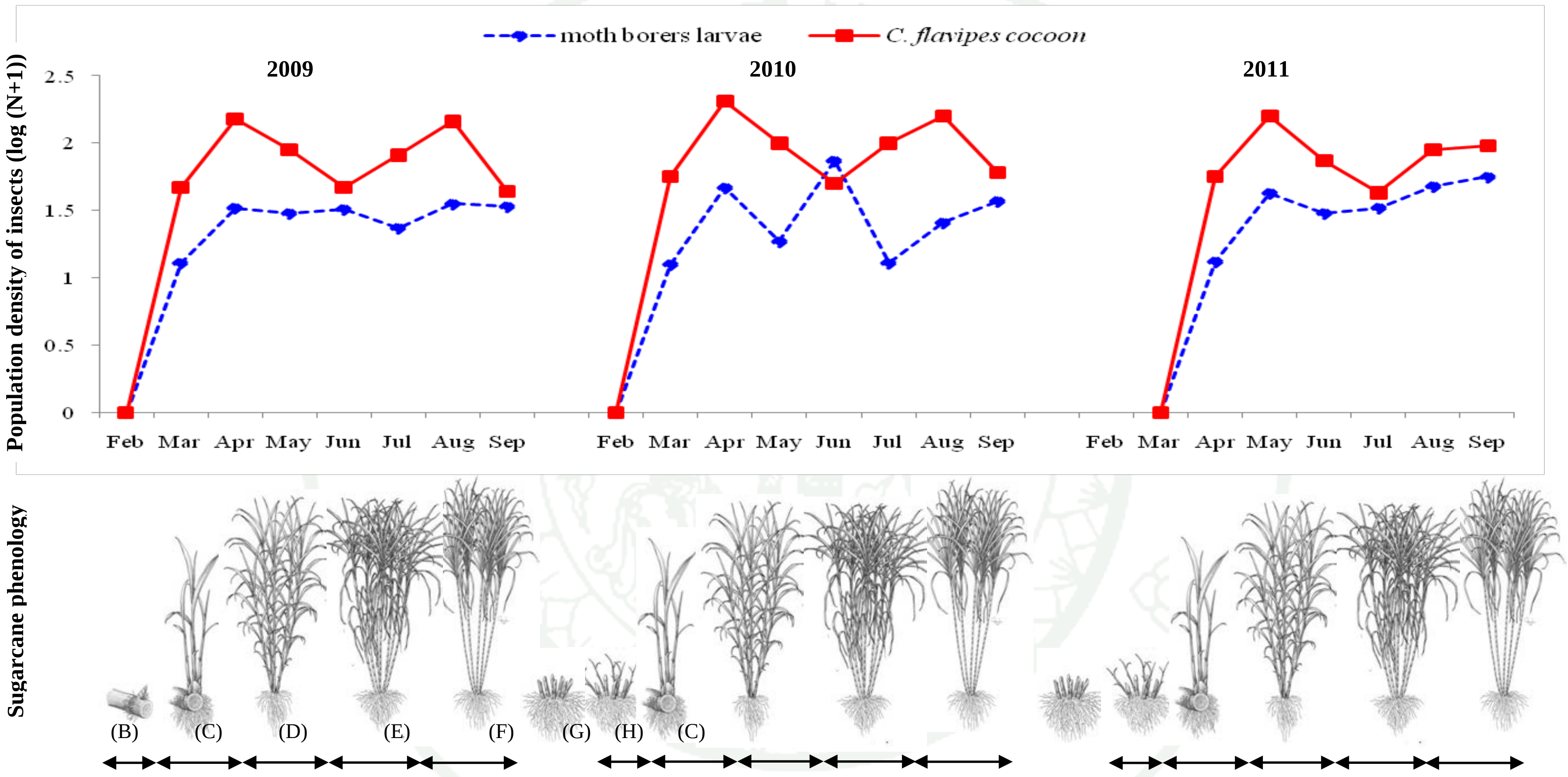


Figure 14 Population trends of the sugarcane moth borer complex and the larval parasitoid, *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) in sugarcane plots at Kamphaeng Saen district, Nakhon Pathom province during February 2009 to September 2011.

Source: Sugarcane phenology modified from Cheavegatti-Gianotto *et al.* (2011)

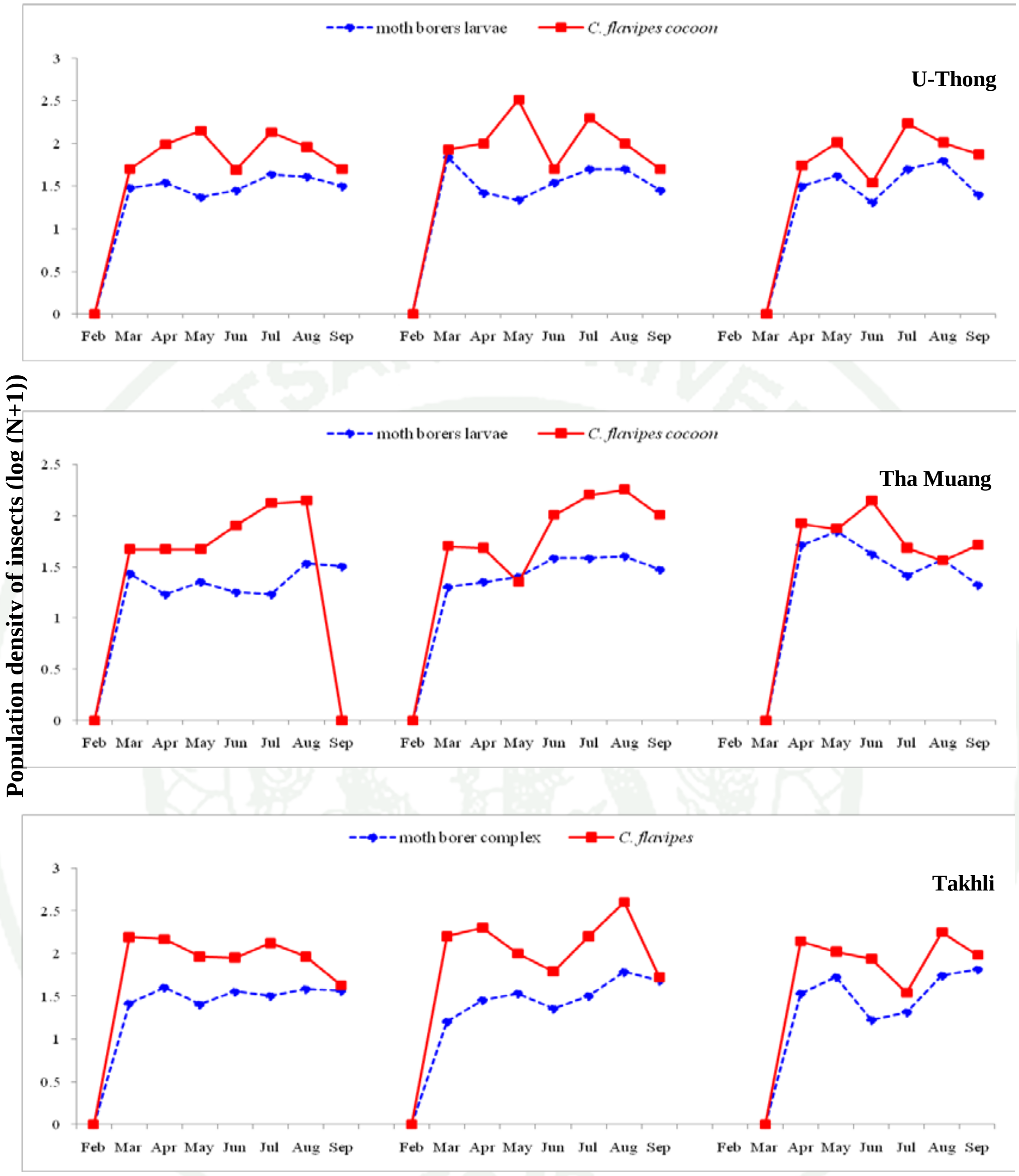


Figure 15 Population trends of the sugarcane moth borer complex and the larval parasitoid, *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) in sugarcane plots at U-Thong district, Suphanburi province, Tha Muang district, Kanchanaburi province and Takhli district, Nakhon Sawan province during February 2009 to September 2011 (See sugarcane phenology in Figure 14).

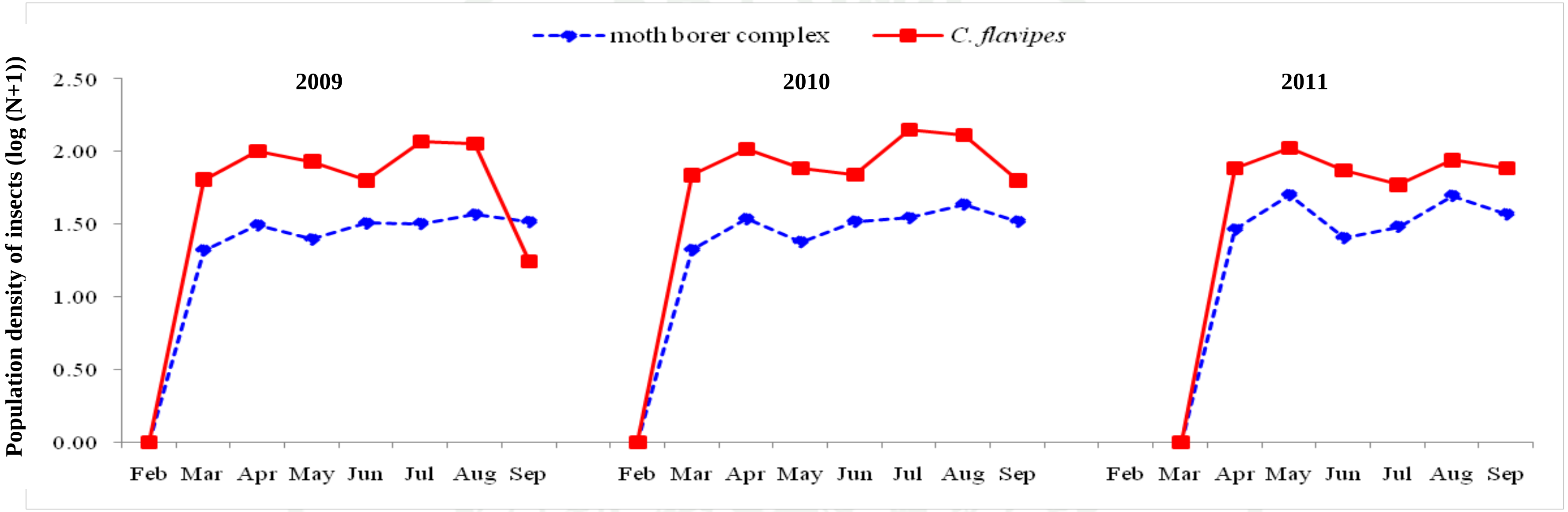


Figure 16 Population trends of the sugarcane moth borer complex and the larval parasitoid, *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae), average of 3 cropping seasons at 4 locations during February 2009 to September 2011 (See sugarcane phenology in Figure 14).

2. *Cotesia flavipes* release for sustainable control the sugarcane moth borer complex in the fields

Prerequisite of the mass-production of the natural enemies for field release is laboratory cultures of the insect hosts which will support the raising of the natural enemies.

Stock cultures of *Sesamia inferens* and *Cotesia flavipes*

Following the methods (Figure 10 and 12) of mass-rearing, both the insect host, *S. inferens*, and its larval parasitoid, *C. flavipes*, a total number of 500 larvae of the insect host and 20,000 adults of the larval parasitoid were mass-produced monthly for the augmentative release plan in the test locations.

Augmentative release of the larval parasitoid, *Cotesia flavipes,* for the control of the sugarcane moth borer complex in sugarcane plots

A series of three years experiment were conducted, beginning in 2009 and ending in 2011, to evaluate the infestation of the sugarcane moth borer complex lavae and parasitization of *C. flavipes* on the sugarcane moth borer complex larvae in release and non release plots.

C. flavipes adults were augmented to control the sugarcane moth borer complex, primary interest was to monitor the infestation of sugarcane planting with the sugarcane moth borer larvae, in 2009 cropping season. Mean values of infestation in release and non release plots cropping seasons and locations, Kamphaeng Saen district, Nakhon Pathom province, U-Thong district, Suphanburi province, Tha Muang district, Kanchanaburi province, and Takhli district, Nakhon Sawan province demonstrated that the infestation of the sugarcane moth borer larvae in release plots in all locations began in tillerting initiation stage (3-month old planting). Space and time of the results is shown in Table 1 and Figure 11-13.

The infestation of the sugarcane moth borer complex of release and non release plots began in tillering initiation stage of sugarcane (2 to 3-month old of sugarcane). *C. flavipes*, in release plots could reduce and supress population infestation of the sugarcane moth borer larvae in 4 to 5-month old of sugarcane (in April to June) until harvesting but the infestation of the sugarcane moth borer larvae showed two peaks, the first one in the tillering initiation to early intense tillering and the second one during last beginning of matuaration to manufactorable stalk in optimal sucrose concentration. Results obtained showed similar trends at all locations and cropping seasons study (Figure 17-19).

Although intensities of infestation from year to year fluctuated, some consistencies were observed. The percentage of infestation of release plots at Tha Muang, U-Thong and Takhli (1.73±0.47-5.55±2.03) were significant lower than non release plots (5.38±3.92-9.82±2.34) ($P<0.01$). Other cropping seasonals of the same location showed similar results, but it was different at Kamphaeng Saen. In the 1st cropping season (new planting) and 3rd cropping season, the infestation of release and non release plots were non-significant different ($P>0.05$) (Table 1).

In terms of percentage of parasitism of *C. flavipes* in both treatments were similar. The parasitism of release plots continually increased (% parasitization range 13.65±7.35-26.66±8.63) and highly significant different ($P<0.01$) than non release plots (% parasitization range 2.91±2.72-6.24±2.73) (Figure 20-22 and Table 2).

The regulation of the population of the sugarcane moth borer complex was triggered by the sugarcane plant phenology as those described in the previous section of population trends study, therefore, it was significant on the population trends of the parasitoids both in release and non release plots.

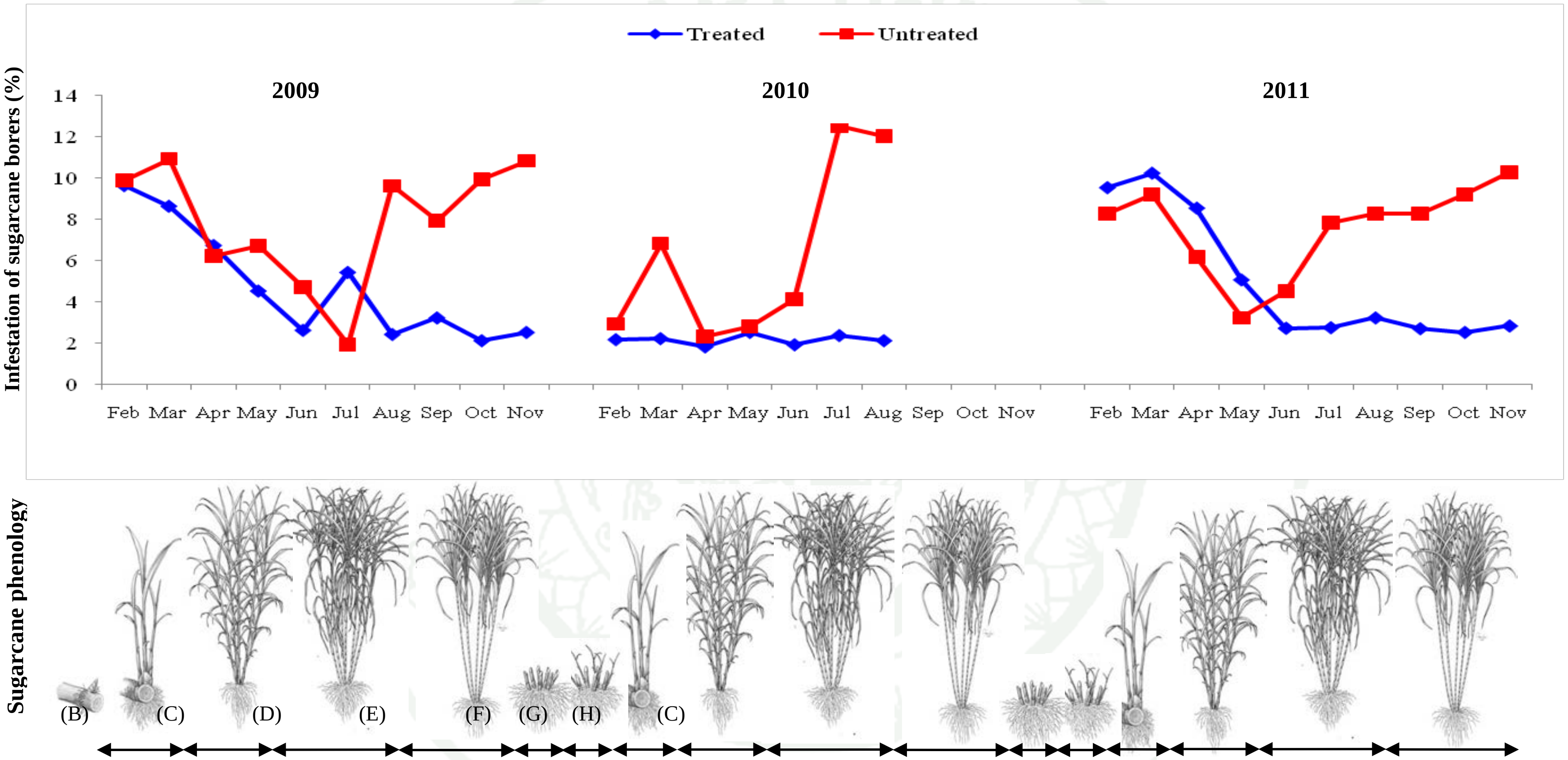


Figure 17 Infestation by the sugarcane moth borers complex in experimental plots at Kamphaeng Saen district, Nakhon Pathom province during February 2009 to September 2011.

Source: Sugarcane phenology modified from Cheavegatti-Gianotto *et al.* (2011)

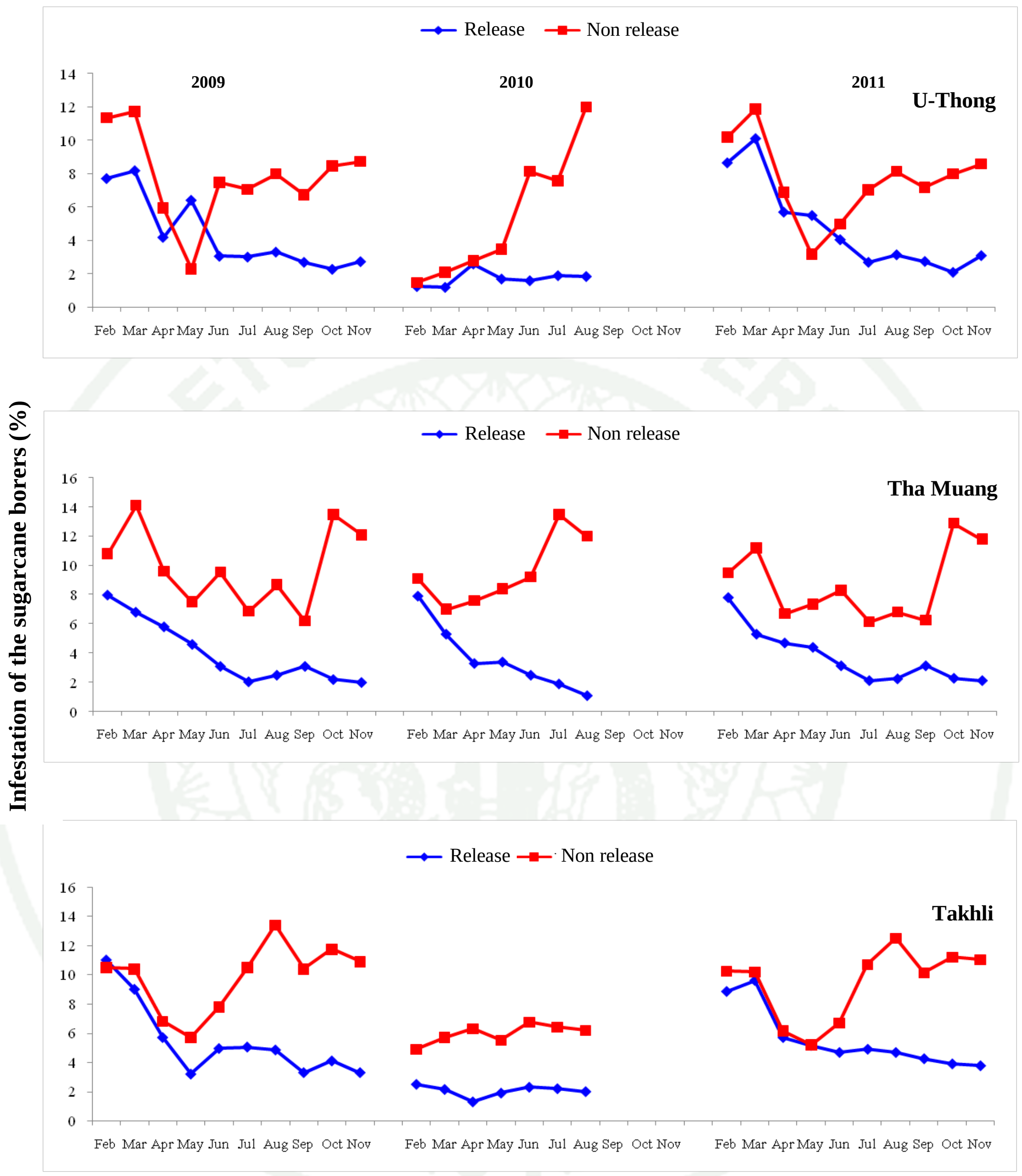


Figure 18 Infestation by the sugarcane moth borer complex in experimental plots at U-Thong district, Suphanburi province, Tha Muang district, Kanchanaburi province and Takhli district, Nakhon Sawan province during February 2009 to September 2011 (See sugarcane phenology in Figure 17).

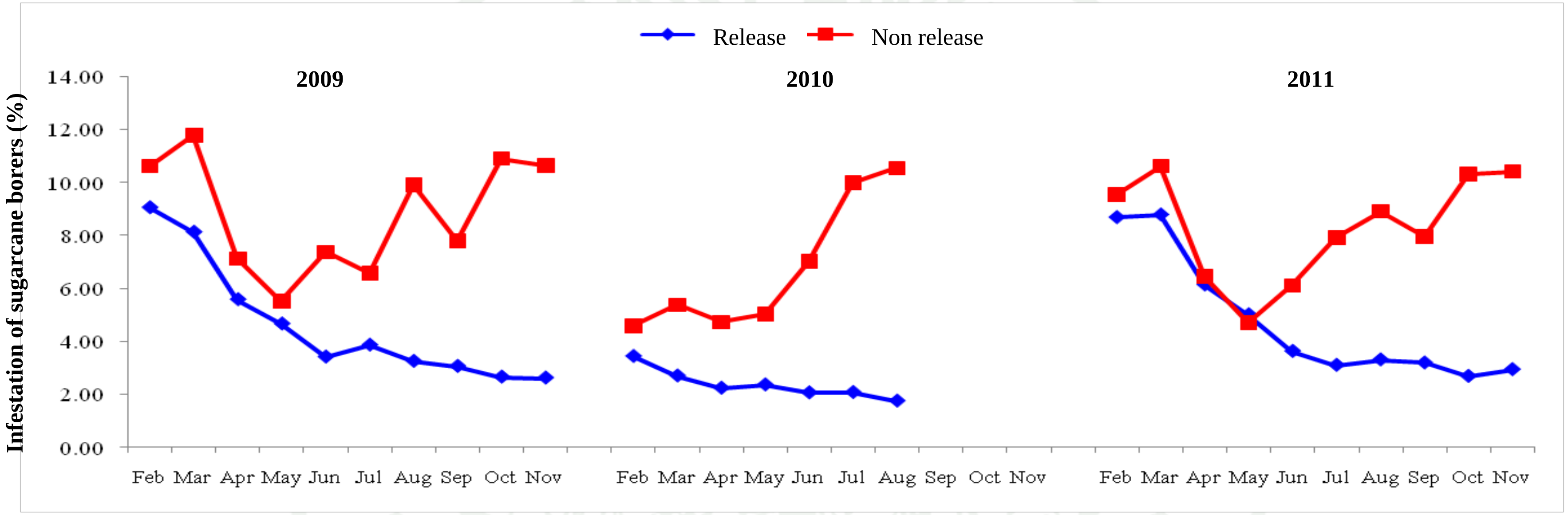


Figure 19 Infestation of the sugarcane moth borer complex and the larval parasitoid, *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae), average of 3 cropping seasons at 4 locations during February 2009 to September 2011 (See sugarcane phenology in Figure 17).

Table 1 Percentage of infestation (Mean±S.D.) of the sugarcane moth borer complex larvae in release and non release plots at4 locations and 3 cropping seasons, during 2009-2011.

Cropping seasons	**Experimental plots**							
	Kamphaeng Saen		**U-Thong**		**Tha Muang**		**Takhli**	
	Release	**Non release**	**Release**	**Non release**	**Release**	**Non release**	**Release**	**Non release**
Cropping season I (2009)	4.90±3.30	7.20±3.01	4.36±2.22	7.63±2.42	4.01±2.16	8.92±2.61	5.45±2.59	9.82±2.34
Cropping season II (2010)	2.14±0.24	6.20±4.39	1.73±0.47	5.38±3.92	3.63±2.30	9.54±2.36	2.05±0.38	5.96±0.63
Cropping season III (2011)	4.99±3.16	7.50±2.22	4.78±2.71	7.62±2.44	3.37±1.84	8.70±2.50	5.55±2.03	9.41±2.46
t test	**		**		**		**	

** Means within location, within cropping seasons were highly significant different (P<0.01) with Dependent-samples *t* test (n=4).

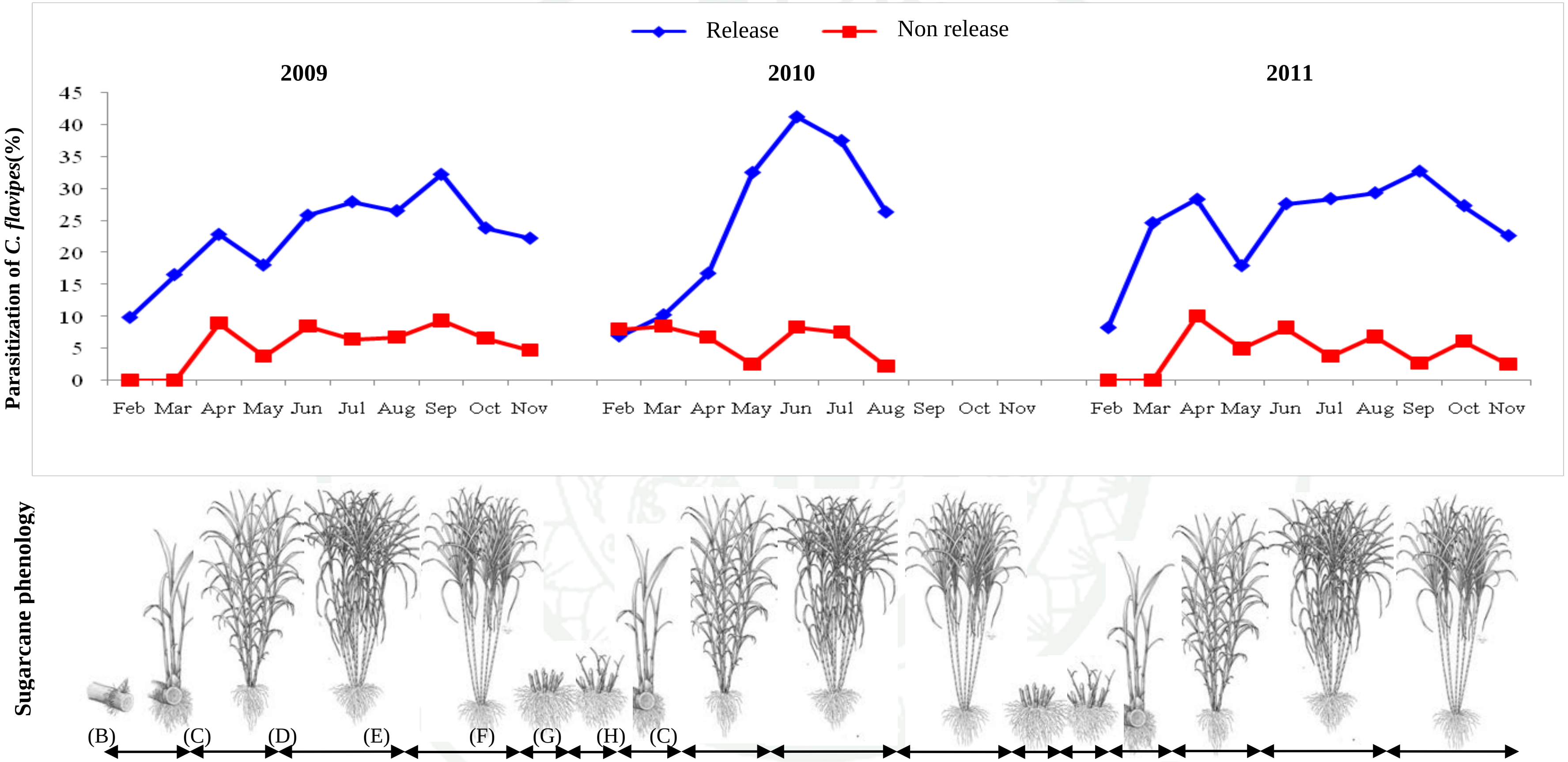


Figure 20 Parasitization of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) on the sugarcane moth borer complex in experimental plots at Kamphaeng Saen district, Nakhon Pathom province during February 2009 to September 2011.

Source: Sugarcane phenology modified from Cheavegatti-Gianotto *et al*. (2011)

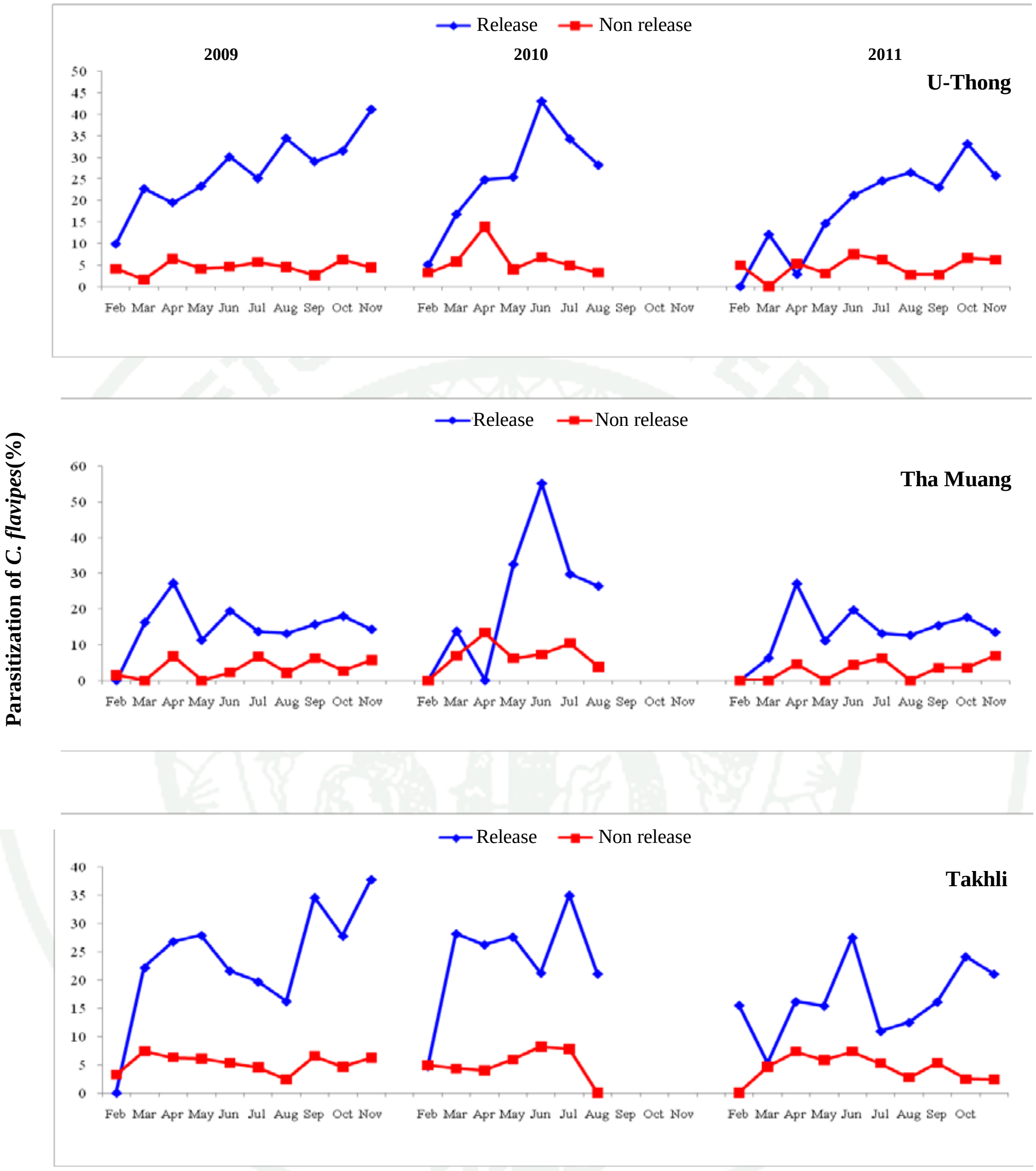


Figure 21 Parasitization of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) on the sugarcane moth borer complex in experimental plots at U-Thong district, Suphanburi province, Tha Muang district, Kanchanaburi province and Takhli district, Nakhon Sawan province during February 2009 to September 2011 (See sugarcane phenology in Figure 20).

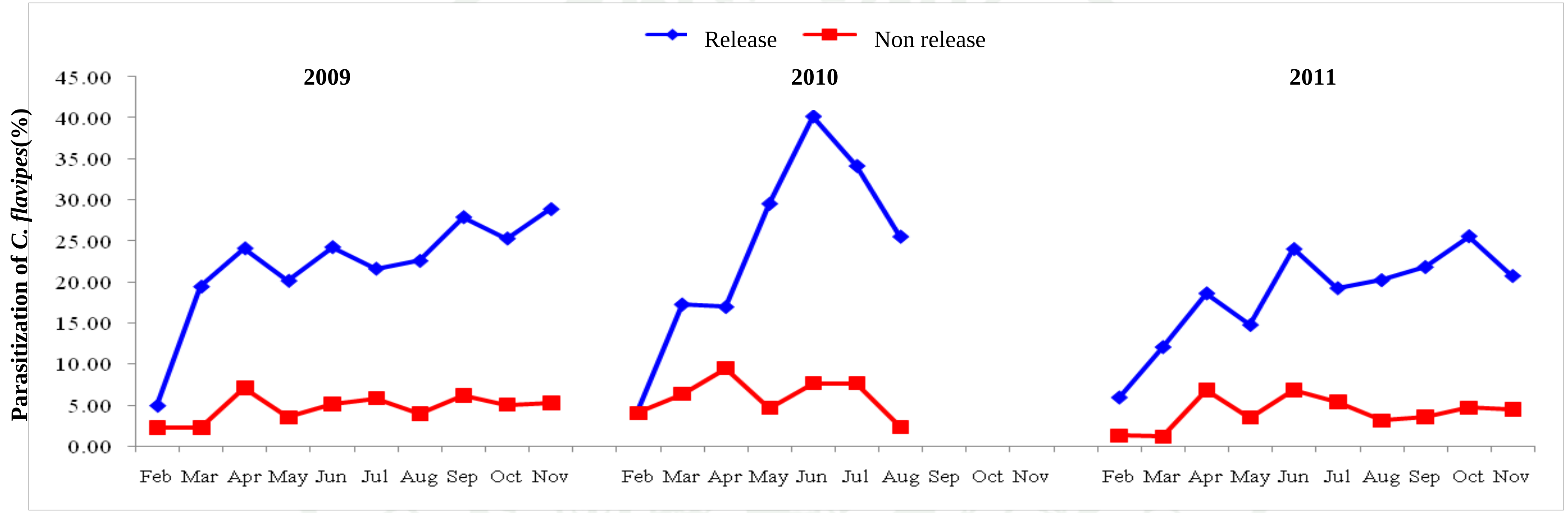


Figure 22 Parasitization of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) on the sugarcane moth borer complex, average of 3 cropping seasons at 4 locations during February 2009 to September 2011(See sugarcane phenology in Figure 20).

Table 2 Percentage of parasitization (Mean±S.D.) of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) on larvae of the sugarcane moth borer complex in release and non release plots at 4 locations and 3 cropping seasons, during 2009–2011.

Cropping seasons	**Experimental plots**							
	Kamphaeng Saen		**U-Thong**		**Tha Muang**		**Takhli**	
	Release	**Non-release**	**Release**	**Non-release**	**Release**	**Non release**	**Release**	**Non-release**
Crop season I (2009)	24.41±6.92	4.72±3.39	26.66±8.63	4.53±2.16	14.90±6.86	3.07±2.90	23.46±10.55	4.31±2.31
Crop seasonII (2010)	24.47±13.48	6.24±2.73	25.36±12.12	5.96±3.74	14.27±7.11	2.99±2.81	23.44±9.51	5.01±2.75
Crop season III (2011)	24.69±7.07	4.50±3.34	18.34±10.72	4.55±2.33	13.65±7.35	2.91±2.72	16.45±6.46	4.30±2.37
t test	**		**		**		**	

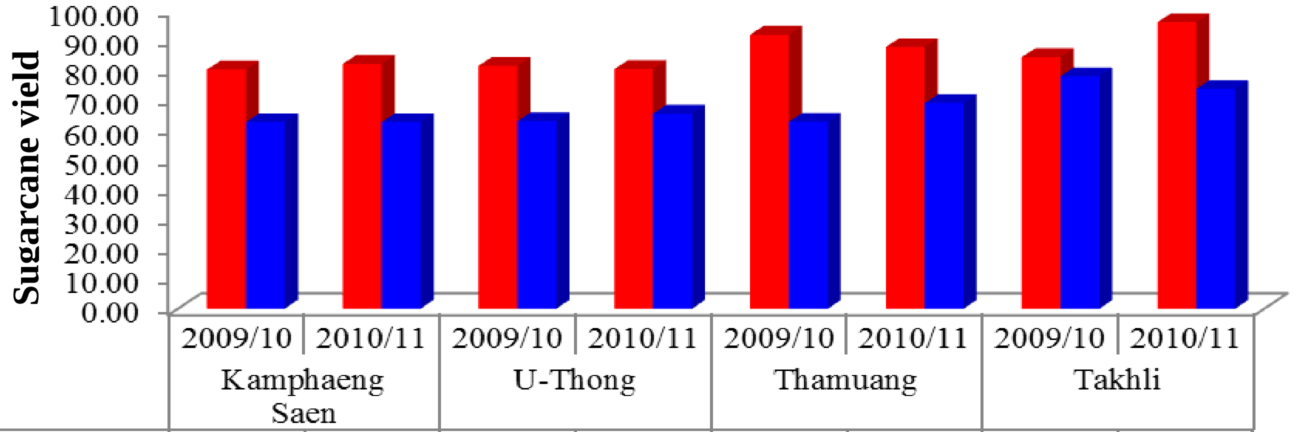
** Means within location, within cropping seasons were highly significant different (P<0.01) with Dependent-samples *t* test (n=4).

3. Comparisons of yield of sugarcane in released and non-released plots with *Cotesia flavipes*

This study were conducted upon 3 years (seasonal 2009/2010, 2010/2011 and 2011/2012) but due to a mistake on the collaborative with the farmer. As a result that, the data of sugarcane product could be collected only 2 cropping seasonals (seasonal 2009/2010 and 2010/2011).

Observations on yearly basis were recorded on impact of the sugarcane moth borer complex infestation on sugarcane yield of all locations during 2009/2010 and 2010/2011. It was observed that in 2009/2010 cropping season, sugarcane production of release experimental plots of all locations (yield range 80.13-91.75 t/ha) significantly ($P<0.05$) greater than non release experimental plots (yield range 62.50-77.88 t/ha). The yield production analysis of experimental plots in Nakhon Sawan were different from another locations because yield of release and non release plots were non-significant ($P>0.05$). Just as the sugarcane production in 2010/2011 cropping season, release experiment plots at all location were 1.5 times significantly better ($P<0.05$) than non release treatment (Figure 23).

It revealed that sugarcane yield obtained from the release plots (average 84.38 t/ha), higher than both central region and National Thailand sugarcane yield, 69.61 and 66.59 t/ha (Office of Agricultural Economics, 2010), respectively, as well as in non release plots (average 66.45 t/ha (Office of Agricultural Economics, 2010)). Additional in 2010/2011 it also gave similar results as those in 2009/2010, the average sugarcane yield in release plot, non release plot, central region (Office of Agricultural Economics, 2011) and Nation Thailand (Office of Agricultural Economics, 2011) yield were 86.47, 67.59, 66.88 and 63.91 t/ha, respectively.



	Kamphaeng Saen		U-Thong		Thamuang		Takhli	
	2009/10	2010/11	2009/10	2010/11	2009/10	2010/11	2009/10	2010/11
Release	80.13	82.00	81.38	80.25	91.75	87.69	84.25	95.94
Non release	62.50	62.50	62.81	65.38	62.63	68.88	77.88	73.63
t test	*	*	*	*	*	*	ns	*
yield increased (%)	28.20	31.20	29.55	22.75	46.51	27.31	8.19	30.31

[*] Means within the same group of bar chart were significantly different (P<0.05) with Dependent-samples *t* test.

[ns]Means within the same group of bar chart were not significantly different (P>0.05) with Dependent-samples *t* test.

Figure 23 Average sugarcane yields (t/ha) in 2009/2010 and 2010/2011 cropping seasons in release (with natural enemies) and non release plots (without natural enemies) in 4 locations.

It significant showed that augmentative release of *C. flavipes* effectively control the sugarcane moth borer complex decreasing percentage of the sugarcane moth borer complex infestation, increasing percentage of parasitzation and yielding higher when augmentative release was performance.

Results of the field studies revealed that under non release area of *Trichogramma chilonis* Ishii, the infestation of borers was 11.65 % and in the release area it was 2.74 % (Hussnain *et al.*, 2007). Another example is Brazil which successfully controlled *Diatraea saccharalis* by using two parasitoids: *C. flavipes* and *Trichogramma galloi* Zucchi (Botelho *et al.*, 1992). This is noteworthy as it is an example where key parasitoids are used in working togeter. It demonstrates that it preferred additional pressure on stem borer populations (Goebel *et al.*, 2010).

The initial achievement by releasing the larval parasitoid, *C. flavipes*, provided more effective control the sugarcane moth borer complex in many countries such as *C. infuscatellus* (Fatima *et al.*, 2009), *Diatraea saccharalis* (Fuchs *et.al.*, 1979), and *Eoreuma loftini* (Legaspi *et al.*, 1997), which shown the infestation of these insects reduced from moreover 10 % to less than 3 %.

CONCLUSION AND RECOMMENDATIONS

Conclusion

The results of the research has highlighted the field population trends of the sugarcane moth borer larvae and their natural enemies, *C. flavipes*, in four provinces and three consecutive cropping seasons (including new stalk crop, 2009, 1st ratoon crop, 2010 and 2nd ratoon crop, 2011). The plant phenology may be crucial factor affecting the populations of the sugarcane moth borer complex and the parasitism. The larval parasitoid *C. flavipes* gave significant result to regulate the sugarcane moth borer complex population in field, subsequently it suggests to be a good scope of the augmentative biological control of the sugarcane moth borer complex by *C. flavipes.*

Afterwards augmentative release of the parasitoid, *C. flavipes* was done by comparing release and non release of the parasitoids under sugarcane field condition. All experimental plots with release of the parasitoid gave lower percentage of the sugarcane moth borer complex. It is in line with the plots with the release of the parasitoids generally gave higher parasitization than those with non release plots. Finally sugarcane in release plots, by average, provided significant higher yield than those non release ones.

Recommendations

For further research works possibly focus on some missing points in this research.

Individual population trend of the five species of the sugarcane moth borers should be conducted separately. Additional sugarcane plant and the sugarcane moth borer complex phenology also can be determined. In this way, field augmentative release of the parasitoids will be clearly defined; hence known species damage on what plant developmental stages can be confirmed.

Importantly future research on augmentative biological control of the sugarcane moth borer complex must identify the systems in which augmentative releases can work in a cost-effective manner, as well as residues of insecticide applied in farmers' sugarcane plots should be compared with the non chemical ones (with release of the parasitoids). Results to be obtained from the study should reveal the utility of this approach.

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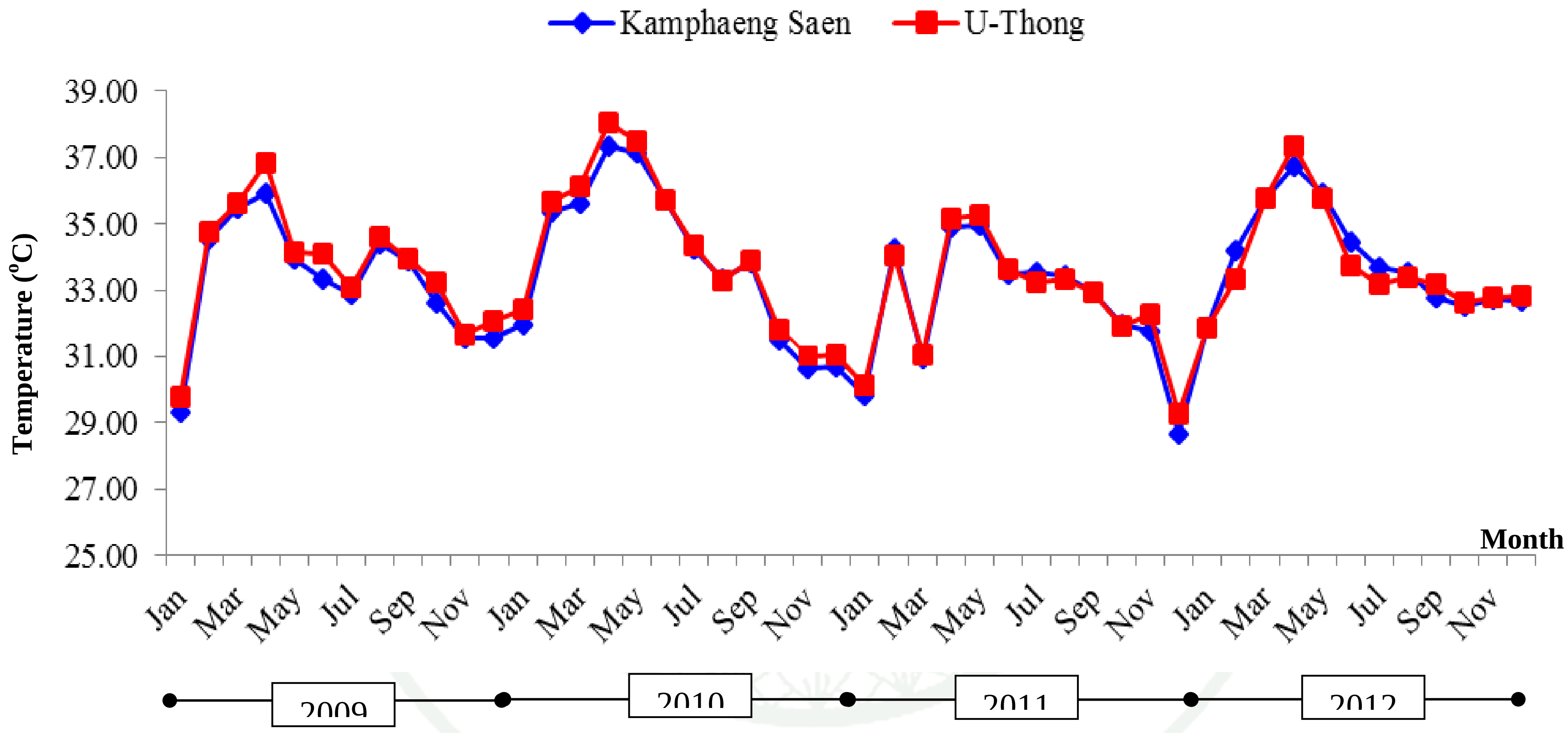
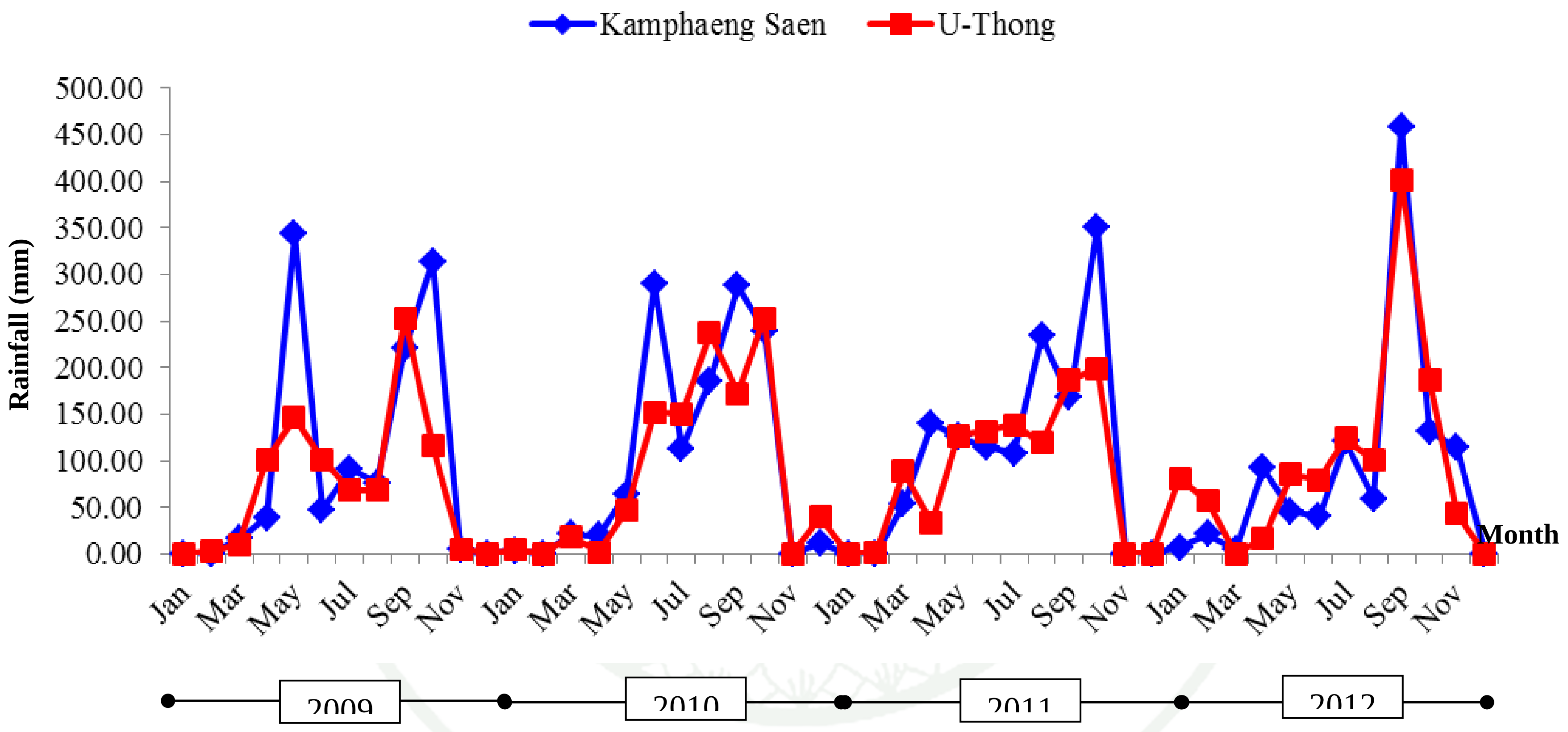
APPENDIX



Appendix Figure A1 Annual temperature for Kamphaeng Saen district, Nakhon Pathom and U-Thong district, Suphanburi (2009-2012).



Appendix Figure A2 Climatological series of annual rainfall (mm) for Kamphaeng Saen district, Nakhon Pathom and U-Thong district, Suphanburi (2009-2012).

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PUBLICATION : Kamata, N., K. Iguchi, S. Sanguansub and **T. Maneerat**. 2014. Infestation by insect borers on *Betula maximowicziana* following successive years of severe defoliation by *Caligula japonica* (Lepidoptera: Saturniidae) with special reference to *Xyleborus seriatus* Blandford (Coleoptera: Curculionidae: Scolytinae). **Forest Pests** 63(5): 18-22.