INSTITUTE OF PLANT PATHOLOGY AND PLANT PROTECTION
GEORG-AUGUST-UNIVERSITY-GOETTINGEN

New Aspects of Biological Control of *Helicoverpa armigera* in Organic Cotton Production

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By
Nabil Mohamed Emara El-Wakeil

M.Sc. Cairo University, Cairo, Egypt
Born in Egypt

Göttingen, Germany

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Referee: Prof. Dr. Stefan Vidal

Co-referee: Prof. Dr. Teja Tscharntke

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Abbreviations

(P: H)	Parasitoid female: Host egg
EW	Epicuticular Wax
LOX:	Lipoxygenase Pathway
BT/JA	BT Cotton /Jasmonic Acid
BT/NJA	BT Cotton /Non-Jasmonic Acid
NBT/JA	Non-BT Cotton /Jasmonic Acid
NBT/NJA	Non-BT Cotton / Non-Jasmonic Acid
GC	Gas Chromatography
GC MS	Gas Chromatography Mass Spectra
NIST	National Institute of Standards and Technology

General Introduction

Many people consider cotton to be the purest fibre on earth. Or as a recent ad campaign suggests, "The fabric of our lives." In fact, cotton cultivation inflicts a heavy toll on the environment. But a small number of farmers are now growing cotton "organically." This means they eliminate toxic chemicals in every step of the growing process, by emphasizing natural, biological methods which have far less impact on the environment (Chaudhry 2000).

Cotton is an important fiber crop that contributes significantly to Egyptian economy. Cotton has been grown in Egypt since about 300 BC. Production for export started around 1830 and was improved with the introduction of foreign cultivars around 1860. Egyptian cotton is known over the world for its long to extra-long staple. In the 1970s, 75 % of the world's long staple cotton was produced by Egypt, which at that time produced about 5% of total global production. Textile manufacturing is the single largest industry in the country and textile is the most important export commodity (Myers & Stolton 1999).

The Problem with Conventional Cotton Production

Cotton provides about half of all global fibre requirements. In addition to the ten big producers (USA, China, India, Pakistan, Uzbekistan, Turkey, Brazil, Australia, Greece and Egypt) cotton is produced in over 60 countries. It is an important source of cash for millions of small farmers and contributes significantly to the national economy of many developing countries (USDA 2002). The size of the global cotton-growing area has not changed much since the 1930s but average yields have increased threefold through the intensive use of synthetic chemicals, irrigation and the use of higher-yielding plant cultivars. Conventional cotton is very prone to insect attacks and large quantities of the most toxic insecticides are used in its production (Cramer 1998). Cotton accounts for about 25% of the global insecticides market by value and about 10% of the pesticides market. The intensive use of toxic insecticides and other toxic chemicals in cotton has caused serious health and environmental impacts, including farmer and farm worker poisonings, water contamination and bird and fish kills (Myers 2000). Additional to reducing soil fertility, there are also salinization, a loss of biodiversity, water pollution, adverse changes in water balance, and resistance-insect to pesticides. Social costs include, health problems related to the heavy use of acutely toxic pesticides (Maeder *et al.* 2002)

Organic cotton

Cotton is grown in a farm system. However obvious this statement may be, it is an approach that is much neglected by cotton specialists who tend to concentrate on cotton alone. Success in organic growing is influenced by the other crops in the rotation, by the presence of farm animals, by the availability of land and labor and by the level of training of the farmer, his or her family and other workers. Growing cotton as a single or mono crop has created many of the problems associated with conventional cotton production (Alfoeldi 2001). Successful farming requires a holistic approach. This is where the word ‘organic’ originates. Sustainable organic farming is not just about organic fertilizers and certainly not about organic pesticides. Many define organic farming by a simple formula: No synthetic fertilizers + no synthetic pesticides = fewer yields x higher price (Fliessbach and Maeder 2000).

Organic cotton is now grown in more than 12 countries but still represents only a tiny fraction of the total global cotton production - less than 0.1%. According to recent surveys, there was a steady increase of organic cotton production throughout the early 1990s. Production dropped in 1995/6 but appears to have increased dramatically between 1997 and 1999 - up 80% to around 14,000 (metric) tonnes. The biggest producers in 1999 were Turkey, the United States, India, Peru and Egypt. Smaller experimental projects are also expanding in size and number (USDA 2002).

Organic Cotton Can Bring Health and Environmental Improvements

So serious are the negative impacts of chemical-intensive agricultural production that momentum for change has grown considerably in recent years. The trend toward more environmentally friendly production methods is supported by a cultivar of interests including farmers wanting to escape the chemical treadmill, enlightened companies under pressure of increased environmental regulation and competition, and informed consumers calling for greater social and environmental accountability. The burgeoning consumer interest in organic food production has now expanded into other areas of organic production including cotton fibre produced in organic systems (Maeder *et al.* 2002)

Organic cotton is produced in organic agricultural systems that produce food and fibre according to clearly established standards. Organic agriculture prohibits the use of toxic and persistent chemical pesticides and fertilizers, as well as genetically modified organisms (Alfoeldi 2001). It seeks to build biologically diverse agricultural systems, replenish and maintain soil fertility, and promote a healthy environment (Stokstad 2002).

Organic cotton in Egypt

Organic cotton production started in Egypt in 1991 on a small area of 20ha in the Gharbia governorate (120 KM north Cairo) in the Nile Delta. It was a successful trial and in the following year more than 120 ha were under organic cotton. By 1996, there were more than 400 ha of organic cotton in Fayoum and Kliubia (70 KM north Cairo) and in Abou Matameer in the north of Delta. The main incentive for farmers to convert to organic production is the promise of higher prices for their products. Farmers in Egypt need the full three-year conversion period to establish well-developed organic farm systems. Cotton is generally grown by small-scale farmers. From an environmental point of view, organic and biodynamic growing has clear advantages in Egypt. In areas under organic cotton production systems, field workers and villagers are no longer exposed to pesticides; drainage water and surface water run-off are no longer contaminated (Myers and Stolton 1999).

Helicoverpa armigera

American Bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is one of the most serious insect pests in many parts of the World. Research now focuses on alternative control methods such as cultural and biological control, and host plant resistance as the major components of integrated pest management programs (Shanower *et al.* 1997). Promising biocontrol agents against *Helicoverpa* are egg parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae). These parasitoids have been successfully released in biological control programs against *Helicoverpa* for example in cotton (Romeis and Shanower 1996). However, many questions remain to be answered: *Trichogramma* and *Chrysoperla carnea* are the effective natural enemies for controlling eggs and larvae of *H. armigera*. The combination between *Trichogramma* spp. and *C. carnea* could be an option for increasing the mortality of the American bollworm.

Trichogramma species

The egg parasitoid, *Trichogramma* species have been widely used as biocontrol agents in inoculative release programs against many Lepidopteran pests; however, programs involving inundative releases of *Trichogramma* species have given varying results (Ashraf *et al.*1993; Duffield 1994). There are some questions need answering: (1) What is the efficiency of *Trichogramma* to suppress *Helicoverpa* in laboratory experiment? (2) Which temperature will be optimal for *Trichogramma* spp.? (3) What is the best release ratio of *Trichogramma* to control *Helicoverpa* in the greenhouse? These questions will be addressed in the first chapter.

Chrysoperla carnea

The lacewing, *Chrysoperla* spp. have long been noted as the efficient predators of many important agricultural insect pests (Stark and Whitford 1987). In addition they have many desirable attributes for use in biological control: (1) What is the efficacy of *Chrysoperla* to control *Helicoverpa* in the laboratory? (2) Which temperature will be optimal for *C. carnea*? (3) Does a combination between egg parasitoids and predator increase controlling *Helicoverpa*? These questions will be answered in the second and third chapters.

Host selection behaviour of parasitoids

In the process leading to successful parasitization, both plant and host-derived volatile chemicals are important in both host habitat and host location by parasitoid insects (Vet and Dicke 1992). In host habitat location, volatile chemicals from the food plant of the host are released in relatively large amounts and provide long range, olfactory information to the parasitoid, whereas chemicals involved in host location, once the parasitoid approaches the microhabitat of its hosts, appear to operate short range and may originate from the host itself and from its by-products (Vinson 1976). The release of chemical compounds by the plant that stimulates the attraction of herbivore’s natural enemies are considered an indirect mechanism of plant defense (Takabayashi *et al.* 1995), whereas chemical compounds repellent to herbivores are considered a direct mechanism of plant defense or antixenotic resistance of the plant against herbivores (Petterson *et al.* 1996). Parasitoids of herbivorous hosts face the problem that they have to utilize reliable and detectable cues during host location. The role of induced plant volatiles for egg parasitoids is almost unknown. Its elucidation, however, may contribute to the enhancement of performance of egg parasitoids utilized as biological control agents. Therefore, understanding the behaviour of *Trichogramma* is crucial for host-parasitoid interactions. The question how *Trichogramma* spp. find their hosts and whether plant characteristics affect their behaviour will be addressed in the fourth chapter.

Olfactometer studies

Biological control often involves the ability of one organism to locate, interact with, or avoid another organism. Since olfactory cues often control these interactions, an understanding of olfactory responses is important in effective use of biocontrol agents. Olfactometry is commonly used in investigations of organism behaviour and responses to olfactory stimuli (Martin *et al.* 1990). Four-armed olfactory chambers, (Vet *et al.*1983) provide a relatively unconfined central arena in which organisms can freely move and into which four olfactory treatments or controls can be introduced. The fifth chapter will deal with attractiveness of parasitoids by olfactory cues to emitted volatiles, which had done in Entomology Department, Texas A&M University.

Jasmonic acid in cotton fields

Some field experiments were done in Texas A&M University farm (Texas, USA) dealing with the possibility of controlling cotton pests biologically. Jasmonic acid found in many plant species and is involved in regulating diverse plant functions, including plant resistance and senescence (Creelman and Mullet 1997). The plant produces Jasmonic acid after caterpillar damage and this result in increased production of compounds involved in resistance (Constabel *et al.* 1995; Thaler *et al.* 1996). In tomatoes, application of jasmonic acid results in induction of proteinase inhibitors and polyphenol oxidase and in a decrease in the preference, performance, and abundance of many common herbivores in the field, including *Frankliniella occidentalis*, *Spodoptera exigua*, *Trichoplusia ni*, and *Myzus persicae* (Thaler *et al.* 1996). These organisms and *S. exigua* in particular, can be important yield-reducing pests of tomato plants (Lange and Bronson 1981). Jasmonic acid affects plant development and physiology, which could in turn affect plant, yield irrespective of effects on herbivory. For example, exogenous jasmonic acid decreases the photosynthetic rate of plants (Metodiev *et al.* 1996), causes a reduction in bud formation and causes an increase in ethylene, a hormone involved in fruit ripening (Saniewski *et al.* 1987). This regard to a jasmonic acid application in cotton fields, I address the following questions: Does Jasmonate affect cotton insects and their natural enemies? What are the benefits in biocontrol programs in organic cotton production? These questions will be addressed in the sixth chapter.

The main objectives of this study are

1. Efficiency of *Trichogramma* spp. to control *Helicoverpa* under laboratory, at different temperature regimes and greenhouse conditions.

2. Efficacy of *Chrysoperla carnea* for controlling *Helicoverpa* in the laboratory, at different temperatures and in the greenhouse.
3. Does *Chrysoperla* prey on *Helicoverpa* eggs parasitized by *Trichogramma* spp. in the laboratory and the greenhouse?
4. Impacts of cotton cultivar characteristics on the parasitization and behaviour of *Trichogramma* on *Helicoverpa* eggs
5. Effects of Jasmonate induction on the behaviour of *Cotesia marginiventris* in olfactometer studies using BT- and non-BT-cotton plants.
6. Effects of Jasmonate applications on pest and natural enemy recruitment in cotton fields

Biology of organisms

Helicoverpa armigera

H. armigera is widely distributed from the Cape Verda Islands in the Atlantic Ocean, through Africa, Asia and Australia to the south Pacific Islands, and from southern Europe to New Zealand (Reed and Pawar 1982). The pest status of *H. armigera* is due mainly to its migratory ability, high fecundity, facultative diapauses and polyphagy (Fitt 1989). The larvae cause major crop damages; feeding on plant parts rich in nitrogen such as reproductive structures and growing tips (Fitt 1989). The management of *H. armigera* in many crops, including cotton and pigeonpea, relies heavily on the use of insecticides (King 1994; Shanower *et al.* 1997). This has led to high levels of resistance to major groups of insecticides (Armes *et al.* 1996). *H .armigera* is a strong flier and the mobility and rapid colonization of new habitats is a major factor limiting the effectiveness of most natural enemies (Fitt 1989).

Trichogramma species

Trichogramma species have a short generation time and can be easily mass-produced and they could kill the Lepidopteran pests during the egg stage before caterpillars can emerge and damage the crop (King *et al.* 1986; Hassan 1993). *Trichogramma* is solitary endoparasitoids, they seek out and parasitize host eggs, more than one egg may be inserted into each host egg and this is based, at least in part, on the egg size. After hatching, the parasitoid larvae feed on the contents of the host egg. The wasps pupate within the egg and adults chew an emergence hole to escape. At a constant 27°C, it takes about 10 days from the time of parasitism to emergence of wasps (Hoffmann *et al.* 1995)

Trichogramma spp. attack more than 400 host species, mostly Lepidopterans and some 32 million ha of agricultural and forest land are treated with *Trichogramma* spp. annually (Li 1994). *Trichogramma* are used against different species of noctuids on cotton in North and South America, South East Asia, Middle Asia, Middle East countries and Australia (Hassan 1993, 1996). Biological control of the bollworm *H. zea* (Boddie), and tobacco budworm, *H. virescens* (Fabricius) in cotton by augmentative releases of *Trichogramma* in the United States, particularly *T. pretiosum* (Riley), is summarized in King *et al.* (1985 a).

Chrysoperla carnea

Chrysoperla spp. inhabit many different agro ecosystems, and they are tolerant to many insecticides (Wetzel *et al.* 1991) and they are quite easily mass reared (Hasegawa *et al.* 1989). The lacewings adults feed only on nectar, pollen, and aphid honeydew, but their larvae are active predators. *C. carnea* occurs in a wide range of habitats; cotton, sweet corn, potatoes and tomatoes (King *et al.* 1985 b). Adults are active fliers, particularly during the evening and night and have a characteristic, fluttering flight. Oval shaped eggs are laid singly at the end of long silken stalks and are pale green. The larvae, which are very active, are gray or brownish and alligator-like with well-developed legs and large pincers with which they suck the body fluids from prey. Larvae grow from <1 mm to 6-8 mm (Henn and Weinzierl 1990).

Insect species and cotton plants

Helicoverpa rearing

The eggs used throughout this study were obtained from a culture of *Helicoverpa* in the laboratory. Larvae were reared on a modified diet (Table 1) according to (Shorey and Hala 1965), at a regime of 27 C°, 70% RH and a photoperiod of 16:8 (L: D). Single larvae were reared in polyester cells (23 X 23 X 20 mm) containing sufficient diet for whole larval development. Pupae were kept in plastic containers; newly emerged adults were transformed to cylinders with filter paper and feed on 10% sugar solution. Daily, the eggs were collected; *Helicoverpa* eggs on filter paper were cut into paper cards to introduce them to *Trichogramma* for rearing or carry out the experiments and also to use these eggs to rerear *Helicoverpa* again.

Table 1: Components of *H. armigera* artificial diet

Components	Quantity (g)	Chemical Structure	Producing Company
Agar	20	Agar-Agar	Loewe Biochemica
Wheat germ oil	40	Vit.B,vit.E,mg,f2,k Ballast substance	Dr. Grandel GmbH
Bean flour	125	Grounded Bean	Personal
Nipagin (Methyl-4-hydroxybenzoate)	3	$C_8H_8O_3$	Fluka chemika
Streptomycin Sulphate	2	$(C_{21}H_{39}N_7O_{12})_2(H_2SO_4)_3$	Fluka biochemika
Vitamin mixtures	8	Vit. A, B1, B2, B6, B12 C, E, Rutin, Biotin, Nicotinamide	Sigma
Oil	4	Sun flower oil	Plus Vertriebs GmbH
Wesson's salt mixture	2	25% $NaHCO_3$ 25% KH_2PO_4 40% $Ca_3O_8P_2$ 10% $MgSO_4 . 7H_2O$	Sigma
Ascorbic acid	6	$C_6H_8O_6$	Merck
Distilled water	800 ml	H_2O	Our Institute

Preparation of medium

Nipagin is dissolved in 5ml alcohol (Ethanol 96%). Water is boiled and Nipagin added, then mixed with agar and boiled again. Afterwards, wheat germ oil, bean flour, oil and Wesson's salt mixture is added. The medium is cooled to about 70°C, followed by adding ascorbic acid, yeast, vitamin mixtures and streptomycin sulphate. Finally, the media is poured into small containers (23 x 23 x 20 mm) and left over night to dry. The containers are stored in the refrigerator until use.

Trichogramma rearing

T. pretiosum and *T. minutum* were provided by the Institute of Biological Plant Protection, Darmstadt. *Trichogramma* spp. were reared on *Helicoverpa armigera* eggs as well as on *Sitotroga* eggs. In vivo rearing was conducted in 50 ml glass tubes at 27±1°C, 70% RH and a 14 L: 10 D photoperiod. Eggs of *Sitotroga* were attached to paper strips with glue TRAGANT (Fluka Company); 6 g was dissolved into 100 ml distilled water. These paper cards are 0.5 cm in width and 7 cm in long.

Also, *Helicoverpa* eggs on the filter papers were cut into paper cards ca. 1.5 cm wide and 7 cm long. These cards were inserted into glass vials (50 ml) with parasitoids 24 hours old. Vials were taped hereafters. Parasitoids were exposed at a ratio of ca. 1 parasitoid female per 10 host eggs for ca. 24 hours. The life cycle of *Trichogramma* ranged from 8-10 days.

***Chrysoperla* rearing**

Green lacewing larvae were obtained from Öre Bio Protect company (Germany). Larvae were reared singly in individual wells, or in a group in a large Tupperware-like container with Vaseline or Fluon painted on the inside upper edge to prevent escape. In the latter situation, crumpled paper towel was used to increase surface area and to establish hiding places as larvae are cannibalisation. Purchased larvae were shipped in a container of corrugated cardboard with silkscreen glued to either side. A single larva resides in each of the small cells or corrugations provided by the cardboard. Larvae were shipped by the company one day before starting the experiment to avoid the hassle of providing food for the hungry larvae and kept in a humid environment until use.

Cotton plants

Four- to six-week-old cotton, *Gossypium barbadense* cv. Giza 89 Giza 86 and Alex 4 plants and *G. hirsutum* cv. DPL 422B/R (BT) and DPL 420R (non-BT) with four to six fully expanded true leaves were used in all experiments. Cotton was grown in controlled greenhouse conditions; plants were grown in 13 cm diameter pots (Sand: Clay 60: 40) for greenhouse experiments and 10 cm for volatile collection and olfactometer experiments. All plants used in the experiments were free of insects. Seeds of *G. barbadense* were provided from Egypt and *G. hirsutum* from USA.

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Chapter 1

Influence of temperatures and cotton cultivars on the efficacy of *Trichogramma pretiosum* and *T. minutum* on *Helicoverpa armigera* eggs

ABSTRACT

Worldwide organic cotton production and processing is still at an experimental level. Although occupying a niche market, organic cotton production increases steadily and this also increases the demand for additional biocontrol measures. The release of mass propagated eggs parasitoids could be one option for farmers to cope with lepidopteran pests. This study aimed at assessing the ability of *Trichogramma pretiosum* (Riley) and *T. minutum* (Riley) (Hymenoptera: Trichogrammatidae) to parasitize *Helicoverpa armigera* (Hüb.) (Lepidoptera: Noctuidae) (One of the most important pests in cotton) under laboratory and greenhouse conditions. The parasitoid strains used in the experiments emerged from *Sitotroga cerealella* or from *H. armigera* eggs. *H. armigera* eggs were exposed at a ratio of 0.5 and 1.0 parasitoid female per host egg for 24h in the laboratory or in the greenhouse. Parasitism rate, adult emergence rate, and longevity were used to test for the effects of temperatures and host density or influence of cotton cultivars (Giza 89, Giza 86 and Alex 4) on *Trichogramma* efficiency.

Parasitism rates of *Trichogramma* species on *Helicoverpa* eggs were higher at 25° and 30°C than at 20°C, and were higher in *T. pretiosum* than in *T. minutum* for 24 hours. Total Parasitism rates of *Trichogramma* species for adult parasitoid lifespan were slightly higher for *T. minutum* compared to *T. pretiosum*. Longevity of *Trichogramma* spp reared on *Helicoverpa* eggs were longer than reared on *Sitotroga* eggs. Longevity of *Trichogramma* species was significantly different between different temperatures and also between species. Longevity of *T. minutum* wasps was longer than of *T. pretiosum.* In most experiments *T. pretiosum* parasitized as many eggs as *T. minutum*. Emergence rates and sex ratio were greater at 25°C compared to at 20° and 30°C. Emergence rates were higher at high release rates than at low ones. Cotton cultivars significantly influenced the parasitism rates, indicating differences in host location or host handling capabilities by the searching parasitoid species. The results are discussed with regard to the potential of these parasitoid species as biocontrol agents to be used in organic cotton fields.

Keywords: *Trichogramma pretiosum, T. minutum,* organic cotton, *Helicoverpa*, temperature, laboratory, greenhouse.

INTRODUCTION

Organic cotton production is farming in harmony with nature, aimed at providing products of a high quality without the aid of artificial fertilizers, pesticides and growth regulation and without genetic manipulation of living organisms. The aim is to achieve optimum harvest levels without exploiting the natural base (the soil) or degrading the local environment (Alfoeldi 2001). Organic cotton provides important environmental, economic and social benefits compared to conventional cotton production (Myers and Stolton 1999).

Cotton is very prone to insect attacks. Large quantities of the most toxic insecticides have to be used for its production (Cramer 1998). The intensive use of toxic insecticides and other toxic chemicals in cotton has caused serious health and environmental impacts, including farmer and farm worker poisonings, water contamination and bird and fish kills (Myers 2000). This background requires the need for the development of alternative protection strategies including American bollworm *Helicoverpa armigera.*

H. armigera is a highly polyphagous agricultural pest. Host species for *H. armigera* come from a broad spectrum of families and include important agricultural crops such as cotton, maize, chickpea, pigeonpea, sorghum, sunflower, soybean and groundnuts (Fitt 1989). Females lay eggs on the flowering and fruiting structures of these crops, where voracious larval feeding leads to substantial economic loss (Reed and Pawar 1982). The ability of ovipositing females to locate and utilize a wide range of hosts from a number of plant families is one of the major factors contributing to the pest status of this moth (Fitt 1989). The management of *H. armigera* is difficult and in many crops, including cotton and pigeonpea, relies heavily on the use of insecticides (King 1994; Shanower *et al.* 1997). This has led to high levels of resistance to major groups of insecticides (Armes *et al.* 1996). Thus, research now focuses on alternative control methods such as cultural and biological control, and host plant resistance as the major components for integrated pest management programs (Shanower *et al.* 1997).

Promising biocontrol agents against *Helicoverpa* spp. are egg parasitoids belonging to the genus *Trichogramma* (Hymenoptera: Trichogrammatidae). These parasitoids have been mass produced and released against at least 28 herbivorous pest species on 20 crops (Li-Ying 1994; Smith 1996; Hassan 1998). *Trichogramma* spp. wasps have been successfully released in biological control programs against *Helicoverpa* spp. and other lepidopteran species world-wide in cotton (Stinner *et al.* 1974; King *et al.* 1986; Hassan 1993; Romeis and Shanower 1996; Pinto 1998).

King *et al.* (1985) reported the release of *T. pretiosum* in cotton fields (413.000 adult *Trichogramma* per ha). In their experiments egg parasitism increased as a consequence of parasitoid releases, but parasitism rates could not be correlated with larval suppression. According to Kovalenkov (1992), releasing *T. pintoi* (Voegele) on cotton in Tajikistan (Middle Asia) against *H. armigera* for 3-4 times every 4-5 days in one generation eliminated an average of 50% of host eggs. After augmentative releases of *T. confusum* (Viggiani) in China, *H. armigera* egg parasitism was 68.4% (Zhou 1988). Weekly releases of 100.000-200.000 specimens/ ha of *T. pretiosum* in Australian cotton resulted in an average of 49.0% parasitism of *H. armigera* eggs (Twine and Lloyd 1982). Initial inundative releases of *Trichogramma* spp. in Australian grown cotton were encouraging, with levels of egg parasitism approaching 33.0 and 76.0% in non release and release sites, respectively (Scholz and Murray 1995). Stinner *et al.* (1974) released *Trichogramma* which resulted in an average of 51% parasitism of *H. zea* and *H. virescens* eggs and a reduction of 66-80% in the number of larvae on five Texas cotton farms.

Organic cotton is grown in a wide rang of climatic conditions and agricultural systems. These different conditions will both affect *Helicoverpa* abundance and efficiency of *Trichogramma* in cotton fields. Life history parameters of *Trichogramma* and temperatures are very important with regard to mass production and efficiency of *Trichogramma* for controlling *Helicoverpa* (Smith 1996; Hansen and Jensen 2002).

Inoue and Parra (1998) studied parasitization of *T. pretiosum* at six constant temperatures, demonstrated that the highest temperatures were the best for *T. pretiosum* parasitization. However fecundity was highest at intermediate temperatures. Contrary, the number of host eggs killed by host-feeding was highest at the two lower temperatures. Navarro and Marcano (1997) evaluated effects of constant temperatures on life history parameters of *T. pretiosum* and *T. caiaposi,* and found that longevity and oviposition periods were negatively correlated with temperature.

Cultivar selection is one of the most important decisions that growers must make annually. To become familiar with selecting the best cultivar, growers have to evaluate the new cultivars on their own farms on experimental level or have to rely on research (Barron and Liptay 1997). Cotton genotype variation may affect the efficiency of *Trichogramma* in terms of morphology and chemical composition of these genotypes*.* The influence of the plant species on the efficiency of *Trichogramma* spp. has been reviewed by Vinson and Barbosa (1987). Therefore, the main objectives of this study were

- To evaluate the influence of environmental conditions on *Trichogramma* efficiency prevailing in the regions often organic cotton is grown in Egypt.
- To evaluate the differences in the efficacy of different *Trichogramma* species
- To evaluate the parasitism rates and life history parameters of *Trichogramma* species on *Helicoverpa* eggs at 3 different temperatures.
- To study the effect of 3 different cotton cultivars on the behaviour and efficiency of these *Trichogramma* species.

MATERAIL AND METHODS

Plant and insect species: See general introduction.

1. Laboratory experiments

a) Parasitism rates by *T. pretiosum* and *minutum* on *H. armigera* eggs

In the laboratory experiments, paper cards with a known number of *H. armigera* eggs were exposed to 1-day–old adult *T. pretiosum* and *T.minutum* at a ratio of 0.5 and 1 parasitoid females per host egg in 12 x 2 cm glass tubes for 24 hours at 27°C, 70% RH and L: D (16:8). The parasitism rates were recorded for *T. pretiosum* and *T.minutum* reared on *H. armigera* eggs and compared with those reared on *Sitotroga* eggs (a well know artificial host for *Trichogramma* spp.)*.* In these experiments, vials were replicated 6 times. Parasitism rates were determined by counting the black eggs in each treatment. The eggs were and classified as parasitized (black eggs) or unparasitized; hatch or desiccated eggs (eggs didn't parasite or hatch). Total mortality comprised parasitized and desiccated eggs. In the control group, the eggs of *Helicoverpa* were kept without parasitoid exposure.

b) Life history parameters of *Trichogramma* species

Life history parameters comprised adult emergence rates, longevity and sex ratio. These parameters were evaluated for *Trichogramma* species reared on *H. armigera* eggs and compared with those reared on *Sitotroga* eggs*.* The experimental vials were replicated 6 times. Emergence rates were determined in each treatment by counting emergence holes from black eggs. The sex ratio and percentage of females with deformed wings or apterous forms were determined by examining the dead individuals under a microscope. Longevity of wasps was measured by keeping the adults, individually in a 1 x 5 cm glass tube without food (from the time of emergence until death at intervals of 24 hours).

2. Temperatures experiments

a) Parasitism rates of *T. pretiosum* and *T. minutum* on *H. armigera* eggs

Paper cards with a known number of *H. armigera* eggs were exposed to 1-day –old adult *T. pretiosum* and *T.minutum* at a ratio of 0.5 and 1 parasitoid females per host egg (P: H) in 12 x 2 cm glass tube for 24 hours at 3 different temperatures; 20°, 25° and 30°C, RH 70 ± 5% and 16: 8 L: D. The parasitism rates were recorded for *Trichogramma* species reared on *H. armigera* eggs and *Sitotroga* eggs*.* Parasitism rates were determined by counting the black eggs in each treatment. The experimental vials were replicated 6 times.

b) Life history parameters of *Trichogramma* spp.

The same life history parameters as mentioned in the laboratory experiments, were studied at 3 different temperatures; 20°, 25° and 30°C.

c) Effects of longevity (adult parasitoid lifespan) of *T. pretiosum* and *T. minutum* on parasitism rates on *Helicoverpa* eggs

Ten fresh *Helicoverpa* eggs were exposed to 10 *Trichogramma* spp. females. *Helicoverpa* eggs were exchanged daily. Starting from emergence of *Trichogramma* as adults until their death, parasitism rates were calculated for *T. pretiosum and T. minutum* at 3 different temperatures 20°, 25°, 30°C. In this experiment, vials were replicated 6 times.

3. Greenhouse experiments

Parasitism rates of *T. pretiosum* and *T. minutum* on 3 cotton cultivars

Ten *Helicoverpa* eggs were attached by TRAGANT glue (Fluka, Germany) on the lower surface of cotton leaves (on 1st, 3rd and 5 th leaves) and these cotton plants were placed into wood cages. These experiments were set up as 1 plant / cage (30x 30x 60 cm), 3 plants / cage (60x 100x 70 cm) and 6 plants / cage (60x 100x 70 cm). *H. armigera* eggs were exposed to 1-day –old adult *T. pretiosum* and *T.minutum. Trichogramma* were released at different ratios (1 or 2 parasitoid females per host egg) for 24 hours in the cages. Thereafter, the eggs were collected from the cotton plants and incubated in climatic chamber (27 C°, 70 % RH and 16: 8 L: D). After 4-5 days, black eggs were counted to calculate parasitism rates. The same life history parameters were recorded as in the laboratory experiments and compared with those reared on *Sitotroga* eggs. Three cotton cultivars were used (Giza 89, Giza 86 and Alex 4). These experiments were replicated 3 times in each cotton cultivar and in each cage.

Statistical analysis

Means were compared with ANOVA and followed by Bonferroni correction, using the program SYSTAT 8.0 (Wilkinson *et al.* 1998). Percentage data were arcsine transformed prior to analyses to homogenize variances.

RESULTS

1) Laboratory experiments

a) Parasitism rates of *T. pretiosum* and *T. minutum* on *Helicoverpa* eggs

Parasitism rates were 60.0, 90.0, 63.3 and 98.8% for *T. pretiosum* reared on *Sitotroga* or *Helicoverpa* eggs with a ratio of 0.5 or 1.0: 1 (P: H), respectively (Fig.1). Parasitism rates in *T. minutum* were 57.2, 85.6, 60.5 and 87.2% respectively. Parasitism rates in *T. minutum* were significantly lower (df= 1; F= 18.6; P< 0.003) than in *T. pretiosum*. The total mortality was significantly different (df= 1; F= 35.5; P< 0.04) between *Trichogramma* species. The total mortality was the highest for *T. pretiosum* (99.4%) reared on *Helicoverpa* eggs with a release rate of 1: 1. Total mortality was the least for *T. minutum* (66.6%) reared on *Sitotroga* eggs and with a release rate of 0.5: 1 (P: H) (Fig. 2).

b) Life history parameters of *Trichogramma* spp

Emergence rates differed significantly (df= 1; F= 14.0; P< 0.05) between *T. pretiosum* and *T. minutum*. The emergence rates were the highest for *T. pretiosum* (99.8%) reared on *Helicoverpa* eggs at release rate of 1: 1 (P: H). The lowest ones were 83.6% for *T. minutum* reared on *Sitotroga* at release rate of 0.5: 1 (P: H) (Fig. 3). Longevity by days was significantly different (df= 1; F= 3.8; P< 0.001) between *Trichogramma* species. Longevity of *T. pretiosum* wasps was 2.0 days and of *T. minutum* was 2.8 days (Fig. 4). Sex ratio was slightly higher in *T. pretiosum* than in *T. minutum* (Fig. 5).

■ **Release rate 0.5** □ **Release rate 1.0**

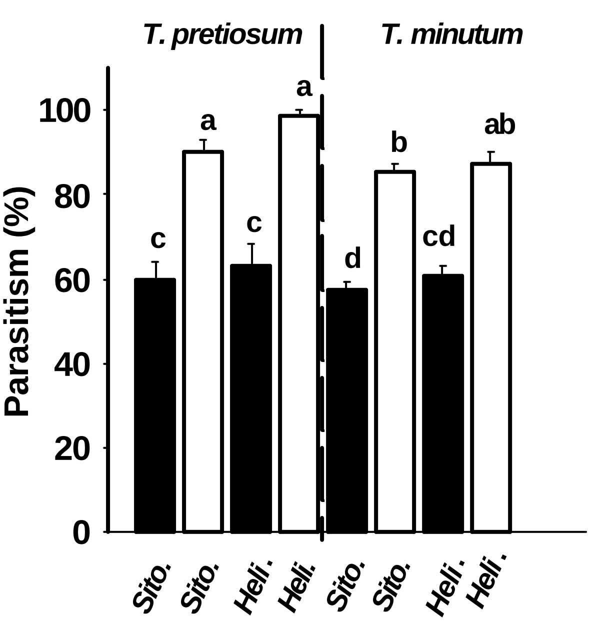

Fig. (1) Parasitism rates of *T. pretiosum & T. minutum* at 2 release rates and 2 cultural hosts

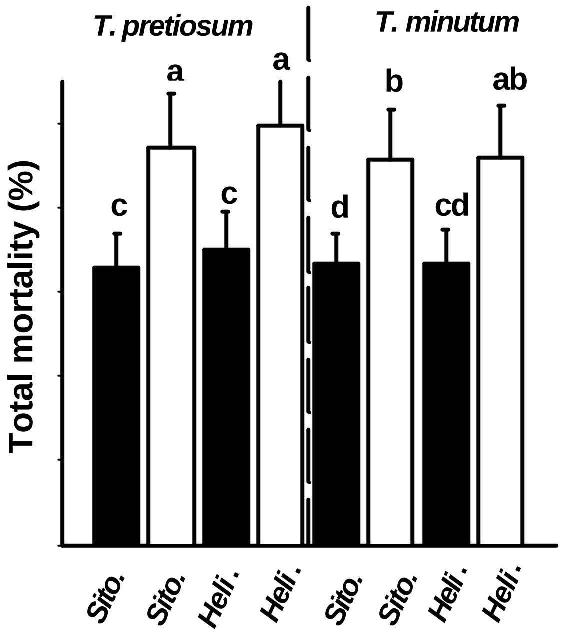

Fig. (2) Total mortaltiy of *Helicoverpa* eggs at 2 realease rates and 2 cultural hosts

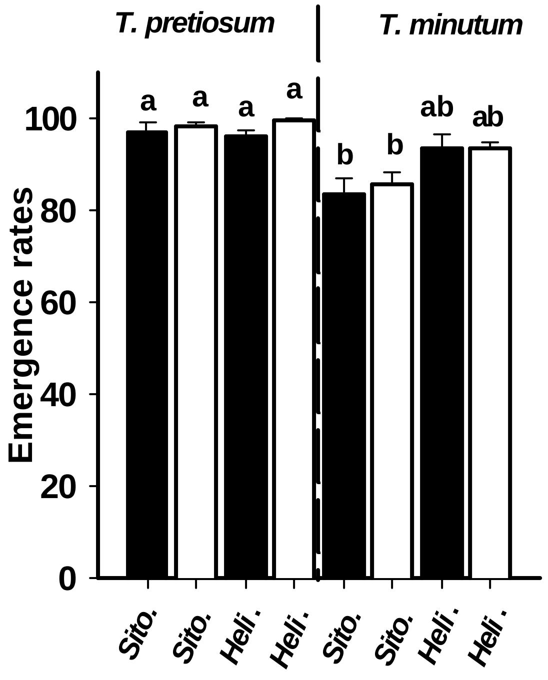

Fig. (3) Emergence rates of *T. pretiosum* & *T. minutum* at 2 release rates and 2 cultural hosts

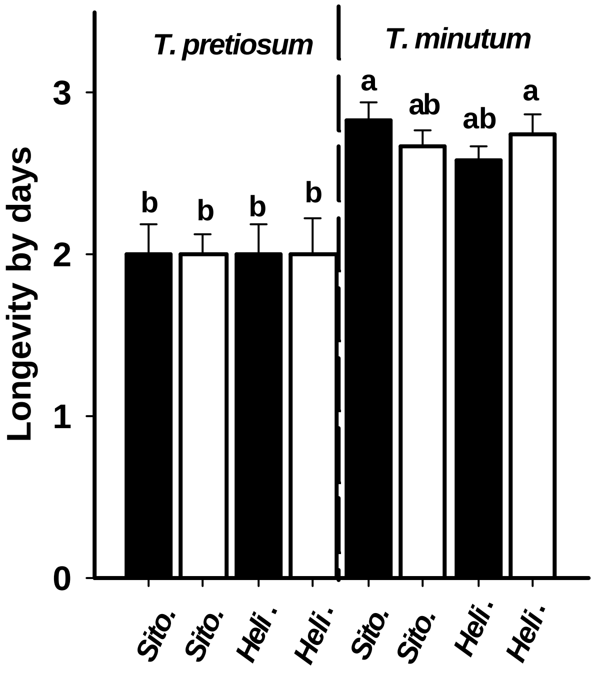

Fig. (4) Longevity of *T. pretiosum* & *T. minutum* at 2 release rates and 2 cultural hosts

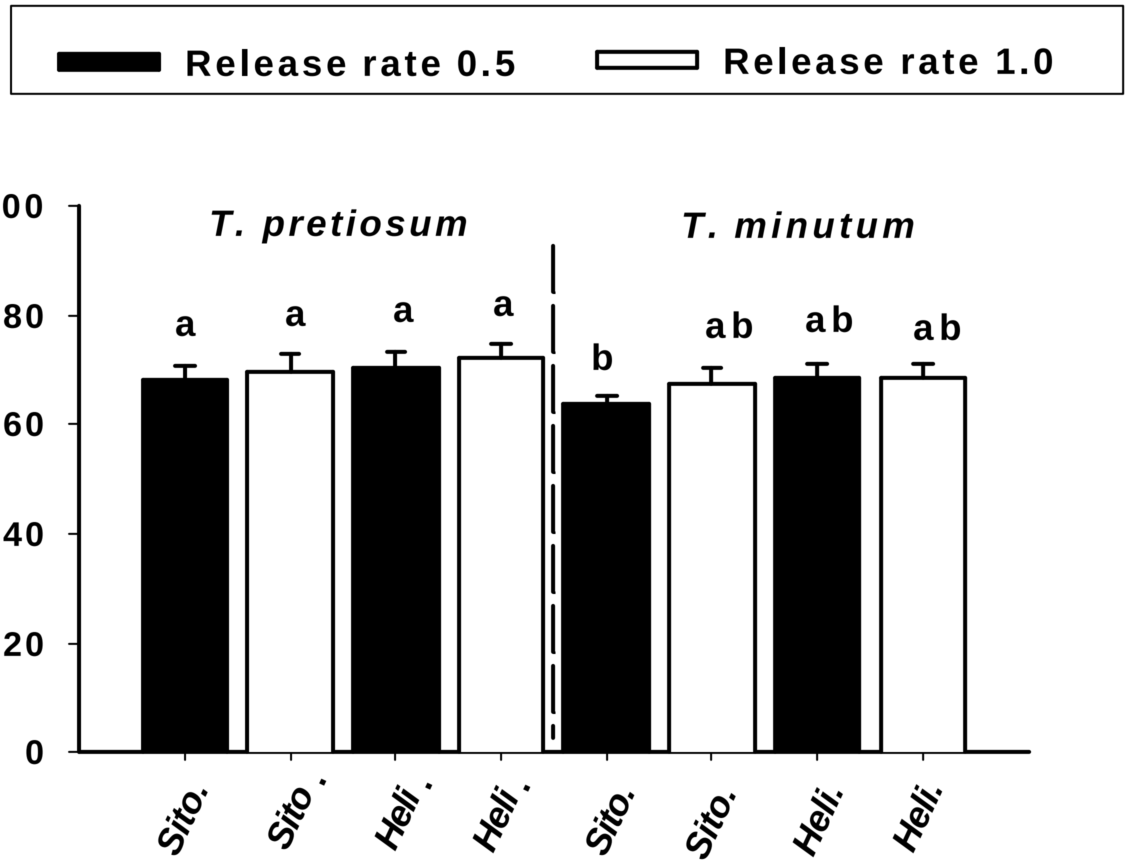

Fig. (5) Sex ratio of *T. pretiosum* and *T. minutum* at 2 release rates and 2 cultural hosts

2) Temperatures experiments

a) Parasitism rates of *T. pretiosum* and *minutum* on *H. armigera* eggs

Parasitization rates were the highest at 25°C reaching 98.8% in *T. pretiosum* and 87.2% in *T. minutum*. The lowest rates were 60.5 and 57.2 % at 20°C in *T. pretiosum* and *T. minutum* respectively. Parasitization rates significantly differed (df= 2; F= 12.7; P< 0.004) between the three temperatures. The parasitism rates were the highest at 25°C followed by 30°C and the lowest one at 20°C. Parasitism rates differed significantly (df= 1; F= 18.2; P< 0.04) between *Trichogramma* species. Parasitism rates was different significantly (df= 1; F= 7.9; P> 0.001) between 2 release rates (Fig 6). Parasitism rates were higher at release rates of 1: 1 (P: H) than at release rates of 0.5: 1 (P: H). The total mortality differed significantly (df= 1; F= 9.6; P< 0.003) between *Trichogramma* species and (df= 1; F= 3.9; P< 0.001) between release rates. Total mortality was higher for *T. pretiosum* than for *T. minutum.* It was higher at release rates of 1: 1 (P: H) than at release rates of 0.5: 1 (P: H) (Fig. 7).

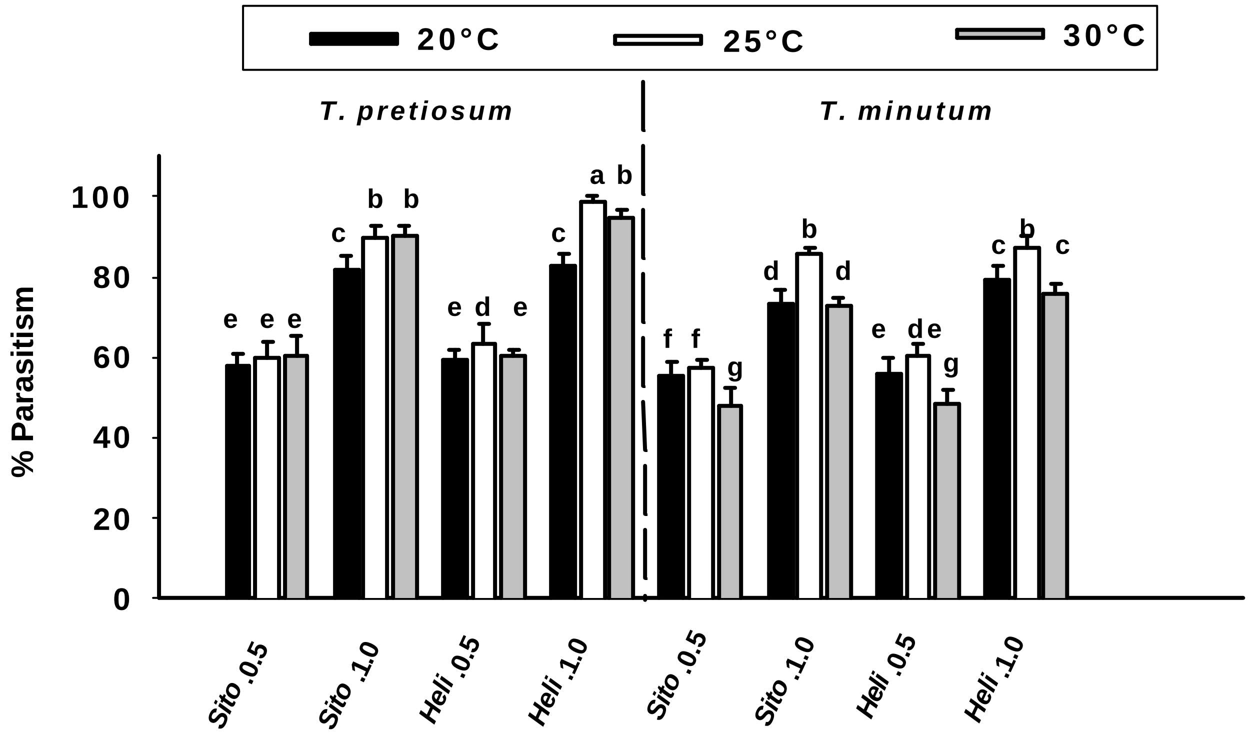

Fig. (6) Parasitism rates of *T. pretiosum* & *T. minutum* at 3 differents temperatures, at 2 release rates and 2 cultural hosts

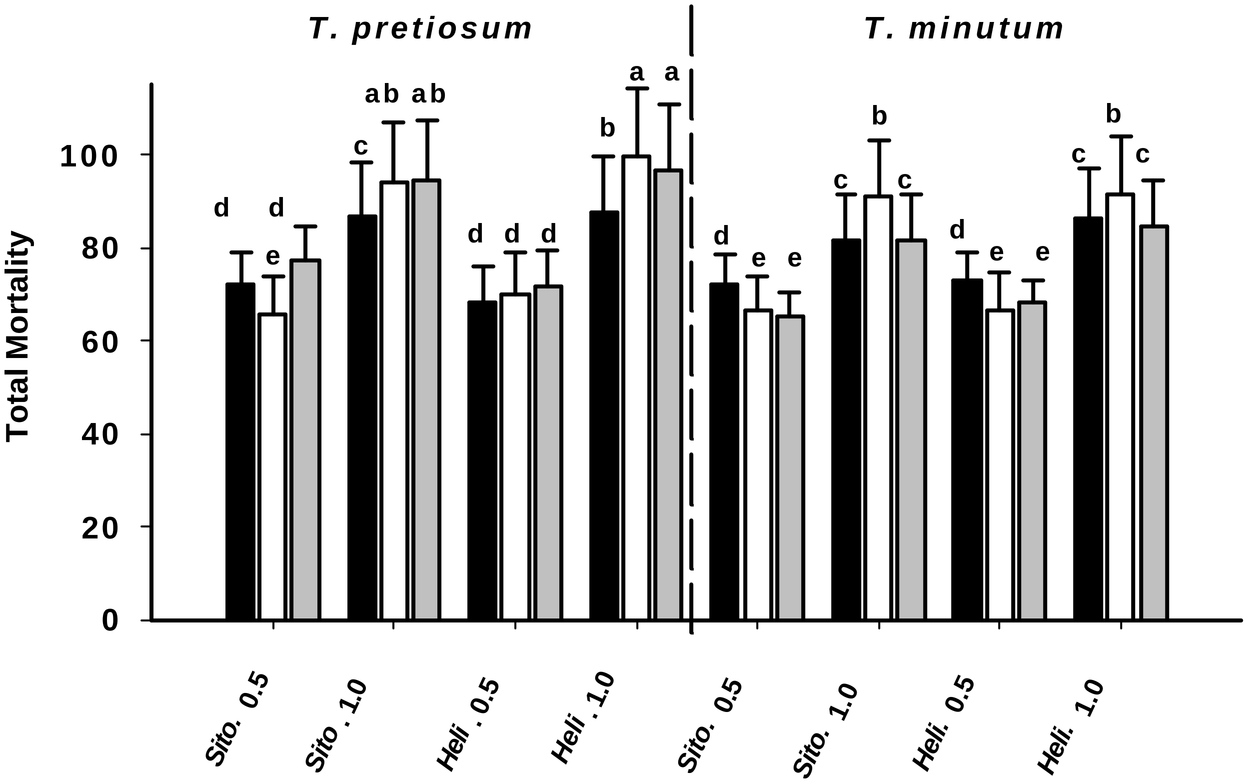

Fig. (7) Total mortaltiy of *Helicoverpa* eggs at 3 different temperatures, at 2 release rates and 2 cultural hosts

b) Life history parameters of *Trichogramma* spp.

Emergence rates for *T. pretiosum* reared on *Sitotroga* or *Helicoverpa* eggs with a ratio of 0.5 or 1: 1 (P: H) were 96.9, 98.3, 95.9 and 99.8%, respectively. Emergence rates for *T. minutum* were 83.6, 85.7, 93.5 and 93.7% respectively. Emergence rates for *T. minutum* were significantly lower (df= 1; F= 19.7; P< 0.04) than for *T. pretiosum*. Emergence rates differed significantly (df= 2; F= 9.3; P< 0.009) between the three temperatures (Fig. 8). The longevity was significantly shorter in *T. pretiosum* than in *T. minutum*. The longevity differed significantly (df= 2; F= 14.8; P< 0.03) between the three temperatures. Longevity was the longer at 25°C compared to 20° and 30°C (Fig. 9). The sex ratio was significantly different (df= 2; F= 35.6; P> 0.05) between the three temperatures. The highest sex ratio was at 25°C followed by at 30°C and the lowest one at 20°C. Sex ratio differed significantly (df= 1; F= 13.7; P> 0.035) between *Trichogramma* species. Sex ratio was higher in *T. pretiosum* than in *T. minutum* (Fig. 10).

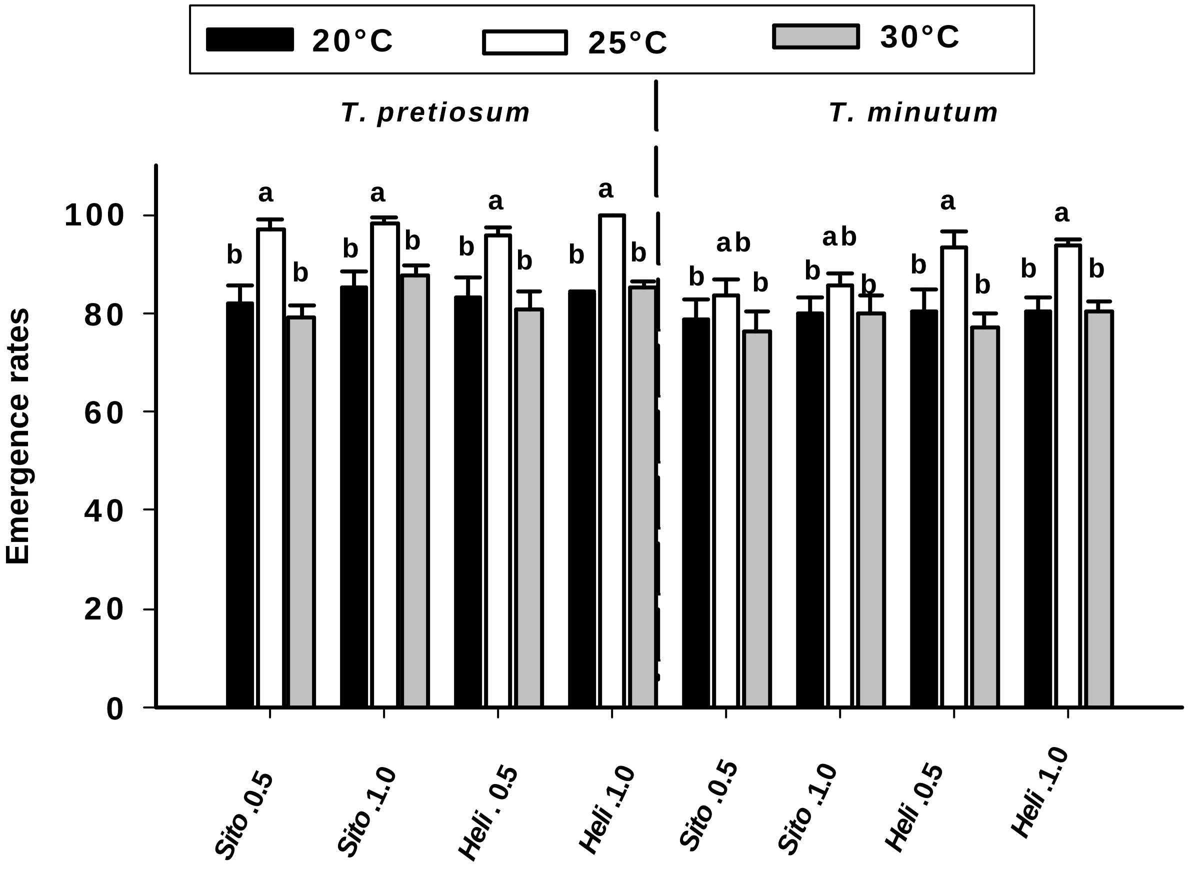

Fig. (8) Emergence rates of *T. pretiosum & T. minutum* at 3 different temperatures, at 2 release rates and 2 cultural hosts

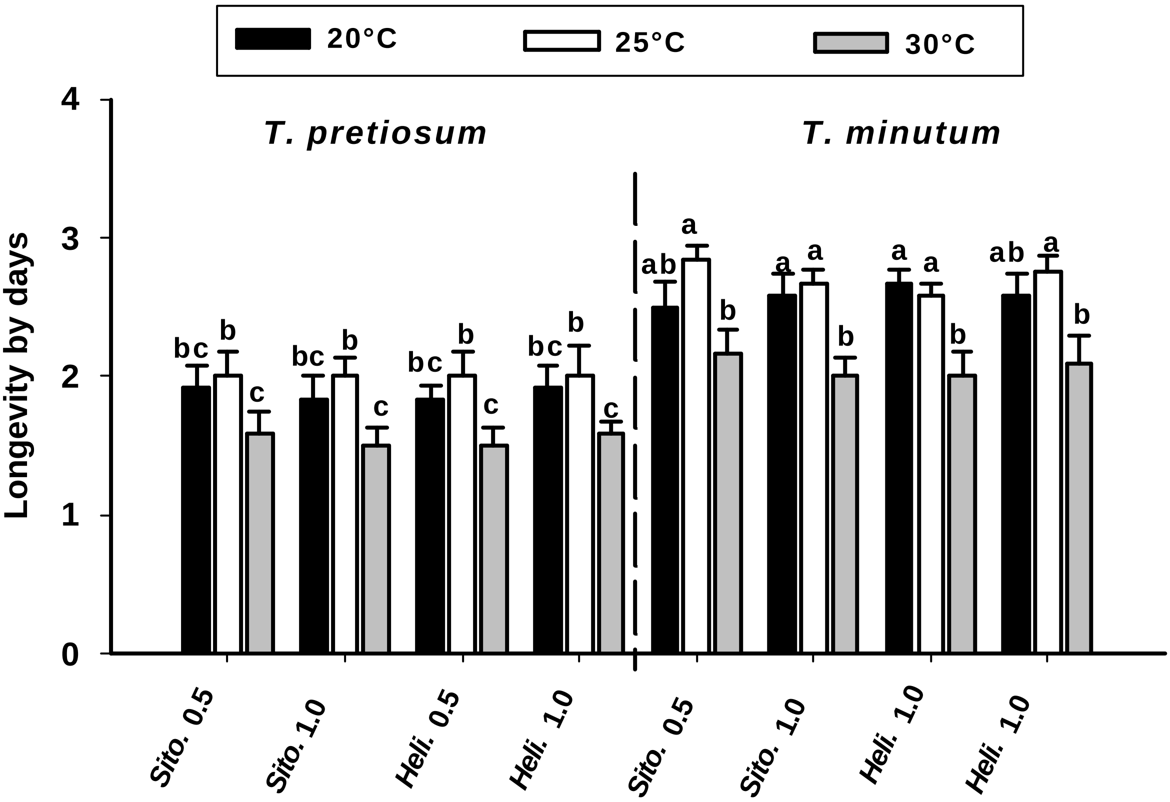

Fig. (9) Longevity of *T. pretiosum* and *T. minutum* at 3 different temperature, at 2 release rates and 2 cultural hosts

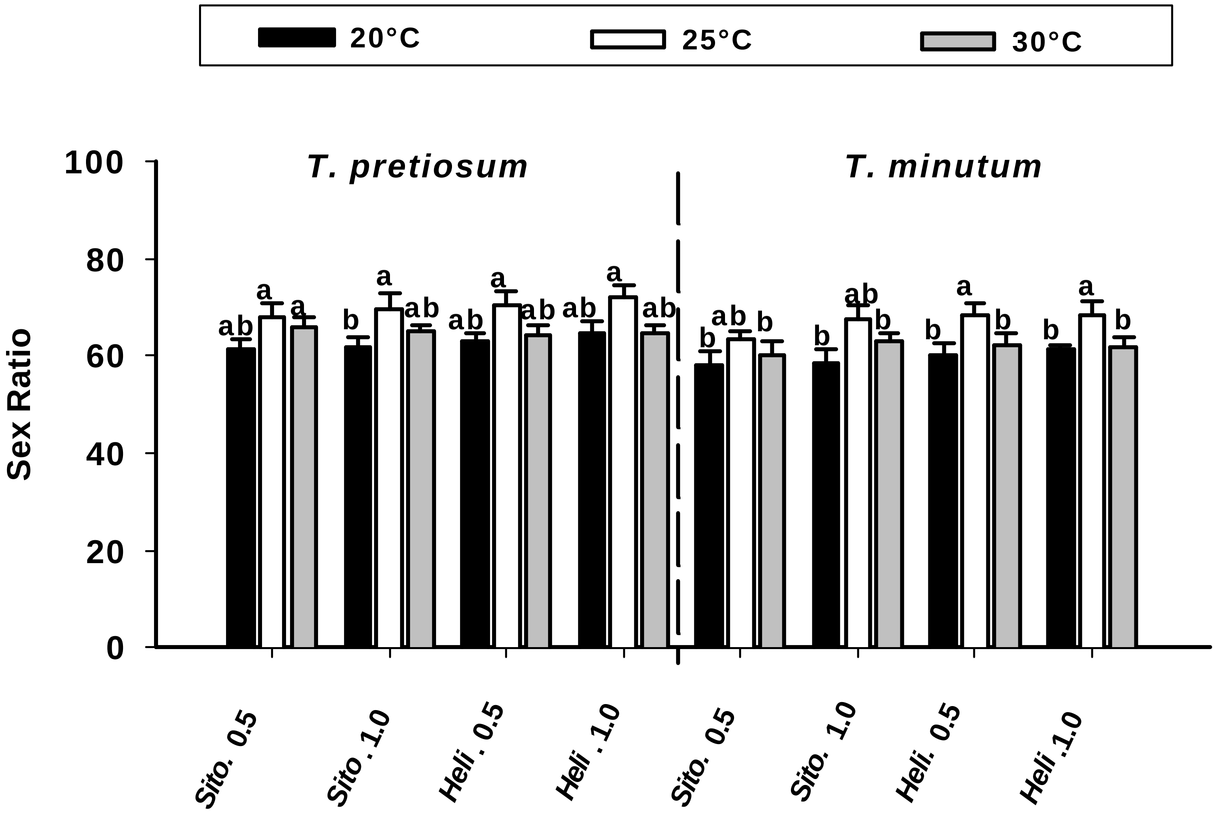

Fig. (10) Sex ratio of *T. pretiosim* & *T. minutum* at 3 different temperatures, at 2 release rates and 2 cultural hosts

c) Influence of temperatures on adult parasitoid lifespan and parasitism rates of *T. pretiosum* and *T. minutum*

The parasitism rates were significantly different (df= 1; F= 7.5; P< 0.009) between *T. pretiosum* and *T. minutum*. Parasitism rates in *T. pretiosum* were 86.7, 86.7 and 100% at 20°, 25° and 30°C respectively. Conversely, parasitism rates in *T. minutum* were 60.0, 80.0 and 70% at 20°, 25° and 30°C respectively. Generally, parasitism rates for whole lifespan were higher in *T. minutum* than in *T. pretiosum* (Fig. 11). Adult parasitoid lifespan differed significantly (df= 1; F= 29.3; P< 0.05) between *Trichogramma* species. Adult parasitoid lifespan was the shortest at 30C° (4.0 and 5.0 days for *T. pretiosum* and *T. minutum*). It was the longest at 20C° (5.4 and 7.0 days for *T. pretiosum* and *T. minutum*). The lifespan was a medium at 25C° (5.0 and 6.0 days for *T. pretiosum* and *T. minutum*). The parasitism rates were significantly different (df= 6; F= 3.8; P< 0.001) between lifespan days (from adult emergence to death of parasitoid). Parasitism rates decreased with aging of the parasitoids (Fig.11).

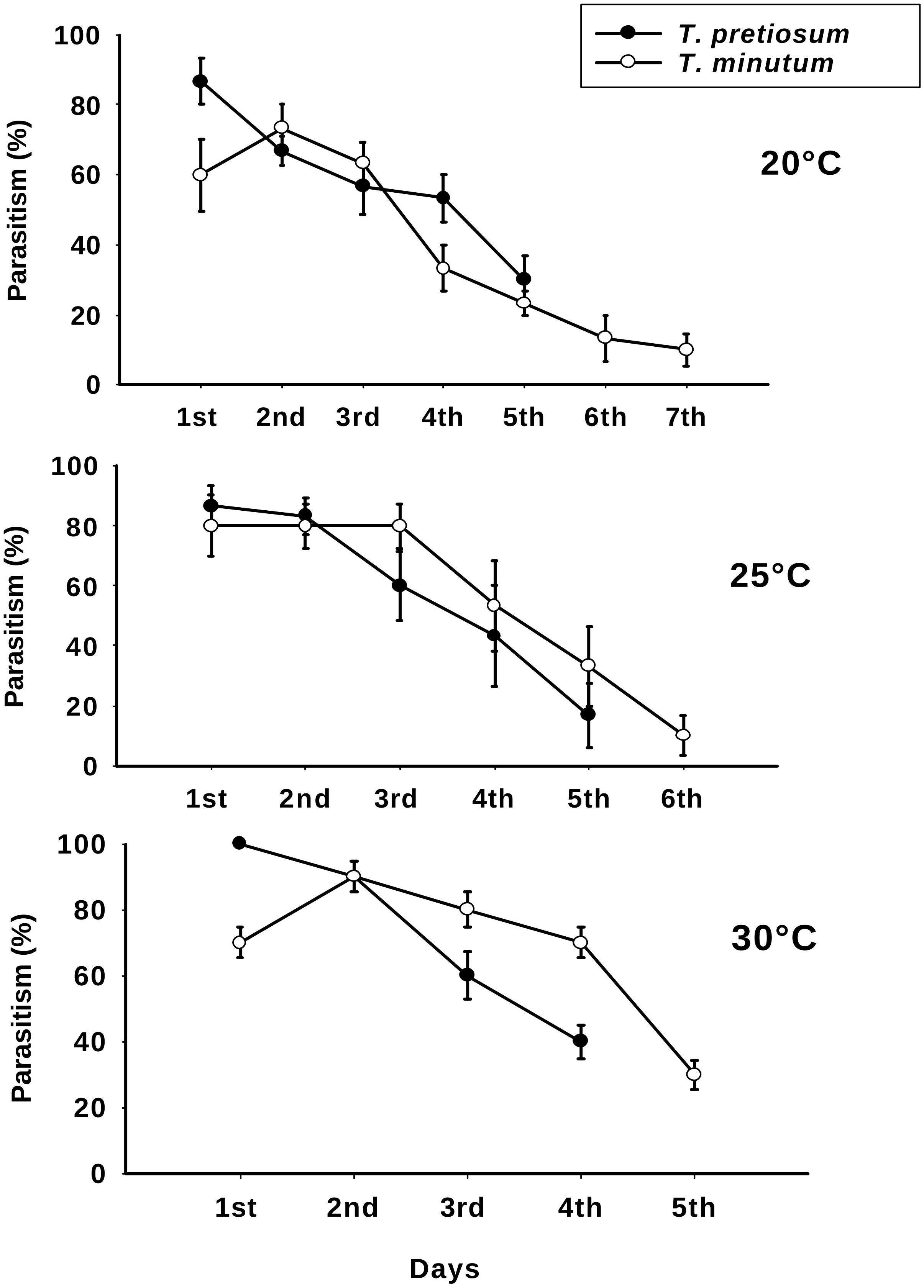

Fig. (11) Parasitism rates of *T. pretiosum & T. minutum* for adult parasitoid lifespan at 3 different temperatures

3) Greenhouse Experiments

Effects of different cotton cultivars and plant densities on parasitism rates of *T. pretiosum* and *T. minutum*

Parasitization rates for *T. pretiosum* and *T. minutum* on *Helicoverpa* eggs were significantly different (df= 2; F= 8.7; P < 0.005) between three different cotton cultivars. The highest parasitism rates were on Alex 4 cultivar followed by Giza 89 and the lowest cultivar was Giza 86. There was a significant difference (df= 1; F= 16.2; P< 0.01) between *Trichogramma* species. Parasitism rates in *T. pretiosum* were higher than in *T. minutum*. Parasitism rates for *Trichogramma* spp. were significantly different (df= 1; F= 12.9; P< 0.05) between those parasitoids reared on *Sitotroga* and *Helicoverpa* eggs. Parasitism rates were higher for *Trichogramma* reared on *Helicoverpa* (92.8%) than on *Sitotroga* eggs (86.7%). The parasitism rates were significantly different (df= 1; F= 3.4; P< 0.001) between 2 release rates; 1 and 2: 1 (P: H). Parasitization rates at release rate of 2:1 (P: H) were 90.6, 87.2 and 92.8% on Giza 89, Giza 86 and Alex 4 cultivars. Conversely, parasitism rates at release rate of 1: 1 (P: H) were 49.3, 45.1 and 50.5% on Giza 89, Giza 86 and Alex 4, respectively (Fig. 12).

Parasitism rates were significantly different (df= 2; F= 36.5; P< 0.04) between plant densities (plant numbers in cages). Parasitism rates were 93.3, 90.0 and 95.5% on Giza 89, Giza 86 and Alex 4 in 1 plant / cage at release rate of 1:1 (P: H). Parasitism rates were 86.9, 83.7 and 88.5 % on Giza 89, Giza 86 and Alex 4 in 3 plants / cage at release rate of 1:1 (P: H). Parasitism rates were 91.5, 88.0 and 94.6 on Giza 89, Giza 86 and Alex 4 in 6 plants / cage at release rate of 1:1 (P: H). Conversely, at release rate of 2:1 (P: H) the parasitism rates could be arranged depending on plant densities as the followings: The highest in 6 plants / cage, followed by 1 plant / cage and the lowest ones in 3 plants / cage. Parasitism rates were significantly different (df= 1; F= 4.3; P< 0.001) between 2 release rates (Fig 13; A and B).

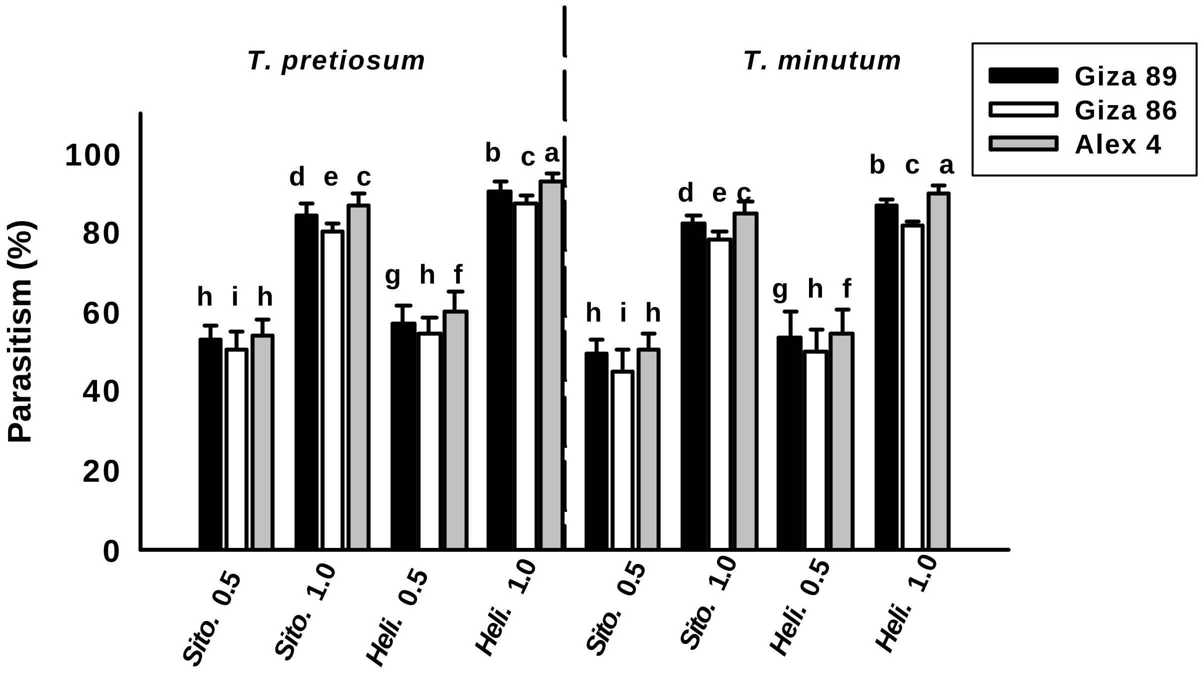

Fig. (12) Parasitism rates of *T. pretiosum* and *T. minutum on* 3 different cotton cultivars

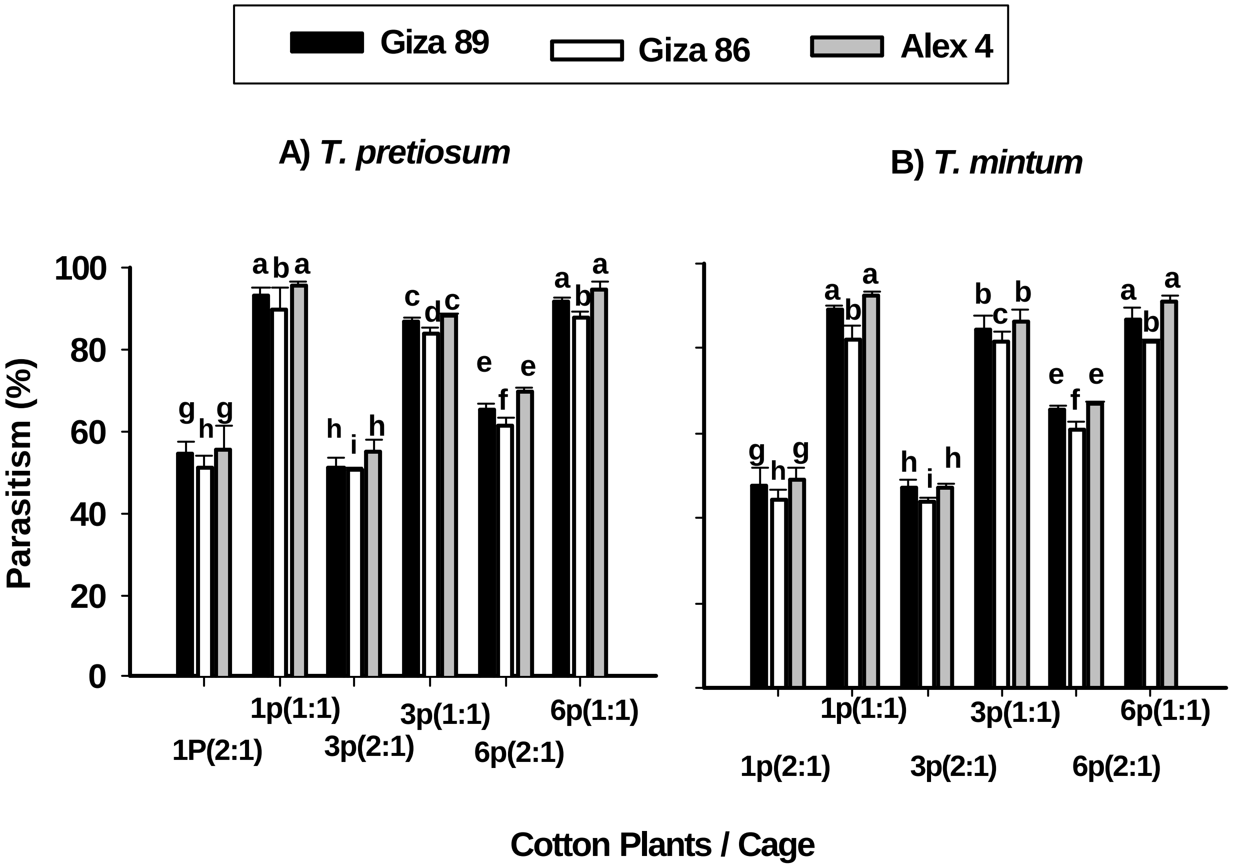

Fig. (13) Effects of plants denstity and release rates on effecincy of *Trichogramma* spp A) *T. pretiosum*, B) *T. minutum* on 3 different cotton cultivars

DISCUSSION

Parasitism rates of *Trichogramma minutum* and *T. pretiosum* were significantly different on *Helicoverpa* eggs with regard to several life history parameters tested, the latter parasitoid species being the more efficient one. These results correspond to King *et al.* (1985, 1986). Contrary to these results Greenberg *et al*. (1998 a, b) reported that parasitism rates of *T. minutum* were higher than of *T. pretiosum* when parasitizing beet armyworm or Mexican rice borer eggs. On the other hand, total mortality was higher in *T. pretiosum* compared to *T. minutum* (Greenberg *et al.* 1998a). Besides different host species studied these difference may be related to a differential quality and quantity of host eggs. For example, Biever (1972) found differences in the parasitism rates between individuals of *T. minutum* reared on two different host species and rearing *T. evanescens* on different host resulted in differences in the parasitism rates as well (Boldt 1974).

Temperatures regimes had a significant influence on parasitism rates and life history parameters in *T. pretiosum* and *T. minutum*. Intermediate temperatures (25° C) were more appropriate for the performance of these parasitoid species compared to 20°C or 30°C. These results seem to highlight a general feature in species of this genus. Navarro and Marcano (1997) evaluated the effect of different temperatures on biological characters of *T. pretiosum* and *T. caiaposi.* According to their results, fecundity and the female: male ratio were maximum at 25C° for *T. pretiosum*. Egg parasitism decreased with temperatures less than 17 °C and increased with higher temperatures up to 27°C. However, the decrease in egg parasitism may not simply result from low temperatures, but also searching behaviour of *Trichogramma* (Biever 1972), such as walking speed of searching wasps changes with temperature (Qian *et al.* 1984).

Longevity is negatively correlated to increasing temperatures; the higher the temperature the lower longevity. For example Gou (1986) showed that longevity will be reduced from 6 days at 20°C to 2 days at 27°C, and to 0.5 day at 35 °C, respectively. The same trend has been reported by Harrison *et al.* (1985). Longevity is also influenced by the cultural host species. Longevity of *Trichogramma* spp. reared on *Helicoverpa* eggs was slightly longer compared to *Sitotroga* eggs, corroborating the results of Butler and Lopez (1980). Therefore, *T. pretiosum* is recommended for the control of *Helicoverpa armigera* for its high efficiency, especially if it is reared on *Helicoverpa* eggs instead of *Sitotroga* eggs.

These differences results from differences in nutritional components of the eggs in these insect hosts. To be an effective biocontrol agent, *Trichogramma* spp. should be reared at a suitable temperature and hosts to gain the highest parasitization rates and life history parameters ensuring high efficient *Trichogramma* specimens and many generations per year.

Parasitism rates on *Helicoverpa* eggs significantly differed between cotton cultivars, *Trichogramma* species, density of cotton plants and release rates. Greenberg *et al.* (1998 a, b) demonstrated the ability of *T. pretiosum* and *T. minutum* to parasitize and develop on noctuids eggs.

Results of the laboratory and greenhouse experiments suggest a potential for these two *Trichogramma* species to be used in field augmentation efforts. However, additional studies are needed to evaluate the potential impact of *Trichogramma* in cotton fields. If used in biocontrol strategies incorporating *Trichogramma* to control *Helicoverpa* eggs, this will reduce the cost of insecticide and man power for pest control by 73.4% and 27% respectively, compared to insecticides (Nurindah and Teger 1993).

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Chapter 2

Predacious efficiency of *Chrysoperla carnea* on *Helicoverpa armigera* at different temperatures and on 3 cotton cultivars

ABSTRACT

The predation rates of *Chrysoperla carnea* (Stephen) (Neuroptera: Chrysopidae) on *Helicoverpa armigera* (Lepidoptera: Noctuidae) eggs and larvae were investigated including the prey's influence on survival and development. The experiments were done in the laboratory in temperature controlled cabinets, using 3 different temperatures (20°, 25° and 30°C) and in the greenhouse. *C. carnea* were fed on eggs and 1st instars of *Helicoverpa* larvae. In both cases, daily predation rates of *C. carnea* increased during the two first instars and reached a peak in the third one. The third instars consumed 85.3 and 79.1 % of the total numbers of *Helicoverpa* eggs and larvae, respectively. *C. carnea* preyed on a mean total of 218 eggs and 182 larvae of *H. armigera* during its larval development. The largest numbers of eggs were consumed by third instar larvae; 67.24, 85.36 and 59.1% of all eggs were preyed. For whole preyed larvae, those were 61.6, 79.1 and 64.9% at 20°, 25° and 30°C, respectively. The developmental time of *C. carnea* fed on *H. armigera* eggs was 36.5, 19.3 and 15.6 days, and for *C. carnea* fed on *H. armigera* larvae it was 36.3, 20.3 and 18.4 days. In the greenhouse, predation rates of *C. carnea* on *H. armigera* eggs reached a peak at the 2nd day and 98.3% of the eggs were consumed. Predation rates differed significantly between the position of leaf on the cotton plant; eggs on the 5th leaf were consumed more often than those on the 1st leaf. Cotton cultivars had no significant influence on the predation rate.

Keywords: *Chrysoperla carnea, Helicoverpa*, cotton cultivars, temperatures

INTRODUCTION

Cotton is grown in over seventy countries and is one of the most important cash crops in the world. Cotton plays a vital multi-sectoral role in the economies of many developing countries, earning foreign exchange and providing employment for millions of people (Reddy *et al.* 1997). Cotton production has suffered setbacks; one important problem is the impact of the American bollworm *Helicoverpa armigera*. This lepidopteran species is a highly polyphagous pest and its management is difficult. Multiple insecticide applications have led to high levels of resistance to major groups of active compounds (Fitt 1989; Armes *et al.* 1996). Pest identification, monitoring, and prevention are essential elements of successful cotton production (Hansen 1996). This is especially true for organic cotton production because most of the pesticides that are currently used by producers of conventionally grown cotton are not approved for use by growers of organic cotton. Moreover, the fewer pest control products legislized in organic cotton are generally not as effective as synthetic pesticides for immediate or acute problems. One promising option could be to manage cotton pests by release of natural enemies. Augmentive release of parasitoids and predators could help to increase the level of naturally established natural enemies additionally those which are already established in cotton fields (Klonsky *et al.* 1996). The development of a strategy that conserve and maximize the abundance as well as effectiveness of natural enemies will be crucial in the management of cotton pests.

Chrysoperla spp. have long been considered an important naturally occurring predators in many agricultural cropping systems, including fiber crops (cotton) vegetables, fruits, and forage crops (Stark and Whitford 1987). Worldwide, *Chrysoperla* spp. is ranked as one of the most commonly used and commercially available natural enemies (Ables and Ridgway 1980; King *et al*. 1985). In addition, *Chrysoperla* larvae have many desirable attributes for use in biological control, and inhabit many different agroecosystems (Ridgway and Murrphy 1984; King *et al.* 1985). *Chrysoperla* spp. are tolerant to many insecticides (Croft 1990; Wetzel *et al*. 1991) and are quite easily mass reared (Tulisalo 1984; Hasegawa *et al.* 1989).

The effectiveness of *C. carnea* in controlling noctuids pests and aphids on various crops has been studied by several authors (Scopes 1969; El-Dakroury *et al.* 1977; Dean 1983; Megahed *et al.* 1984; Sengonca and Grooterhorst 1985; Stark and Whitford 1987; Obrycki *et al.* 1989; Hagely 1989). They reported that *Chrysoperla* larvae achieved high mortality levels of eggs and neonate larvae of noctuids as well as aphid nymphs. However, little information is available on the predation activity of *C. carnea* on lepidopterous eggs and larvae. Obrycki *et al.* (1989) investigated consumption and feeding capacity by *C. carnea* larvae on the noctuid *Agrotis ipsilon* neonates. They reported that *C. carnea* larvae were very effective for controlling this noctuid pest. Klingen *et al.* (1996) stated that the daily predation rate of *C. carnea* on eggs and first instar larvae of *Mamestra brassicae* (Lep.: Noctuidae) increased slowly during the two first instars and reached a peak in the third larval instar.

Cotton and its insects (pests or natural enemies) are subjected to weather effects, especially temperature. These weather conditions may affect the efficiency of lacewings. Life history parameters are very important with regard to mass production and efficiency of *Chrysoperla* (Reddy 1995). Therefore, the main objectives of this study were

- To evaluate the predations rates and life history of *C. carnea* on *Helicoverpa* eggs at 3 different temperatures.
- To study the effect of 3 different cotton cultivars on the efficiency of *C. carnea*.

MATERIAL AND METHODS

Plant and insect species: See general introduction.

1. Predation experiments in laboratory

The predation experiments were conducted in the laboratory in temperature control cabinets and 70% RH and 16: 8 h (L: D) to evaluate the predacious efficiency of larval instars of *Chrysoperla* on *Helicoverpa* eggs and larvae.

a) ***Helicoverpa*** **eggs**

First instars of *C. carnea* larvae were taken from a stock culture immediately after hatching and kept individually in glass vials (50ml) covered with gauze. *Helicoverpa* eggs were provided to *Chrysoperla* larvae, and the consumed eggs were counted and replaced by new eggs at daily intervals. To prevent hatching of the eggs, and to ensure that the eggs had an equal nutritional value, only *H. armigera* eggs not older than 24h were used. The numbers of eggs offered to the predator were in access to what they were able to prey on all predation stages. Ten replicates of *Chrysoperla* larvae were used.

b) ***Helicoverpa*** **larvae**

Newly-hatched *C. carnea* larvae were provided with 2-3 day old larvae of *H. armigera* and kept individually in glass vials (50ml). Numbers of consumed larvae were recorded daily, and all larvae were replaced by new ones. Ensure that *C. carnea* larvae were supplied with enough prey items; they were given 50% more *H. armigera* larvae than *C. carnea* were able to consume. Ten replicates of *Chrysoperla* larvae were used.

2. Predation experiments at 3 different temperatures

The predation experiments were conducted at 3 different temperatures (20°, 25° and 30°C) at 70% RH and 16: 8 h (L: D). These experiments were conducted with *Helicoverpa* eggs and larvae using similar experiments as in laboratory studies. Ten replicates (glass vials) of *Chrysoperla* larvae were used for each temperature.

The two predation experiments using *Helicoverpa* eggs and larvae were conducted during the entire larval development of *C. carnea* to pupation. Predation rates for each instar of *C. carnea* were calculated to estimate the predacious capacity for the entire life cycle of *Chrysoperla* larvae. Developmental time of *Chrysoperla* larvae was recorded for each instar by estimating the days between each molting.

3. Predation experiments in the greenhouse

Based on the laboratory experiments, greenhouse experiments were conducted using 20 *H. armigera* eggs which were attached by TRAGANT glue (Fluka, Germany) on the 1st, 3rd and 5th leaves of 3 different cotton cultivars; Giza 89, Giza 86 and Alex 4. Cotton plants were placed inside wooden cages (60 X 100 X 70cm). The 3rd instar *Chrysoperla* larvae were released into the cages containing the attached *Helicoverpa* eggs (60 eggs/ plant). The host eggs were replaced daily until *C. carnea* pupation. The consumed eggs were counted to calculate the predation rates. Three plants of each cultivar per cage were replicated 6 times.

Statistical analysis

Statistical analyses were conducted using analysis of variance (ANOVA) followed by comparing means of treatments using the Bonferroni correction by Program SYSTAT 8.0 (Wilkinson *et al.*1998). Percentage data were arcsine transformed prior to analyses.

RESULTS

1. Predation experiments in the laboratory

Daily predation rates of C. *carnea* on *H. armigera* eggs and larvae in the laboratory increased gradually with increasing larval age during 3 larval instars until pupation. Predation rates reached a peak at the 10th and 11th day when fed on eggs and larvae, respectively. Mean number of *H. armigera* eggs and larvae consumed by all three larval instars of *C. carnea* were 218.7 and 182.2, respectively (Fig. 1).

Predation rates of *Chrysoperla* on eggs of *H. armigera* were 5.2, 9.5 and 85.3% for 1st, 2nd and 3rd instars, respectively. Predation rates on *Helicoverpa* larvae were 5.3, 15.6 and 79.1% for 1st, 2nd and 3rd instars of lacewing larvae (Fig. 2). Predation rates were significantly different (df= 2; F= 9.7; P< 0.001) between the 3 larval instars of *C. carnea.*

H. armigera eggs and larvae were equally suitable as preys for *C. carnea* larvae. The developmental time of *C. carnea* took 19.1 and 19.8 days to complete their life cycle when fed on *H. armigera* eggs and larvae, respectively (Fig. 3) Developmental time was 2.2, 2.0 and 5.2 days for 1st, 2nd and 3rd larval instars of *C. carnea* fed on eggs, while it was 2.5, 3.7 and 7.0 days for 1st, 2nd and 3rd instars fed *H. armigera* larvae. No lacewing larvae died during the predation experiments in the laboratory.

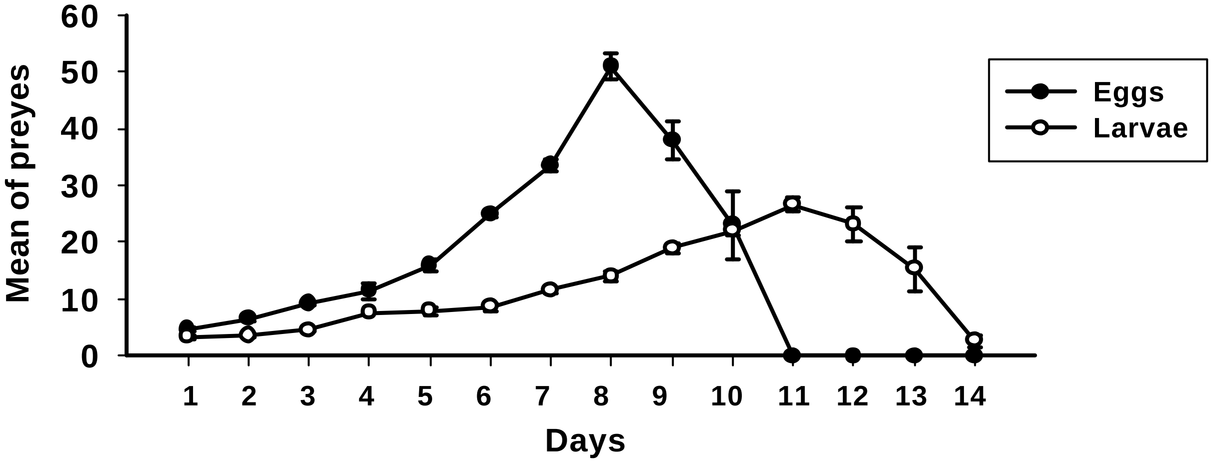

Fig. (1) Mean number of *Helicoverpa* eggs and larvae preyed by *C. carnea* larvae during development from 1st larval instar to pupation

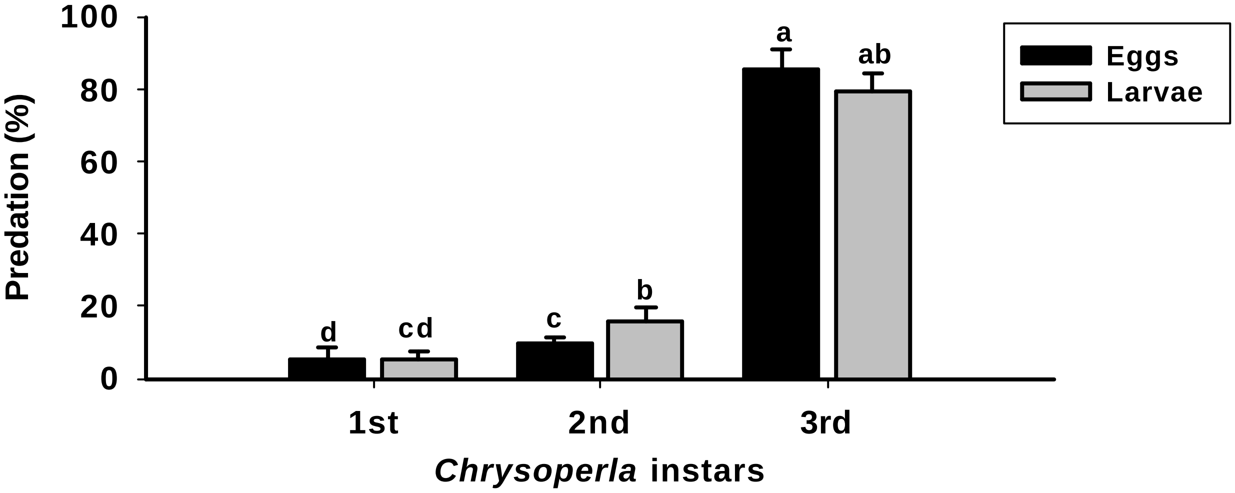

Fig. (2) Predation rates of the 3 larval instars of *C. carnea* on *Helicoverpa* eggs and larvae

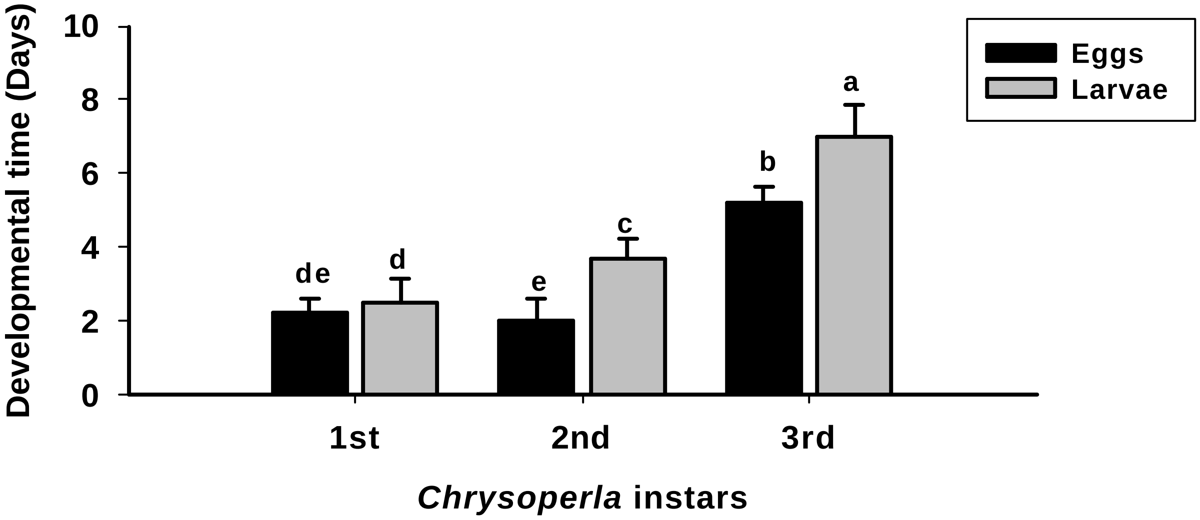

Fig. (3) Developmental time of the 3 larval instars of *C. carnea* fed on *Helicoverpa* eggs and larvae

2. Predation experiments at 3 different temperatures

a) ***Helicoverpa* eggs**

Daily predation rates of C*. carnea* larvae on *H. armigera* eggs increased gradually with increasing larval age, reach a peak on the 17th, 10th and 8th day at 20°, 25° and 30°C, respectively. Mean total *H. armigera* eggs consumed by all three larval instars of *C. carnea* were 204.5, 217.9 and 203.0 eggs at 20°, 25° and 30°C. The highest number of eggs eaten by third instars were 67.24, 85.36 and 59.1% at 20°, 25° and 30°C, respectively (Fig. 4). The eggs consumed differed significantly (df= 2; F= 7.6; P< 0.001) between 1st, 2nd and 3rd larval instars.

Predation rates of *C. carnea* on *H. armigera* eggs were 9.5, 23.2 and 67.3% for 1st, 2nd and 3rd instars at 20°C. At 25°C, they preyed on 5.2, 19.5 and 75.3% for 1st, 2nd and 3rd instars. Predation rates were 10.6, 30.3 and 59.1% for 1st, 2nd and 3rd instars at 30°C. Predacious efficacy was significantly different (df= 2; F= 21.7; P< 0.005) between the 3 larval instars. Moreover, predation rates differed significantly (df= 2; F= 19.7; P< 0.05) between the three temperatures (Fig. 5).

Developmental time of *C. carnea* fed on *H. armigera* eggs at 3 different temperatures was significantly different (df= 2; F= 2.4; P< 0.001) between the 3 larval instars. For *C. carnea* larvae at 20°C it took 36.5 days to complete their life cycle compared to 19.3 and 15.6 days at 25° and 30°C, respectively. The first and second instars needed less time (10.3, 4.2 and 5.0 days at 20°, 25° and 30°C, respectively). On other hand, the third instars took 12.1, 5.2 and 3.3 days, while the pupae took 14.1, 9.9 and 7.3 days at 20°, 25° and 30°C (Fig. 6). None of the larvae died during the egg predation experiment.

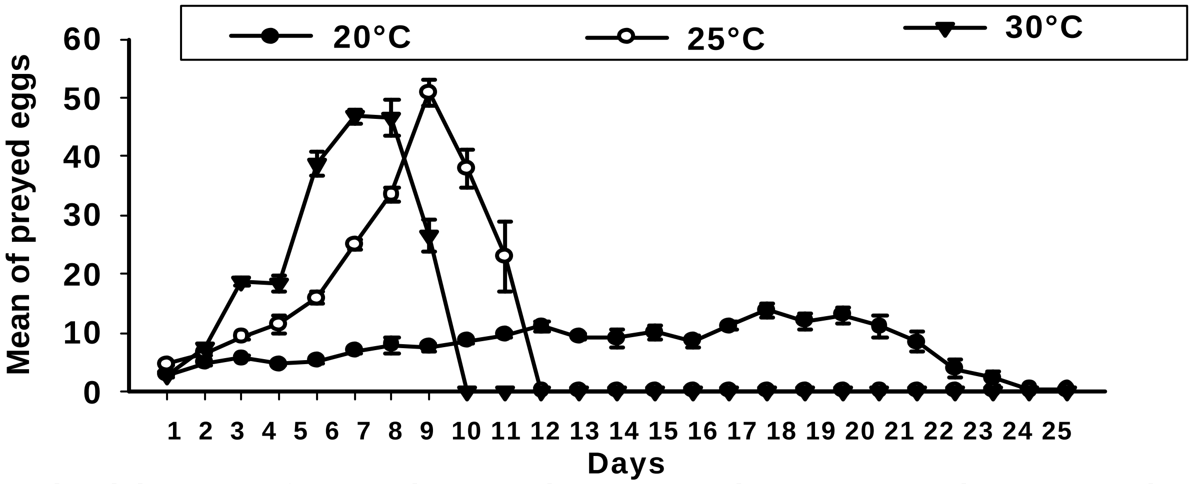

Fig. (4) Mean of preyed *H. armigera* eggs by *C. carnea larvae* per day to pupation at 3 temperatures

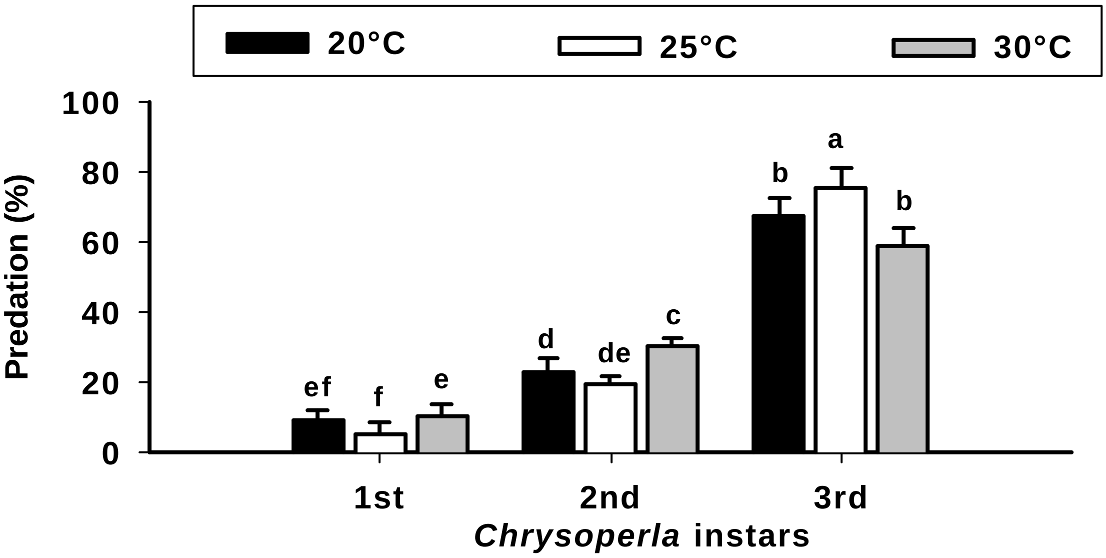

Fig. (5) Predation rates of the 3 larval instars of *C. carnea* on *H. armigera* eggs at 3 different tempertures

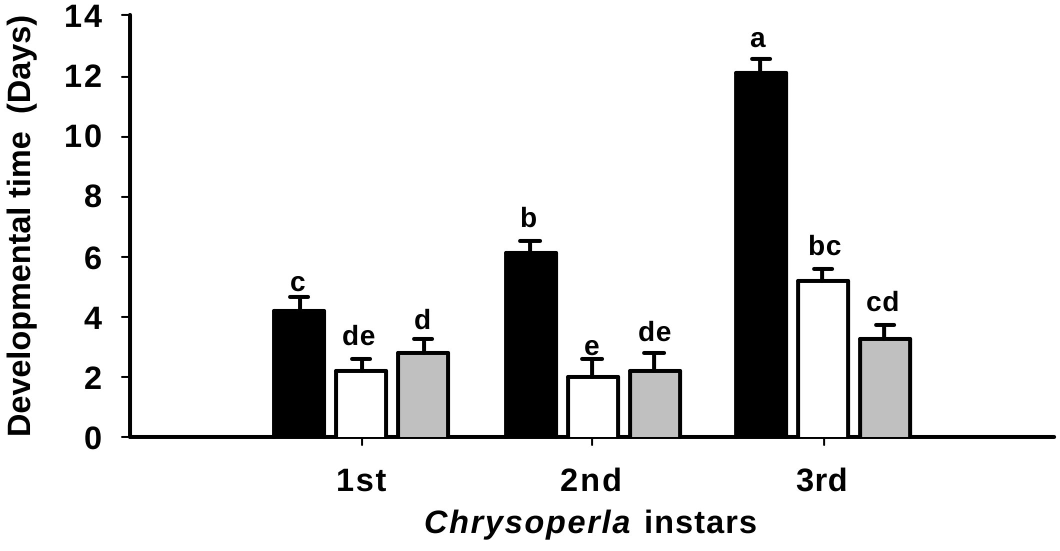

Fig. (6) Developmental time of the 3 larval instars of *C. carnea* fed on *H. armigera* eggs at 3 different temperatures

b) ***Helicoverpa* larvae**

The daily predation rates of *C. carnea* on *H. armigera* larvae increased gradually with increasing *Chrysoperla* larval age. They reached a peak on the 19th, 11th and 8th day at 20°, 25° and 30°C, respectively. *Chrysoperla* larvae consumed during all three larval instars 164.0, 180.5 and 161.4 *H. armigera* larvae at 20°, 25° and 30°C, respectively. The largest numbers of larvae eaten by third instar were 61.6, 79.1 and 64.9% at 20°, 25° and 30°C, respectively (Fig. 7). The *H. armigera* larvae consumed were significantly different (df= 2; F= 11.3; P< 0.005) between *Chrysoperla* larval instars.

Predation rates of *C. carnea* on *H. armigera* larvae were 11.2, 27.2 and 61.6% for 1st, 2nd and 3rd instars at 20°C. They were 5.3, 21.6 and 74.1% at 25°C for 1st, 2nd and 3rd instars. At 30°C, predation rates were 9.6, 25.5 and 64.9% for 1st, 2nd and 3rd instars. Predation rates were significantly different (df= 2; F= 8.7; P< 0.001) between the 3 larval instars. Moreover, predation rates significantly differed (df= 2; F= 27.0; P< 0.02) between the three different temperatures (Fig. 8).

H. armigera larvae were equally suitable as prey for *C. carnea* larvae. Figure (9) shows the developmental time of *C. carnea* fed on *H. armigera* larvae at 3 different temperatures. *C. carnea* larvae took the longest period at 20°C to complete their life cycle (36.3 days), compared to 20.3 and 18.4 days at 25° and 30°C, respectively. The first and second instars took the shortest time (10.5, 6.2 and 4.5 days at 20°, 25° and 30°C, respectively). The third instar took 11.7, 7.0 and 6.7 days, while the pupae took 14.1, 7.1 and 7.2 days at different temperatures, respectively (Fig. 9). Developmental time was significantly different (df= 2; F= 8.3; P< 0.003) between the 3 different temperatures. One *Chrysoperla* larva died during the larval predation experiment at 20°C.

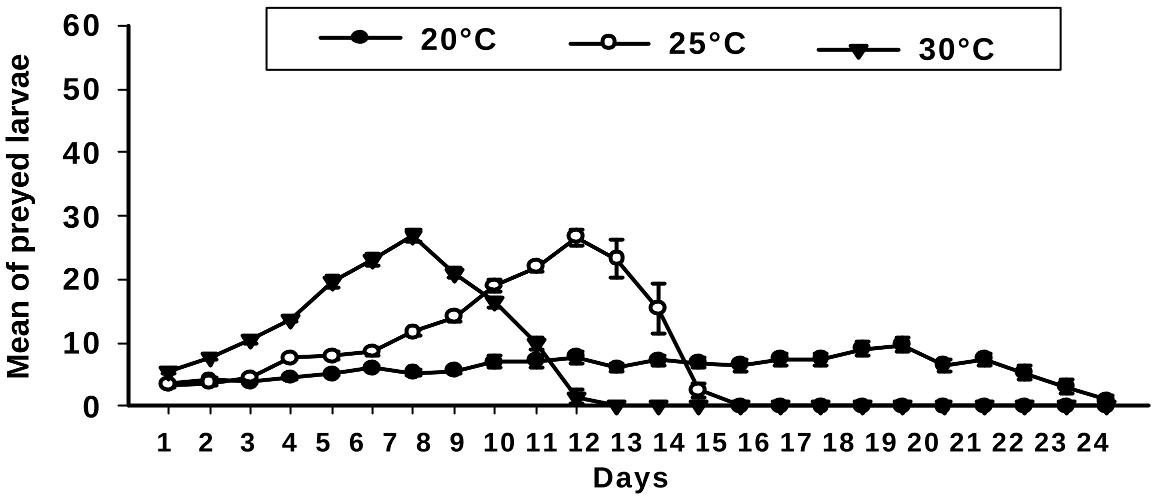

Fig. (7) Mean of preyed *H. armigera* larvae by *C. carnea* larave per day to pupation at 3 temperatures

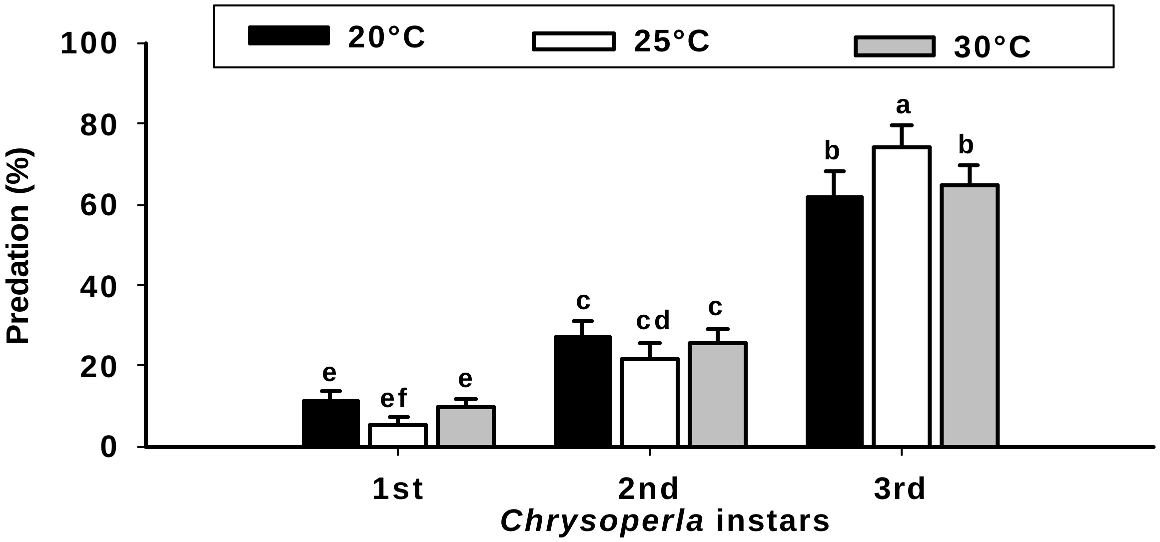

Fig. (8) Predation rates of the 3 larval instars of *C. carnea* on *H. armigera* larvae at 3 different tempertures

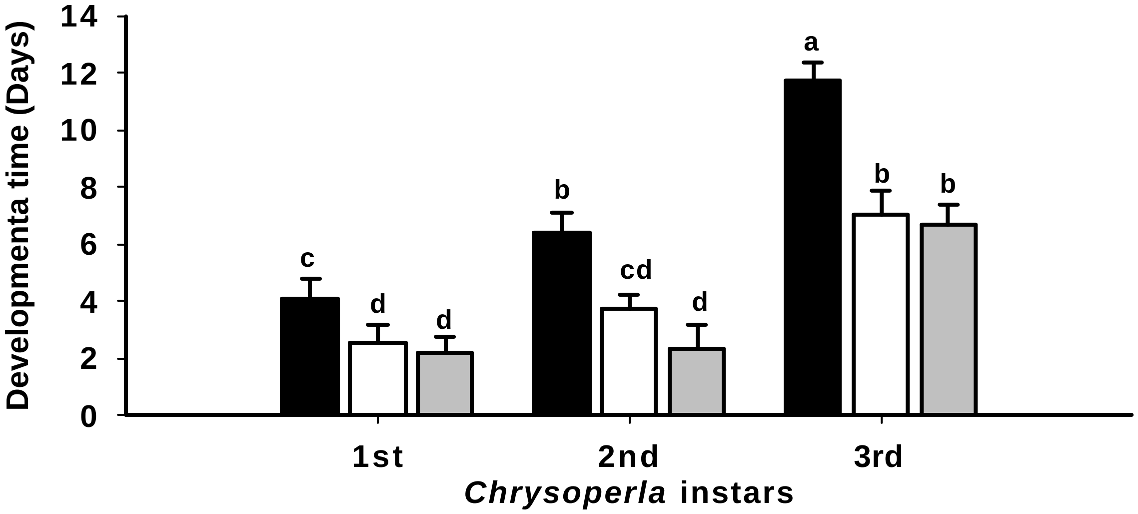

Fig. (9) Developmental time of the 3 larval instars of *C. carnea* fed on *H. armigera* larvae at 3 different temperatures

3. Predation experiments in the greenhouse

Based on the laboratory results, the third larval instar of *C. carnea* were chosen for the greenhouse experiments due to their higher predacious efficiency on American bollworm eggs. Predation rates of C*. carnea* on *H. armigera* eggs increased gradually, reached a maximum at the 2nd day to consume 98.3% of the eggs provided. Predation rates were high during the first two days (98.3%), and were moderate at 3rd day (58.0 to 77.0%). During the 4th day, predation rates decreased (10 to 27%).

The percentages of consumed *H. armigera* eggs by 3rd larval instars of *C. carnea* differed significantly (df= 2; F= 16.8; P< 0.05) between the positions of eggs on cotton leaves. *C. carnea* larvae consumed more eggs on the 5th leaf than on the 1st leaf. Predation rates were 33.3, 90.8, 68.3 and 17.5% for 1st, 2nd, 3rd and 4th day on the 1st leaf. On the 3rd leaf, they were 38.3, 95.0, 67.5 and 21.3% for the same days, while on the 5th leaf, predation rates were 50.0, 98.3, 69.2 and 27.5% for 1st, 2nd, 3rd and 4th day. Predation rates of *H. armigera* eggs did not differ significantly (df= 2; F= 37.1; P> 0.06) on the different cotton cultivars (Fig. 9). There were no dead *Chrysoperla* larvae in this experiment.

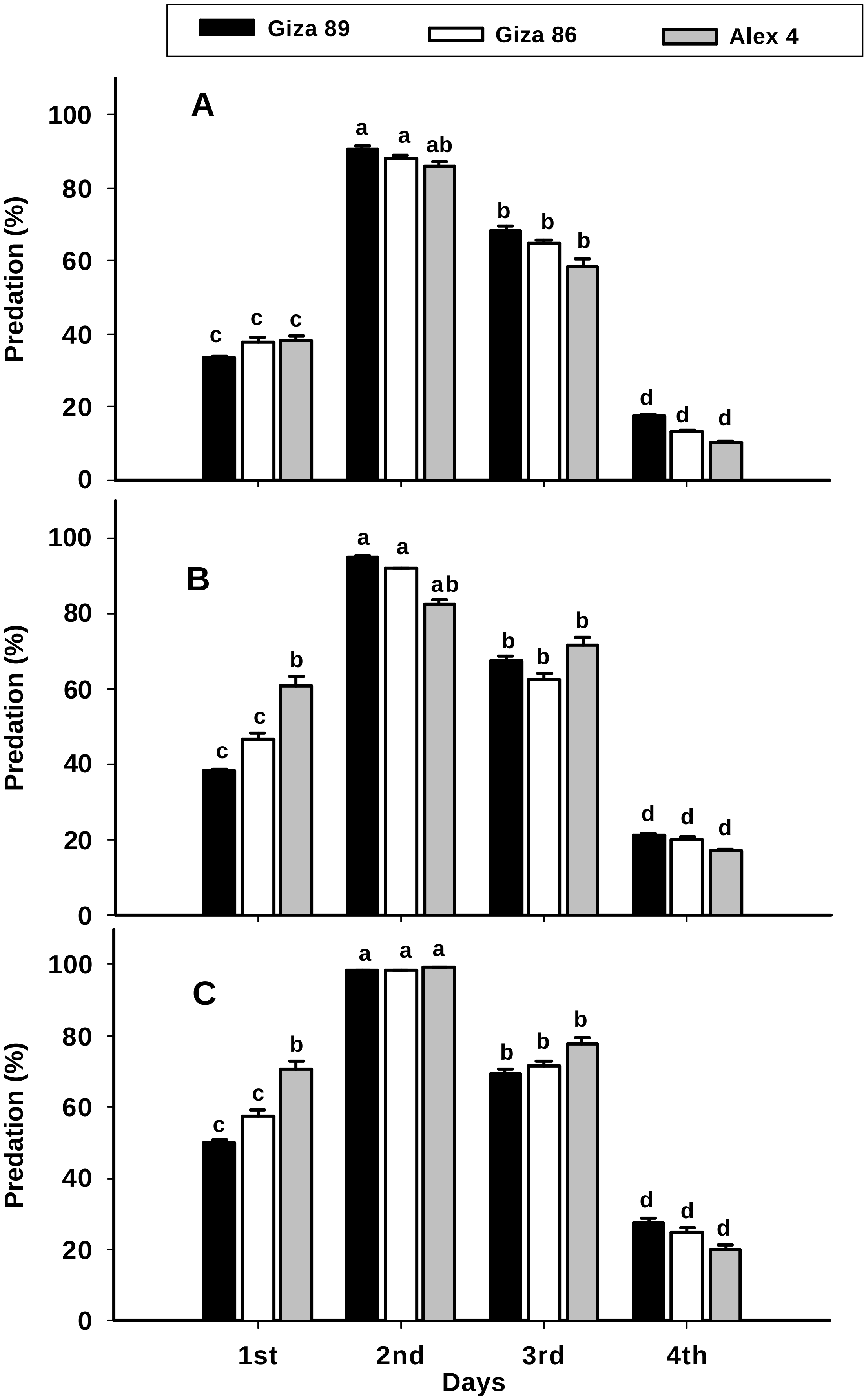

Fig. (10) Predacious effeciency of *C. carnea* on *Helicoverpa* eggs on 3 different cotton cultivars A) on 1st leaf, B) on 3rd leaf and C) on 5th leaf

DISCUSSION

The daily predation behaviour of *C. carnea* showed much the same pattern both when fed on eggs and larvae of *H. armigera.* The two feeding experiments showed that the consumption rate reached a peak in the third larval instar, consuming about 85% of the total number of eggs and larvae. This feeding behaviour was consistent with results on other host species given by several authors (Scopes 1969; Arzet 1972; El-Dakroury *et al.* 1977; Sengonca and Grooterhorst 1985; Obrycki *et al.* 1989; Hegde and Lingappa 1998). The third instars consumed more than the first two instars. The third instar consumed 85.4 compared to 14.6 by the first two instars on *H. armigera* eggs. These results are consistently with Obrycki *et al.* (1989). They used noctuids eggs and larvae as prey compared with aphids. They found that third instar of *C. carnea* larvae consumed more than 72% larvae of *Agrotis ipsilon*.

The developmental time of *C. carnea* fed on *H. armigera* eggs and larvae at 3 different temperatures showed that *Chrysoperla* larvae took the longest period to complete their life cycle at 20°C compared to 25° and 30°C. The first and second instars needed a shorter time, while the third instar took a longer time. According to New (1984) the 3rd instars *C. carnea* live longer than the first two instars.

The results of these experiments showed a difference in the total number of consumed eggs compared to consumed larvae by *C. carnea,* taking into consideration that weight of *Helicoverpa* eggs is less than that larvae. This difference in egg and larval consumption indicate that *Helicoverpa* eggs are of better nutritional quality as a prey than larvae. These results are consistent with Sengonca and Grooterhorst (1985). They found a difference between consumed eggs and larvae by *Chrysoperla.* They reported that the difference in consumption may indicate that the larval exoskeletons might represent a higher proportion per unit weight than the egg shell, assuming that neither the egg shell nor the exoskeletons are consumed. The difference in eggs and larvae consumed may be related to the developmental time of *C. carnea* larvae. Developmental time of *C. carnea* fed on *Helicoverpa* eggs was shorter than fed on *Helicoverpa* larvae. These results are consistent with that of Obrycki *et al.* (1989) and Klingen *et al.* (1996). They mentioned that the developmental time of *C. carnea* larvae fed on *M. brassicae* eggs was significantly shorter than fed on *M. brassicae* larvae.

The results indicate that the 3rd instars of *C. carnea* are sufficient to control *Helicoverpa* eggs in the greenhouse. Predation rates of C. *carnea* feeding on *H.*

armigera eggs increased gradually, reaching a maximum at the 2nd day with 98.3%. These results correspond with the results of Klingen *et al.* (1996). They reported that the daily predation rate of *C. carnea* on eggs and larvae of *M. brassicae* increased during the two first instars and reached a peak in the third larval instars.

C. carnea larvae are a promising predator of *Helicoverpa* eggs and larvae, specially the third larval instars for their high predaceous efficiency. Therefore, there is a potential for using *C. carnea* in organic cotton production to get good and clean products. Especially in cotton fields, the release of mass propagated *C. carnea* could be an option to maintain cotton production organically and protect our skin from bad residues of pesticides in cotton fibers.

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Chapter 3

Does *Chrysoperla* prey on *Helicoverpa* eggs parasitized by *Trichogramma* spp.?

ABSTRACT

One new approach in biological control of *Helicoverpa armigera* in organic cotton farms is the combined use of natural enemies (parasitoids and predators). The combination between parasitism of *Trichogramma* species and predation of *Chrysoperla carnea* on *Helicoverpa armigera* was investigated in the laboratory and greenhouse. In the laboratory, the effect of preying time of *Chrysoperla* combined with *Trichogramma* on predation of *H. armigera* eggs for 1, 12 and 24 hours was studied. Moreover, effects of *Chrysoperla* predation on parasitized eggs by *Trichogramma* species after 2-3, 5-6 and 7-8 days were investigated. In the greenhouse, combined releases of *Trichogramma* and *Chrysoperla* were studied to control *Helicoverpa* on 4 cotton cultivars. Preying time of *Chrysoperla* both affected predation and parasitism rates. Predation rates increased with increasing preying time of *Chrysoperla*. Predation rates of *Chrysoperla* on parasitized *Helicoverpa* or *Sitotroga* eggs decreased with aging of parasitized eggs. The proportion of *H. armigera* eggs parasitized when both natural enemies were present differed significantly between *Trichogramma* species, being higher in *T. pretiosum* compared to *T. minutum*. On the other hand, the proportion of *H. armigera* eggs preyed in greenhouse cages was higher in *T. minutum* compared to *T. pretiosum*. Combining *Trichogramma* species and *C. carnea* for controlling *H. armigera* eggs resulted in higher mortality rates than *Trichogramma* alone. Predation and parasitism rates were affected by different cotton cultivars; they were higher on Giza 89 than on Giza 86, and they were also higher on a *Bacillus thuringiensis* cultivar (BT) than on non-*Bacillus thuringiensis* cultivar (non-BT). In most of the experiments *Chrysoperla* did not prey on parasitized eggs

***Key words**: Trichogramma* spp., *Helicoverpa armigera*, *Chrysoperla carnea*, parasitism, predation, combined release, biological control.

INTRODUCTION

Cotton losses to insects, from the cotyledon stage through boll maturity, can be significant. If not well managed, insect pests can slash yields from about 20 to 85%. Additionally, insect pests can lower lint quality directly by feeding and indirectly by causing maturity delays (Bednarz and Ethridge 1990). The American bollworm, *H. armigera* is a major pest in many places and different agrosystems over the World. The pest has become increasingly difficult to control with the widespread use of insecticides, which made the pest resistant throughout its geographical range (Armes *et al.* 1996). It is damaging or destroying the squares, blooms and bolls of cotton. Injured squares flare and drop from plants (Fitt 1989). Successful cotton production depends on an integrated management strategy that recognizes and adapts to the unique characteristics of cotton yield. Biological control is a method used worldwide in pest management in organic cotton production. It has been considered as a sustainable, economical and environmental attractive alternative for chemical pest control (Hokkanen and Lynch 1995).

The combined release of predators and parasitoids which attack different life history stages of a pest species can improve the efficiency of biological control and help to overcome pest complexes (Keever *et al.* 1986; Press 1989; Brower and Press 1990). On the other hand, beneficial insects may adversely affect each other (Press *et al.* 1977; Verkerk and Wright 1996).

Some predatory arthropods prey on both unparasitized herbivores and herbivores that are parasitized and contain the immature stages of endoparasitoids, a form of intraguild predation. Thus, the biological control of herbivorous arthropods can be either enhanced or disrupted by introducing a predator species to an existing host-parasitoid system (Hairston and Hairston 1997)

Pintureau *et al.* (1999) reported that when *Trichogramma* species reach the prenymph stage, they secrete a substance which darkens the hosts (insect eggs). Several hypotheses have been put forward to explain the role of this dark substance. One hypothesize a protection against predators, another one assumes a reduction of the developmental duration by increasing the temperature. Thus, the dark substance allows the acceleration of the parasitoid development and probably, in an indirect way, the reduction of predation risks (Pintureau *et al.* 1999)

Ciociola *et al.* (1998) exposed *Helicoverpa* eggs to parasitism by *T. pretiosum* alone, and parasitism and predation by *T. pretiosum* and one 3rd instar larva of *Chrysoperla*. Eggs parasitized by *T. pretiosum* were not preyed by *C. carnea* in all treatments, although non-parasitized eggs were preyed. Combining *Trichogramma* and *Chrysoperla* were showing a complementary action in the control of *Helicoverpa* as compared to the one obtained by *Trichogramma* alone (Ciociola *et al.* 1998).

Colfer & Rosenheim (2001) evaluated the impact of introducing *Hippodamia convergens,* on the biological control of *Aphis gossypii,* by the parasitoid *Lysiphlebus testaceipes*. They reported that *H. convergens* did not substantially alter the percentage of aphids mummified by parasitoids and showed a partial feeding preference for unparasitized aphids over aphid mummies.

The objective of this study was to combine an egg parasitoid (*Trichogramma* species) and a predator (*C. carnea*) to increase control rates of *H. armigera*, and to test whether *Chrysoperla* prey on *Helicoverpa* eggs parasitized by *Trichogramma* species. Therefore, the following points were studied

- The effect of preying time of *Chrysoperla* in combination with *Trichogramma* on the predation rate of *Helicoverpa* eggs.
- The effects of *Chrysoperla* predation on emergence rates of parasitized eggs.
- The effect of combining *Trichogramma and Chrysoperla* in controlling *Helicoverpa* on different cotton cultivars.

MATERIAL AND METHODS

Plant and Insect species: see general introduction

1. Laboratory experiments

a. Effect preying time of *Chrysoperla*

Twenty *Helicoverpa* eggs were placed in glass vials (50ml), and were exposed to *Trichogramma* species with a ratio of 1:1 parasitoid: host (P: H), thereafter one larva of second instars of *Chrysoperla* was introduced. The predators were removed after 1 hour, 12 and 24 hours. *Trichogramma* wasps were removed after 24 hours. *Helicoverpa* eggs in the control were exposed once to *Trichogramma* without *C. carnea* and the vice versa. Black eggs and consumed eggs were counted to calculate the parasitism and predation rates. Four parameters were studied in this experiment, predation rates, parasitism rates, number of hatching eggs and remaining eggs that were neither preyed on, nor parasitized or did not hatch. Six vials were used as replications.

b. Effect of *Chrysoperla* predation on parasitized *Sitotroga* eggs by *Trichogramma* species

Twenty parasitized *Sitotroga* eggs (attached on a paper card) were placed in glass vials (50ml). In each vial the 2nd instars larval of *Chrysoperla* were introduced after 2-3, 5-6 and 7-8 days. The predators were removed after 24 hours. In the control fresh eggs were exposed to *Chrysoperla*. Consumed eggs were counted to calculate the predation rates. This experiment was replicated 6 times.

c. Effect of *Chrysoperla* predation on parasitized *Helicoverpa* eggs by *Trichogramma* species

Twenty parasitized *Helicoverpa* eggs were placed in glass vials (50ml) and the 2nd instars larval of *Chrysoperla* were introduced in each vial after 2-3, 5-6 and 7-8 days. The predators were removed after 24 hours. In the control fresh eggs were exposed to *Chrysoperla*. Consumed eggs were counted to calculate the predation rates. This experiment was replicated 6 times.

2. Greenhouse Experiments

Effect of combining *Trichogramma* and *Chrysoperla* in controlling *Helicoverpa* on different cotton cultivars

Twenty *Helicoverpa* eggs were placed in holed Petri dishes (the design allows the parasitoids and the predators to get in contact). One *Chrysoperla* larva (2nd instars) was introduced into the Petri dish, and the dishes were hanged with clips on 1st, 3rd and 5th cotton leaves. *Trichogramma* species were released into greenhouse cages with a ratio of 2: 1 parasitoid females: host egg for 24 hours. Black and consumed eggs were counted to calculate parasitism and predation rates to evaluate the effects of a *Trichogramma* species *C. carnea* combination in controlling *Helicoverpa*. For control 20 *Helicoverpa* eggs were placed in holed Petri dish (the design allows *Trichogramma* to get in contact), and the dishes were hanged with clips on the 1st, 3rd and 5th cotton leaves. *Trichogramma* spp. were released with a ratio of 2:1 parasitoid females: host egg for 24 hours. Black eggs were counted to calculate the parasitism rates in the control cages. Four parameters were studied, predation rates, parasitism rates, number of hatching eggs and remaining eggs that were neither preyed on nor parasitized or did not hatch). Four cotton cultivars were used; *Gossypium hirsutum* DPL 422B/R (BT) and DPL 420R (non-BT) and *G. barbadense* Giza 89 and Giza 86. Three cotton plants were replicated 6 times in each cultivar.

Statistical Analyses

Statistical analyses were conducted using analysis of variance (ANOVA) followed by comparing means of treatments using the Bonferroni test by Program SYSTAT 8.0 (Wilkinson *et al.*1998). The percentage data were arcsine transformed prior to analyses.

RESULTS

1) Laboratory experiments

a) Effect of preying time of *Chrysoperla* with *Trichogramma* spp. on *H. armigera* egg mortality

Predation rates were 12.5, 26.7 and 37.5% for 1 hour, 12 and 24 hours of preying of *Chrysoperla*. Conversely, parasitism rates of *T. pretiosum* combined with *C. carnea* larvae were 86.0, 68.3 and 55.0% for 1 hour, 12 and 24 hours. There were no eggs hatching and the remaining eggs rates were 1.5, 5.0 and 7.5% for 1, 12 and 24 hours. Parasitism rates significantly differed (df=1; F= 2.14; P< 0.001) between *Trichogramma* alone and that combined with *C. carnea* for 24 hours; they were 96.0 and 55%, respectively. Predation rates was significantly different (df=1; F= 9.8; P< 0.02) between *Chrysoperla* alone and that combined with *T. pretiosum* for 24 hours; they were 49.5 and 37.5%, respectively (Fig. 1 A).

Predation rates were 10.8, 24.2 and 36.7% for 1 hour, 12 and 24 hours of preying of *Chrysoperla*. On the other hand, parasitism rates of *T. minutum* combined with *C. carnea* larvae were 85.8, 70.0 and 56.7% for 1 hour, 12 and 24 hours. There were no eggs hatching and the remaining eggs rates were 3.3, 5.8 and 6.7% for 1 hour, 12 and 24 hours. Parasitism rates significantly differed (df=1; F= 3.2; P< 0.001) between *Trichogramma* alone and that combined with *C. carnea* for 24 hours; they were 93.0 and 56.7%, respectively. Predation rates was significantly different (df=1; F= 11.1; P< 0.025) between *Chrysoperla* alone and that combined with *T. minutum* for 24 hours; they were 47.2 and 36.7%, respectively (Fig. 1 B). Predation rates increased with increasing preying time of *Chrysoperla*, and parasitism rates decreased with increasing preying time of *Chrysoperla.*

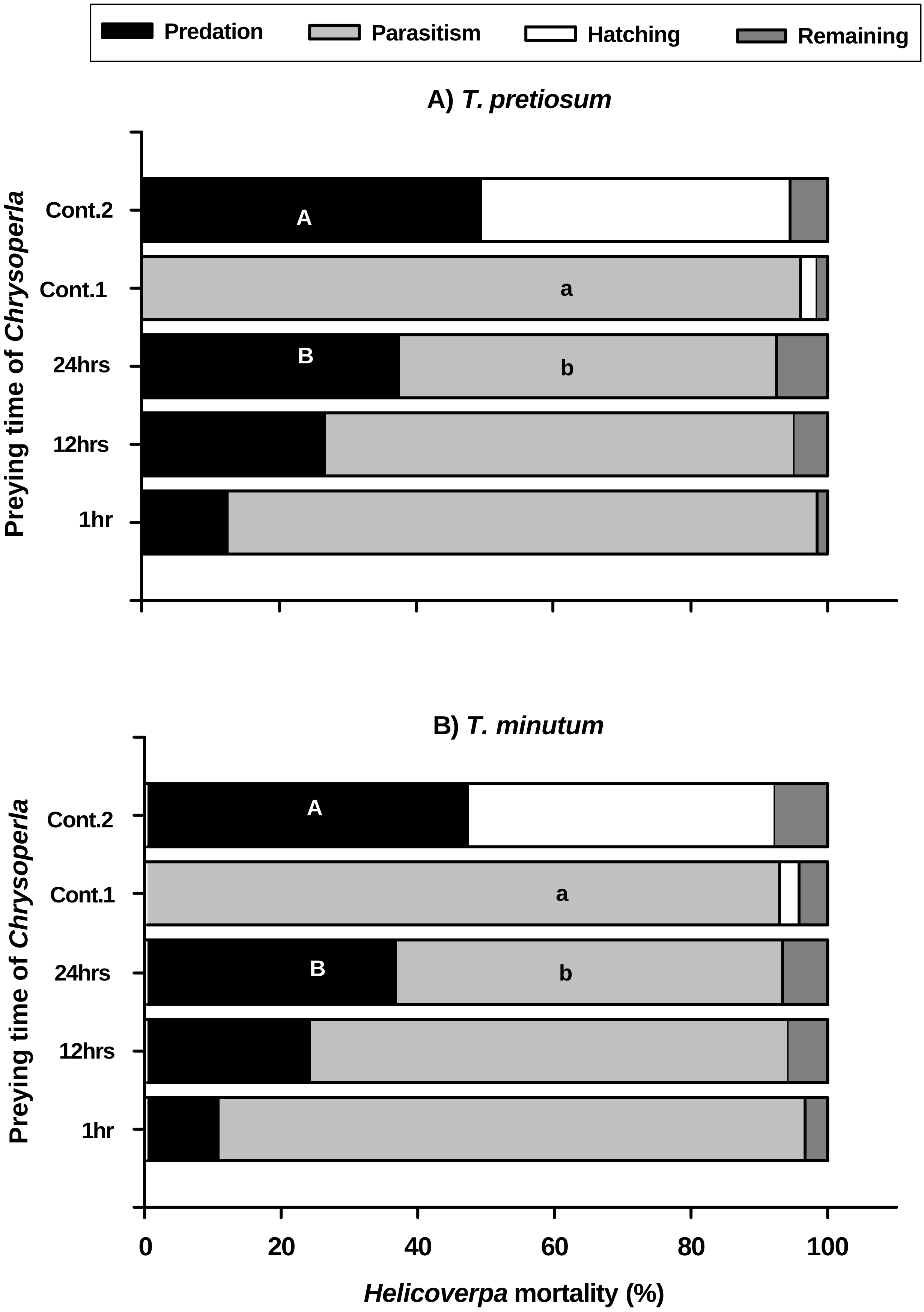

Fig. (1) Effect of preying time of *Chrysoperla* (1, 12 and 24 hours) combined with *Trichogramma* species on *H. armigera* egg mortality

b) Effect of *Chrysoperla* predation on parasitized *Sitotroga* eggs

There was a significant difference (df= 3; F= 15.2; P< 0.002) between predation rates of *Chrysoperla* on parasitized eggs by *T. pretiosum* for 2-3, 5-6 and 7-8 days and control. Predation rates were 36.7, 29.2 and 19.2% for 2-3, 5-6 and 7-8 days, respectively. *Chrysoperla* preyed 46.9% of fresh eggs (control) (Fig. 2 A)

There was a significant difference (df= 3; F= 17.5; P< 0.004) between predation rates of *Chrysoperla* on parasitized eggs by *T. minutum* for 2-3, 5-6 and 7-8 days and control. Predation rates of *Chrysoperla* were 34.2, 25.0 and 20.8% for, 2-3, 5-6 and 7-8 days, respectively. *Chrysoperla* preyed 45.9% of fresh eggs (Fig. 2 B)

c) Effect of *Chrysoperla* predation on parasitized *Helicoverpa* eggs

Predation rates were significantly different (df= 3; F= 16.3; P< 0.001) on parasitized *Helicoverpa* eggs by *T. pretiosum* for 2-3, 5-6 and 7-8 days and control. Predation rates of *Chrysoperla* were 30.8, 18.3 and 11.7% for 2-3, 5-6 and 7-8 days, respectively. *Chrysoperla* preyed 53.7% of fresh eggs (Fig. 2 C).

Predation rates were significantly different (df= 3; F= 19.2; P< 0.003) on parasitized *Helicoverpa* eggs by *T. minutum* for 2-3, 5-6 and 7-8 days and control. Predation rates of *Chrysoperla* were 33.3, 16.7 and 12.5% for 2-3, 5-6 and 7-8 days, respectively. *Chrysoperla* preyed 53.7% of fresh eggs (Fig. 2 D).

Predation rates were affected by age of eggs parasitized. Predation rates decreased with aging the parasitized eggs. Predation rates on parasitized *Helicoverpa* eggs were lower than on parasitized *Sitotroga* eggs. Predation rates on fresh *Helicoverpa* eggs were higher than on fresh *Sitotroga* eggs.

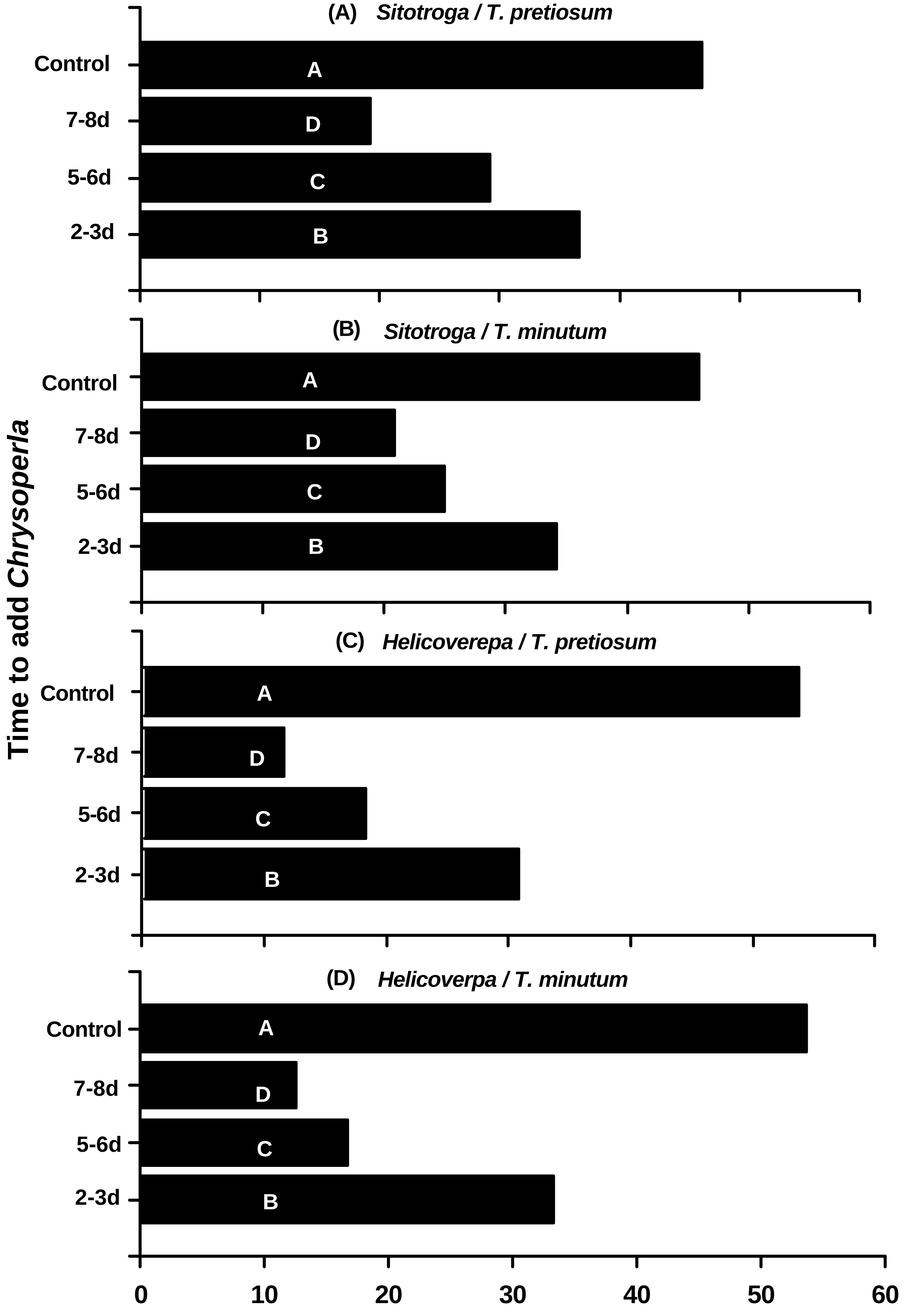

Fig. (2) Effect of *Chrysoperla* predation on *Sitotroga* and *Helicoverepa* eggs parasitized by *Trichogramma* species

2. Greenhouse Experiments

Effect of combination *Trichogramma* species and *C. carnea* in controlling *Helicoverpa* on different cotton cultivars

There was a significant difference (df= 1; F= 18.6; P< 0.05) between predation rates of *Chrysoperla* combined with *Trichogramma* species on Giza 89 and Giza 86 (Fig. 3 A). Predation rates were 39.4 and 37.4% on Giza 89 and Giza 86, respectively. Parasitism rates differed significantly (df= 3; F= 8.2; P< 0.003) between *T. pretiosum* alone and that combined with *C. carnea* larvae. Parasitism rates in *T. pretiosum* combined with *C. carnea* were 55.6 and 53.1% on Giza 89 and Giza 86 cultivars compared to *T. pretiosum* alone which achieved 93.0 and 92.0% on the same cotton cultivars. Hatching rates were 2.7 and 4.6% on Giza 89 and Giza 86 cultivars. The remaining eggs rates were 2.2 and 4.8%, respectively.

Predation rates of *Chrysoperla* were 44.4 and 42.0% for Giza 89 and Giza 86, respectively. Parasitism rates differed significantly (df= 3; F= 14.8; P< 0.004) between *T. minutum* alone and that combined with *C. carnea* larvae. Parasitism rates in *T. minutum* were 49.9 and 48.5% on Giza 89 and Giza 86 cultivars compared to *T. minutum* alone which achieved 90.0 and 89.0 % on Giza 89 and Giza 86 cultivars. Hatching rates were 2.5 and 3.3% on Giza 89 and Giza 86 cultivars. The remaining eggs rates were 3.1 and 6.2% respectively (Fig. 3 B).

Predation rates of *Chrysoperla* combined with *T. pretiosum* significantly differed (df= 1; F= 20.9; P< 0.045) on BT- and non-BT-cotton cultivars (Fig. 4 A). Predation rates of *Chrysoperla* were 43.8 and 41.5% on BT- and non-BT-cultivars, respectively. Parasitism rates differed significantly (df= 3; F= 9.6; P< 0.004) between *T. pretiosum* alone and that combined with *C. carnea* larvae. Parasitism rates in *T. pretiosum* were 53.3 and 51.7% on BT- and non-BT-cultivars compared to *T. pretiosum* alone which achieved 94.0 and 93.0% on BT- and non-BT-cotton cultivars. Hatching rates were 1.7 and 3.8% on BT- and non-BT-cultivars. The remaining eggs rates were 1.1 and 2.8%, respectively (Fig. 4 A).

Predation rates of *Chrysoperla* were 49.7 and 47.5% for BT- and non-BT-cultivars, respectively. Parasitism rates differed significantly (df= 3; F= 17.8; P< 0.006) between *T. minutum* alone and that combined with *C. carnea* larvae. Parasitism rates in *T. minutum* were 45.2 and 43.9% on BT- and non-BT-cotton cultivars compared to *T. minutum* alone which achieved 91.0 and 89.0% on BT- and non-BT-cotton cultivars. Hatching rates were 2.3 and 2.4% on BT- and non-BT-cotton cultivars. The remaining eggs rates were 2.6 and 2.1%, respectively (Fig. 4 B).

Predation rates differed significantly (df= 1; F= 15.7; P< 0.05) in *Trichogramma* species wooden cages. Predation rates were higher in *T. minutum* than in *T. pretiosum* wooden cages, because the later was higher in parasitism rates than *T. minutum*. Combining *T. pretiosum, T. minutum* and *C. carnea* for controlling *H. armigera* eggs achieved higher mortality rates than each of the *Trichogramma* species alone.

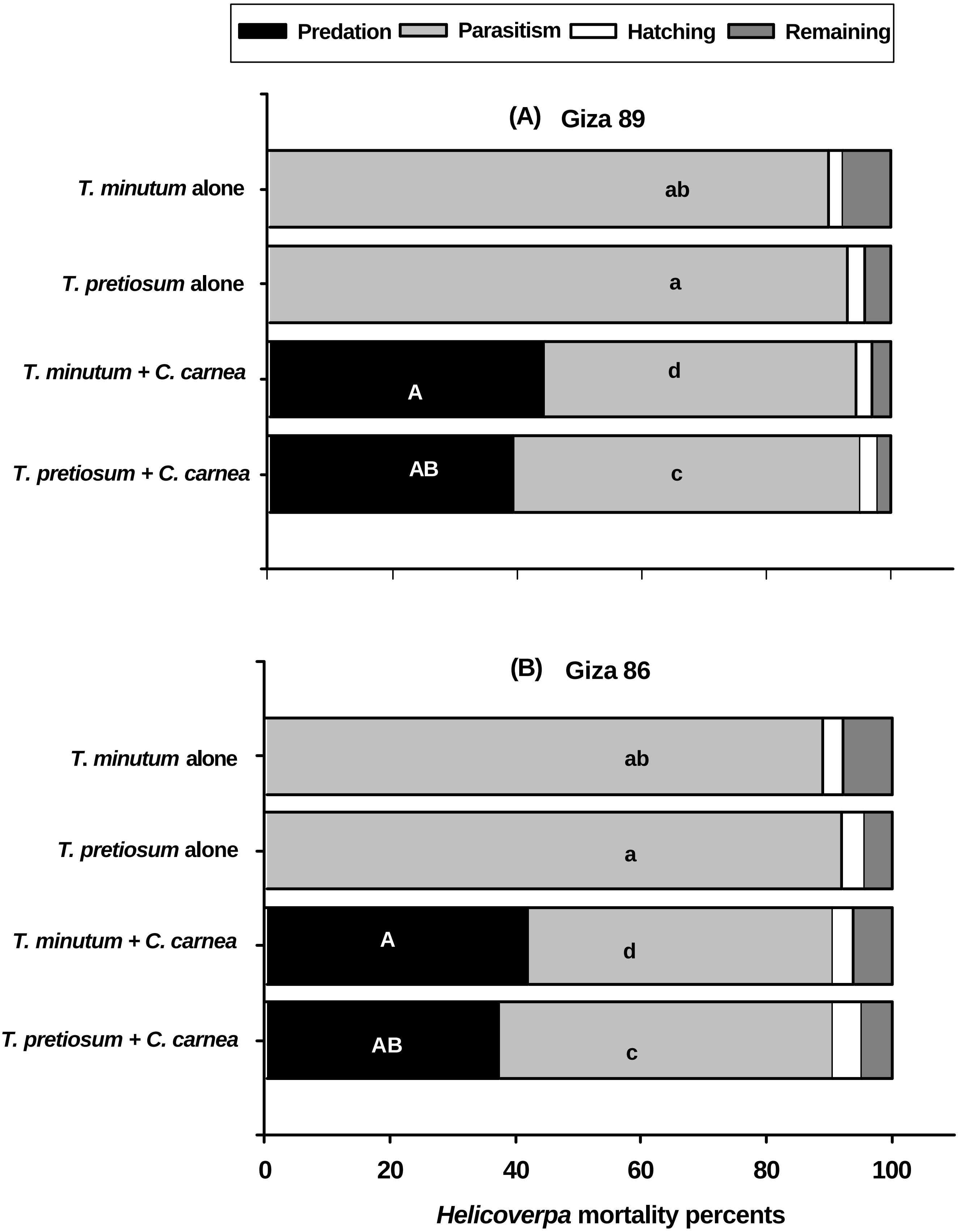

Fig. (3) Effect of combining *Trichogramma* and *Chrysoperla* for controlling *Helicoverpa* on 2 cotton cultivars

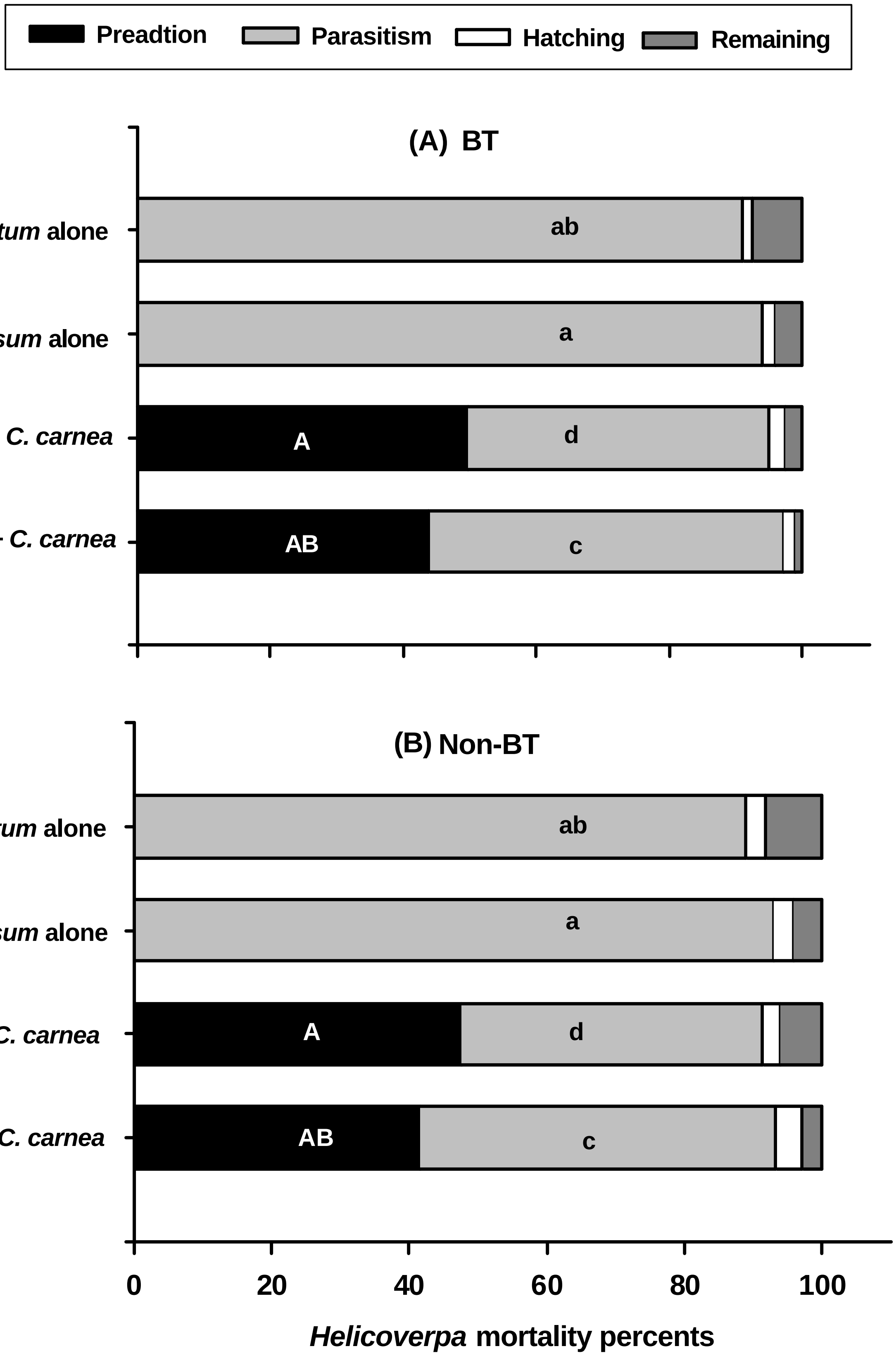

Fig. (4) Effect of combining *Trichogramma* and *Chrysoperla* for controlling *Helicoverpa* on 2 cotton cultivars

DISCUSSIONS

Preying time of *Chrysoperla* was positively related with predation rates and negatively with parasitism rates. Predation rates of *Chrysoperla* were affected by parasitism rates of *Trichogramma* species. Parasitism rates were higher in *T. pretiosum* than in *T. minutum*. *Chrysoperla* combined with *T. minutum* preyed on more eggs than combined with *T. pretiosum.* The differences found between *Trichogramma* species in the proportion of *H. armigera* eggs parasitized are due to differences in host suitability for *Trichogramma* species after combining with *C. carnea* larvae. This result is consistent with Dijken *et al.* (1986) and Pak *et al.* (1990) and Silva and Stouthamer (1998). They reported that variability in parasitism rate is due to host suitability not to host acceptance.

The results indicated that age of parasitized eggs affected predation rates of *Chrysoperla.* Predation rates of *Chrysoperla* were related negatively with age of parasitized *Helicoverpa* eggs. These findings are consistent with Pintureau *et al.* (1999). They reported that the dark substance in parasitized eggs allows the acceleration of the parasitoid development and probably, in an indirect way, the reduction of predation risks. Predation rates of *C. carnea* decreased on parasitized eggs. The results correspond with Stark and Hopper (1988). They indicated that *C. carnea* larvae preferred un-parasitized larvae more than parasitized larvae, when both parasitized and un-parasitized larvae were exposed together to the predator on cotton in field cages. These results are also consistent with Godfray and Pacala (1992). They reported that interactions between predators and parasitoids in heterogeneous environment lead to the persistence of predator-prey and host-parasitoid systems.

The results show that predation rates were higher in *T. minutum* greenhouse cages than in *T. pretiosum* cages, because *T. pretiosum* had higher parasitism rates than *T. minutum*. There was a negative relationship between parasitized eggs and predation of *Chrysoperla*. *Chrysoperla* larvae did not prey on parasitized *Helicoverpa* eggs compared to un-parasitized eggs. This may be due to the black color of parasitized eggs or due to volatile compounds released from these eggs. These results are similar with Ciociola *et al.* (1998). They reported that eggs parasitized by *T. pretiosum* were not preyed compared to un-parasitized eggs in all treatments (either combining with *Chrysoperla* or with *Doru luteipes*). The results indicated that preying non-parasitized eggs compared to parasitized eggs showing a complementary action in the control of *Helicoverpa* combining with *Trichogramma* species.

Combining *T. pretiosum, T. minutum* and *C. carnea* for controlling *H. armigera* eggs resulted in higher mortality rates than each of the *Trichogramma* species alone. These results are consistent with Ciociola *et al.* (1998) and Suh *et al.* (2000). They reported that the percentage of *H. armigera* eggs that hatched in natural enemies release plots (*Trichogramma* species and *C. carnea*) was significantly reduced compared to control plots. The results suggest a potential for using *T. pretiosum* and *T. minutum* in combination with *Chrysoperla* for controlling *H. armigera*. Conceivably, it will be interesting to combine *Trichogramma* species with *C. carnea* in organic cotton fields without risks of predation by *C. carnea* on *H. armigera* eggs parasitized by *Trichogramma* species. However, other environmental factors could also play a role in determine this combination.

Predation and parasitism rates were affected by different cotton cultivars. These rates were higher on Giza 89 than on Giza 86, and they were also higher on BT than on non-BT-cultivar. These results correspond with Silva and Stouthamer (1998). They mentioned that there were differences in predation and parasitism rates depending on different plant hosts and different agroecosystems where Chrysopidae are present in the fields. Some cotton cultivars emitted volatiles more than the other, the parasitoids and predators may be attracted more to those cultivars (Elzen *et al.* 1987).

Parasitoids and predators of pests can affect each other in biological control programs. It should be an incentive to choose carefully from the still many species available the one(s) to release according to the specific characteristics of the agroecological system one is dealing with. Furthermore, biological control should be harmoniously integrated in a total system approach in Integrated Pest Management programs for organic cotton production.

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Chapter 4

Impacts of cotton traits on the parasitization of *Helicoverpa armigera* eggs by *Trichogramma* species

ABSTRACT

The demand for cotton grown organically is rapidly increasing. However, the substantial impact of herbivorous insect pests and the lack of effective and sustainable biological control strategies for most pest species are the main limiting factors for growers to change from conventional production system to organic cotton production system. The large scale application of antagonists such as *Trichogramma* species in the organic cotton production is limited by the scares information about the influence of host plants traits on their efficiency and sustainability in regulation of pest populations.

In greenhouse experiments, the vertical and horizontal distribution of *Helicoverpa armigera* eggs was manipulated. *T. minutum* and *T. pretiosum* significantly differed in searching behaviour as measured by parasitization rates on different cotton cultivars. The parasitization rates were higher on the upper and lower leaves than on the middle leaves of the respective cultivars. Furthermore, parasitization rates were negatively correlated to the distance between the releasing site for *Trichogramma* species and egg batches of the herbivore on the cotton plants. However, the magnitude of variation was strongly influenced by cotton cultivars. Morphological traits, i.e. presence of black glands or trichome densities of the cotton cultivars played a significant role. The parasitization rates on cultivar with glanded and less trichome density were higher than on cultivar with non-glanded and high trichome density.

Moreover, results from GC-MS analysis revealed that, volatiles and the phytosterols composition of leaves are significantly different for cotton cultivars. These chemical traits of host plants are considered in relation to a possible-influencing factor for *Trichogramma* behaviour.

Key words: *Trichogramma pretiosum, T. minutum,* searching behaviour, organic cotton, volatile and sterols compounds, *Helicoverpa armigera*

INTRODUCTION

Cotton production has suffered setbacks; one important problem is the impact of *Helicoverpa armigera*. This insect pest is a highly polyphagous pest and its management is difficult. Multiple insecticide applications have led to high levels of resistance to major groups of active compounds (Fitt 1989). Conceivably, biological control is an alternative method in integrated pest management programs in organic cotton. It is a product without aiding artificial fertilizers, pesticides and growth regulation and without genetic manipulation of living organisms (Alfoeldi 2001). *Trichogramma* with ca. 200 species are the largest genus and the most widely used natural enemies for the controlling of lepidopteran pests (Romeis *et al.* 1997).

Although the genus has attracted considerable attention for over a century and play a significance role in the biological control of insects, there is only limited information regarding the influence of plant physical and chemical plant traits on the behaviour of *Trichogramma* species. Several plant characters have been presumed to affect the searching behaviour and efficiency of *Trichogramma* egg parasitoids (Romeis *et al.* 1998).

Most research on the effect of experience on carnivore foraging behaviour has considered rewarding experiences such as the finding of a host or a host's products (Vet *et al.* 1995, 1998). Previous studies on the effect of physical characteristics on behaviour of parasitoids have focused on a single component, i.e. plant size (Thorpe 1985; Romeis *et al.* 1998). Albes *et al.* (1980) recognized three components to plant structure and studied their effects on searching behaviour of parasitoids. Successful parasitism by parasitoids is preceded by several phases of host searching that lead females into the close vicinity of their potential host (Vinson 1975).

The chemical attribute of host plants may serve as signals for the parasitoids to locate their host eggs (Turlings and Tumlinson 1992; Vet and Dicke 1992). Phytosterols are the integral component of cell membranes, which are also the indispensable nutrition for the herbivores insects (Bilger *et al.* 1997). Chemical stimuli emitted by herbivorous host or the host's food plant are often used in the host-searching process (Price *et al.* 1980; Jacqueline *et al.* 1994; Stout *et al.* 1996; Dicke 1999). Plant volatiles can also serve as a chemical defence by recruiting beneficial insects that are natural enemies of the herbivore, thereby providing an indirect protection to the plant (Turlings *et al.* 1995; Dicke and Vet 1999).

Arthropods are well known for a well-developed sense of smell (Mustaparta 1984; Powell *et al.* 1998). Carnivores can discriminate between plants of different species that are infested by herbivores of the same species (Takabayashi *et al.* 1994). Volatiles emitted from Jasmonate application or larval damage serve as essential host-location cues for parasitic insects in several agricultural species (Thaler *et al.* 1996; Paré and Tumlinson 1998, 1999; Dicke and Vet 1999; Rodriguez-Saona *et al.* 2001). By directly comparing volatile emission patterns of glanded cotton plants treated with jasmonates or damaged by herbivores, compounds induced by jasmonates can be distinguished from those that are emitted due to mechanical damage (Rodriguez-Saona *et al.* 2001).

However, no acute detrimental effects were observed in abundance or biodiversity of beneficial insects associated with transgenic BT-maize and BT-cotton, compared with non-BT-maize and non-BT-cotton (Sims 1995; Pilcher *et al.* 1997; Cannon 2000). Reports of Gould *et al.* (1997) and Heckel *et al.* (1997) strongly suggest that field populations of *Helicoverpa* have the potential to rapidly attain resistance to BT-cotton. Therefore, the main objectives of the present study were

- To test the significance of physical characteristics (leaf position, distance and position of host egg, gland and trichome densities) of cotton cultivars for efficiency *Trichogramma* species.
- To study effect of phytosterols composition of cotton cultivars on parasitization efficiency of *Trichogramma* species.
- To investigate whether either larval damage or jasmonate treatments may influence the plant volatile profile, which alter the behaviour of *Trichogramma* species on transgenic and conventional cotton plants, which rise the necessity for biological control of resistance developed of *H. armigera* in BT-cotton cultivars in the future.

MATERIAL AND METHODS

Cotton plant and insects species: See general introduction.

Effects of vertical host eggs placement manipulation on the parasitism efficiency of *Trichogramma* species

An experiment was conducted whereas the vertical eggs placement on cotton plants was manipulated. Thereby, 10 *Helicoverpa* eggs were fixed with the adhesive martial on each of the six fully expanded leaves of the cotton cultivars (Giza 86 and

Giza 89), plants were then caged and directly exposed to the respective *Trichogramma* species at the ratio of 2:1 (No. of female parasitoids: No. host egg) for parasitism. After four to five days the number of dark coloured *Helicoverpa* eggs was counted and the parasitism rate has been calculated in relation to the number of deposited eggs.

Effects of horizontal host eggs placement manipulation on the parasitization efficiency of *Trichogramma* species

A greenhouse experiment was conducted in which the distance between the placing of host eggs on cotton plants and the releasing site for parasitoids were manipulated. The cotton cultivars, Giza 89, Giza 86 and Alex 4 were arranged each in three rows at 3.5 m interval between the rows. It has been used for each cultivar three plants as replication. The parasitoids were released at either ratio of 2:1 or 5:1 ratio (No. of female parasitoids: No. host egg) at the first row for parasitism. After four to five days the numbers of dark coloured *Helicoverpa* eggs were counted and the parasitization rate has been calculated in relation to the number of deposited eggs.

Effects of gland and trichome intensity of cotton plants on the parasitization rate of *Helicoverpa* eggs by *Trichogramma*

Ten *Helicoverpa* eggs were attached as described above on the 1st, 3rd and 5th leaves of Giza 89, Giza 86 and Alex 4 and exposed to the respective *Trichogramma* species, at the ratio of 2:1 female and host eggs for parasitization. After four to five days the black eggs were counted to calculate the parasitism rates. The numbers of black glands and trichome densities within a circle of one cm diameter at five spots for the respective leaf wise counted and submitted to the correlation analysis with the parasitization rate.

Furthermore, an experiment were conducted with selected plants from the cultivar Alex4 to elucidate whether the gland trait at all do have an effect on the parasitization rate, while some of plants from this cultivar were glandless. The treatments in each experiment were tri-replicated.

Effects of jasmonate treatment and larval damage on parasitization efficiency of *Trichogramma* species

The cultivars used in this experiment were Giza 86, Giza 89 and one BT- and non-BT- cotton cultivars. Plant defenses were either induced by *Helicoverpa* larvae feeding or by treating with Methyl jasmonate.

a) Herbivore-Damaged Plants

A cohort of recently moulted fourth instars *H. armigera* from the colony was transferred from artificial diet cups to cotton plants (2 larvae/plant) 4 hr prior to the experiment to allow the larvae to habituate to the new diet, and placed inside a collection chamber containing a plant and allowed to feed for 24 hr.

b) Methyl Jasmonate-Treated Plants

Cotton was treated with MeJA (Aldrich) overnight. Plants were treated by applying 20 µl of acetone–MeJA (8:1) solution (Thaler *et al.* 1996) onto 15-cm cotton tipped applicator. Control cotton plants were exposed to 40µl acetone.

Thereafter, host eggs were mounted on treated leaves as described above and exposed to the respective *Trichogramma* species for parasitization.

Analysis of free Phytosterols and volatiles

Free phytosterols were extracted from leaves cotton cultivars Giza 86, Giza 89 and Alex4 and quantified by the procedure described in Dugassa *et al.* (1996) applying GC (Shmadzu 14A) equipped with FID, a capillary column (SPB1) and auto sampler. Phytosterols were identified by comparing the mass spectrometry obtained from the measurement by GC/MS at the institute of organic chemistry (Göttingen University) with the mass spectrometry from the online database library for sterols according to National Institute of Standards and Technology (NIST).

Plant volatiles were collected from an individual plant with closed loop stripping method as described by Boland *et al.* (1984) by using the charcoal trapping tube. Volatiles were eluted from the charcoal with 150 µl of Dichloride methane and analysed with a GC. Camphor was used as internal standard (152.4 µg of in 5 µl of Dichloride methane).

The instrument conditions were: Shimadzu model GC-14A; column: (SBP1 30 m x 0.32 mm ID, 0.25 µm film); column temperature program: 60°C for 3 min, then ramps to 280°C at 6°C/min and hold up for 10 min.; carrier gas was He at a linear flow velocity of 40 ml/min.

Furthermore, selected samples were analyzed by TRACE-GC/MS from Thermo Finnegan at the institute for organic chemistry. GC/MS was equipped with the Electron impact ionisation Mass selective detector and the capillary column DB5-MS column (25 m x 0.25 mm ID, with a 0.25-µm-thick film). The temperature program was run with an initial temperature of 60°C for 3 min, then ramps to 280°C at 6°C/min, and hold up for 10 min. The Spectral data of phytosterols and plant volatiles were used for the identification of respective compounds by comparing with commercially available standards spectrum from the National Institute of Standards and Technology (NIST 1995) database.

Statistical Analyses

Means were compared with ANOVA and followed by Bonferroni correction, using the program SYSTAT 8.0 (Wilkinson *et al.* 1998). Percentage data were arcsine transformed prior to analyses. The correlation between parasitization rates and number of glands and trichome densities was also conducted by SYSTAT 8.0.

RESULTS

Vertical host eggs location and parasitizing efficiency of *Trichogramma* spp.

Manipulating the vertical placement of *Helicoverpa* eggs on the cotton plants had an influence on parasitization rates by *Trichogramma* species. *Trichogramma* strongly parasitized *Helicoverpa* eggs on the lower and higher leaves than on the leaves placed at middle position on host plant (Fig. 1). Thereby the *Trichogramma* species differ also in their efficiency of parasitization. *T. minutum* was less in its parasitization efficiency than *T. pretiosum,* while this further influenced by the cotton cultivars. Both *Trichogramma* species shows the higher the parasitization efficiency on the cotton cultivar Giza 89 than Giza 86.

Horizontal host eggs location and parasitism efficiency of *Trichogramma* spp.

There was a negative correlation between the parasitization efficiency of both *Trichogramma* species and the distance to be covered for the locating of host eggs by the parasitoids (Fig.2). Host eggs at the highest rate were at the realign site, i.e. on plants at the first row by both *Trichogramma* species. The number of females released also influenced the parasitization efficiency of both species overall the effect of host location. The lager the number of females was released the higher was that the *Helicoverpa* eggs parasitized. *T. pretiosum* parasitized more *H. armigera* eggs than *T. minutum*. Parasitization rates for both parasitoids were influenced by location of *Helicoverpa* eggs on cotton cultivars. Parasitism rates were the highest on Alex 4 cultivar, followed by Giza 89, and the lowest one was Giza 86 for both *Trichogramma* species on *H. armigera* eggs (Fig. 2).

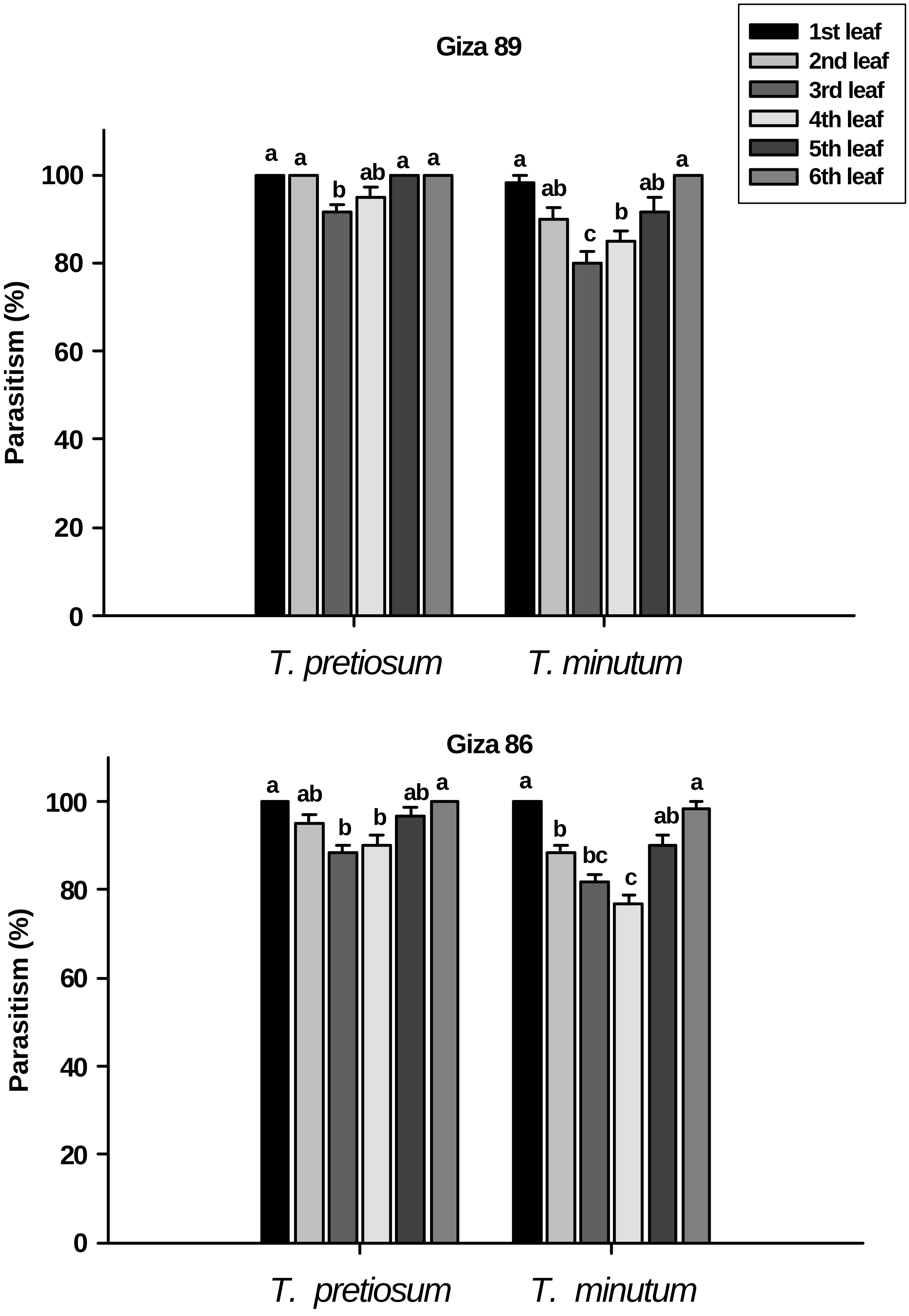
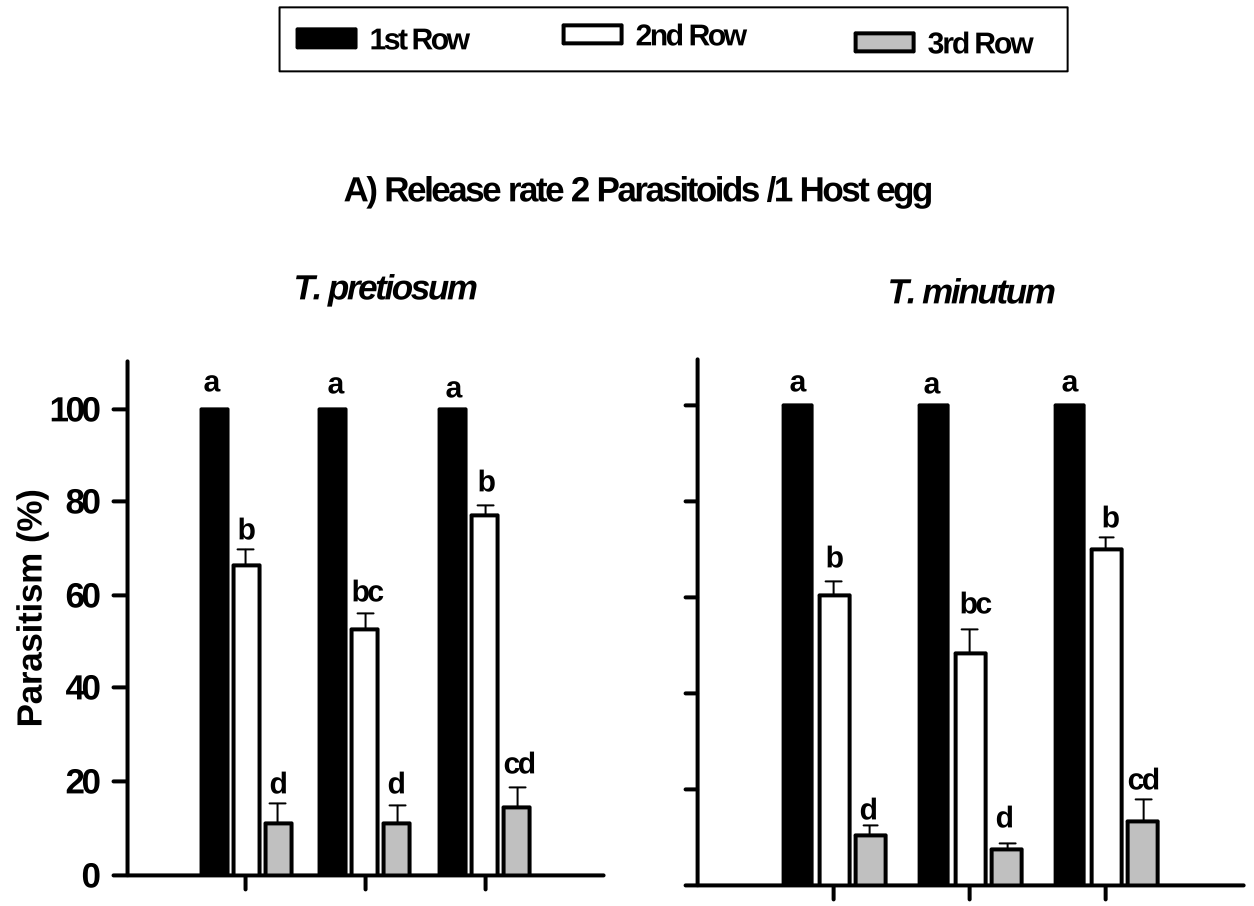


Fig. (1) Effect of cotton leaf positions on *Trichogramma* parasitism on *H. armigera* eggs on 2 cotton cultivars; different letters indicate significant differences



A) Release rate 2 Parasitoids /1 Host egg

B) Release rate 5 Parasitoids /1 Host egg

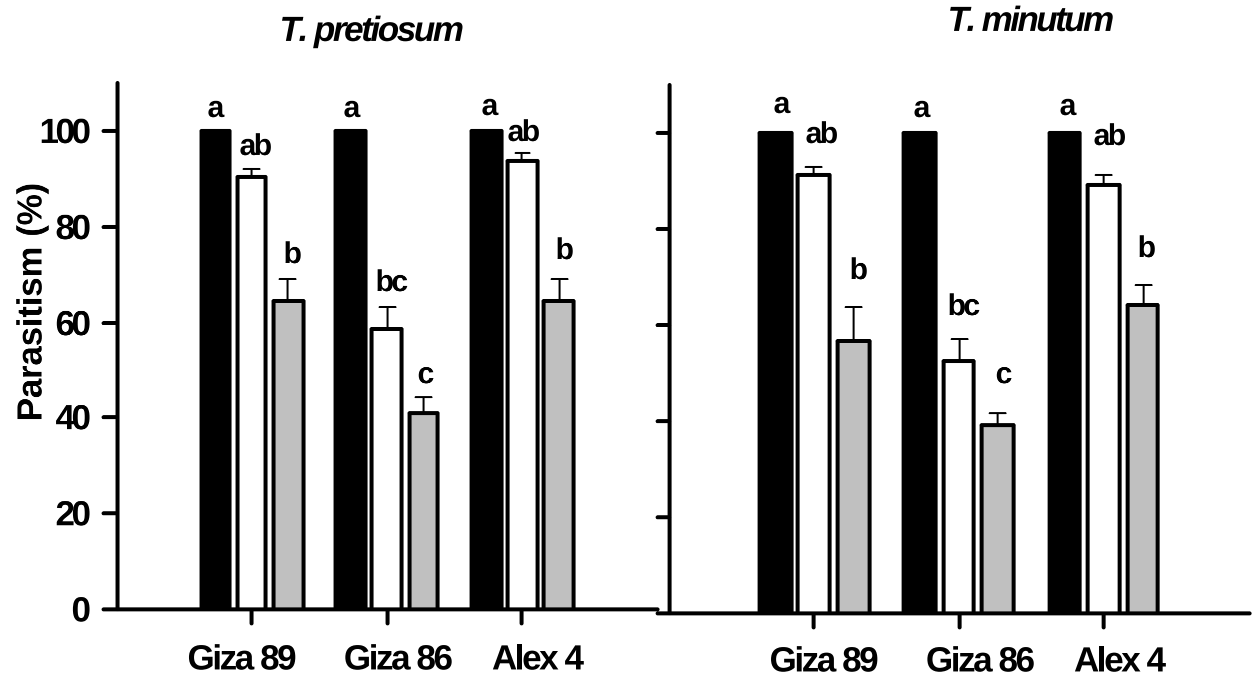


Fig. (2) Effect of distance of *H. armigera* eggs position on parasitism of *Trichogramma* species at 2 release rates and on 3 different cotton cultivars. Different letters indicate significant differences

Cultivars variation in trichome and blank gland densities of leaves

Figure (3) presents the trichome density for cotton cultivars on three different physiologically aged (lower (1st), middle (3rd) and upper (5th)) leaves. The result shows that for all cultivars the trichome density per defined leaf area increases from the lower to the upper leaves. Nevertheless cotton cultivars significantly differ from each other in their trichome density for the respective leaf position. Thus the leaves of Giza 86 were more densely trichome than the leaves of Alex 4 and Giza 89.

Figure (4) shows the number of black glands for three cotton cultivars and respective leaf position. The result shows that the numbers of black glands were also in connection with trichome density and thus increases from the lower leaves to the upper leaves for all cultivars. Although the cotton cultivars shows a significance difference in the number for blank glands, their ranging for trichome density is not in the same line for the number of blank glands. Thus leaves of Alex 4 and Giza 89 holds more blank glands than leaves of Giza 86.

Results presented in Table (1 a and b) reveals that the parasitization rate was low on cultivar (Giza 86) with high trichome density and few number of black glands as compared to cultivars (Alex 4 and Giza 89) with less trichome density and high number blank glands. Nevertheless there is no difference between both species.

Thus trichome and black gland densities on leaves of cotton cultivars strongly influence the efficiency of *Trichogramma* species to parasitize *Helicoverpa* eggs. While it seems us further very interesting to know to which extent the glanding of leaves cultivars affect the parasitization efficiency of *Trichogramma* species. An experiment was conducted with selected glandless cotton plants for the cultivar Alex 4 to compare them with the glanded plants of the same cultivar. The differences in trichome and black glands density may persuade the *Trichogramma* species to parasitize the host eggs.

Both *Trichogramma* species parasitize host eggs at high rate on glanded plants as compared to on glandless plants (Fig. 5). Yet there were significantly difference between *Trichogramma* species in parasitization efficiency, and *T. pretiosum* were more efficient than *T. minutum.*

Table (1a) Mean (±SE) parasitism rates of *Trichogramma pretiosum* and *T. minutum* on *Helicoverpa* eggs on 3 different cotton cultivars on different leaf positions in the greenhouse.

Cultivar	Leaf position	Parasitism rate *	
		T. pretiosum	*T. minutum*
Giza 89	1	84.3 ± 3.1 ab	82.2 ± 2.3 ab
	3	81.0 ± 4.6 abc	79.5 ± 6.4 bc
	5	90.6 ± 2.1 a	86.7 ± 1.9 a
Giza 86	1	80.1 ± 2.0 bc	78.1 ± 2.3 bc
	3	77.4 ± 3.9 c	75.7 ± 5.6 c
	5	87.2 ± 2.3 a	81.7 ± 1.3 abc
Alex 4	1	86.7 ± 3.2 a	84.8 ± 2.8 ab
	3	83.1 ± 5.3 ab	81.3 ± 6.5 abc
	5	92.8 ± 2.2 a	89.8 ± 2.1 a

* Different letters within and between columns indicate significant differences.

Table (1b) Correlation values (r) between parasitism rates and trichome densities and number of glands on different cotton cultivars

Cultivar	Leaf position	Trichome		Glands	
		T. pretiosum	*T. minutum*	*T. pretiosum*	*T. minutum*
Giza 89	1	- 0.41	- 0.44	0.39	0.35
	3	- 0.35	- 0.28	0.28	0.24
	5	- 0.51	- 0.48	0.52	0.51
Giza 86	1	- 0.63	- 0.60	0.36	0.31
	3	- 0.53	- 0.51	0.24	0.19
	5	- 0.59	- 0.62	0.49	0.46
Alex 4	1	- 0.43	- 0.40	0.46	0.42
	3	- 0.38	- 0.32	0.37	0.35
	5	- 0.48	- 0.43	0.69	0.62

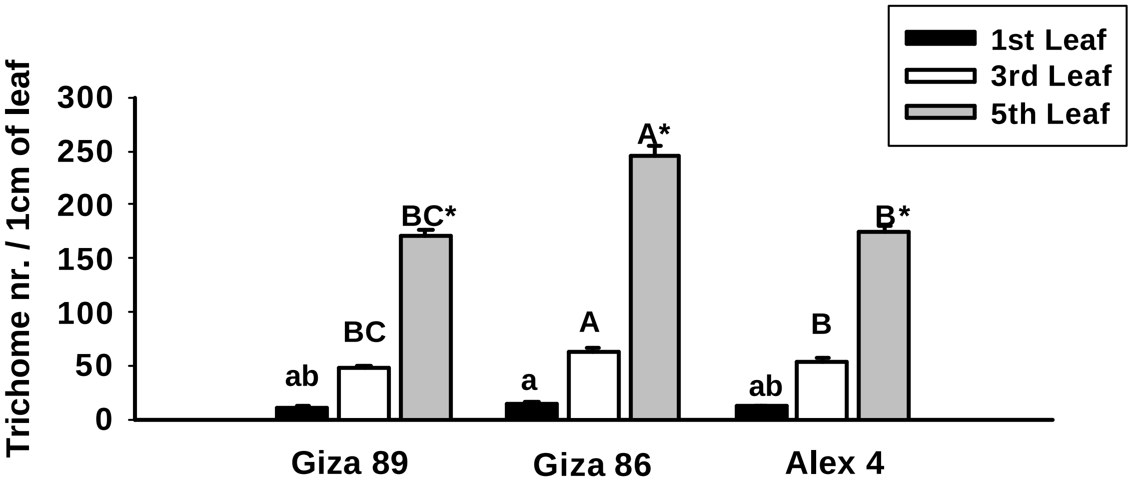


Fig. (3) Numbers of trichome on cotton leaves on 3 different cotton cultivars. Difference letters indicate significant differences

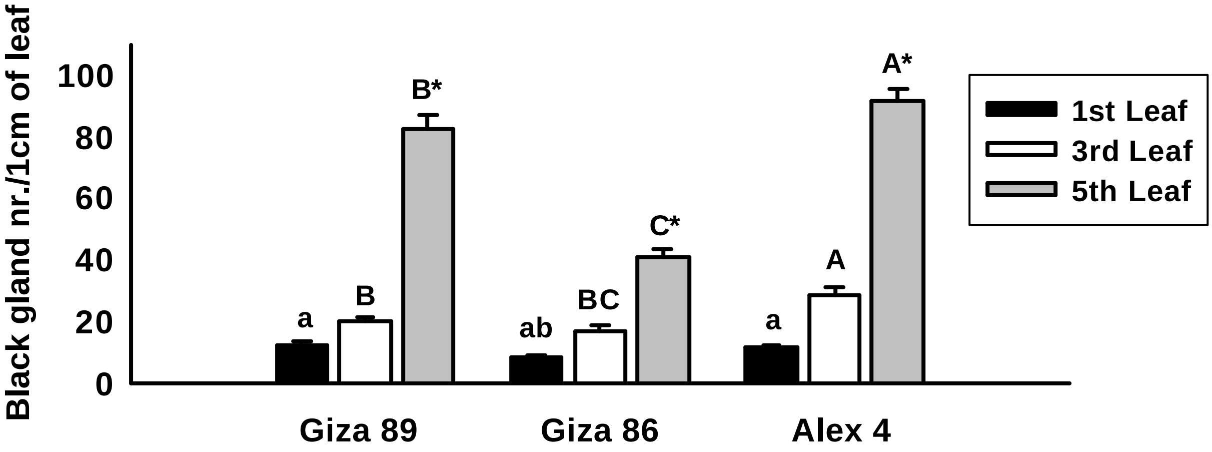


Fig. (4) Number of black glands on cotton leaves on 3 different cotton cultivars. Different letters indicate significant differences

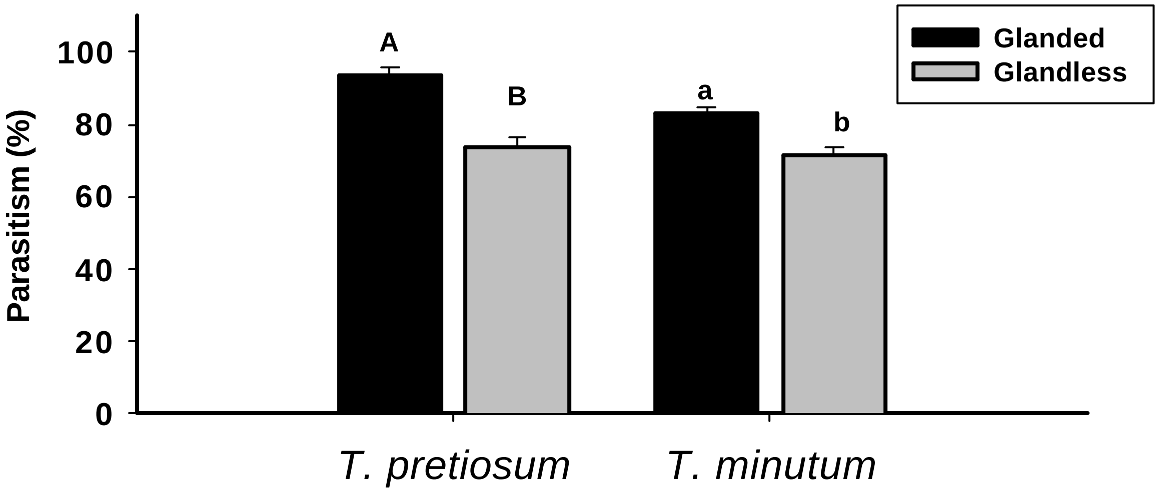


Fig. (5) Effect of black glands on parasitism rates of *Trichogramma* species on *Helicoverpa* eggs. Different letter indicate significant differences

Phytosterols compositions of cotton leaves

The results revealed that all cotton cultivars restrain the same Phytosterols profile where the ß-sitosterol was the major component. Hexamethyl, ß-Methyl-Cholesterol, Stigmasterol, ß-Amyrin and Viminalol were also detected in less quantity as compared to ß-sitosterol. Linolenic acid, which was co-detected with phytosterols, was present in leaf extracts in a higher concentration than all respective sterol (Fig. 6).

Although the cotton cultivars possess a similar phytosterol profile their content were significantly different for cultivars as well as for various leaf positions. Thus the cultivar Giza 86 retains for all leaf positions the lowest content of all phytosterols than Giza 89 and Alex 4 except for the ß-Amyrin and ß-Methyl-Cholesterol. ß-Amyrin was detected at a high concentration for the cultivar Giza 86 especially in the younger 5th leaf as compared to both the cultivars Giza 89 and Alex 4 for the respective leaf position. Independent of the cotton cultivars the elder leaves shows significantly low content of phytosterols than the young leaves. The high content of phytosterols in the younger leaves were strongly evident for ß-sitosterol, ß-Amyrin and Viminalol than the remainder phytosterols.

The cotton cultivars show also a significant difference in the content of linolenic acid. In the same line with phytosterol the lowest concentration of linolenic acid were also detected for the cotton cultivar Giza 86. Therefore, the phytosterols composition of cotton cultivars were investigated in these experiments in attempting to explain the variation of parasitization efficiency of *Trichogramma* species on *Helicoverpa* eggs Table (1a).

Volatiles induced by larval damage and Methyl Jasmonate (MeJA) treatments

Differences in volatile emissions were observed for cotton plants damaged by *H. armigera* larvae, treated with MeJA, or undamaged controls. Control plants emitted much lower volatiles as compared to herbivore-damaged plants or MeJA treated plants. There is also a great variation of volatiles emitted by larvae damaged and MeJA treated plants. Plants treated with herbivores or MeJA emitted a blend that consisted of Hexanal-2ethyl; ß Myrcene, Cis-3-hexenyl acetate, ß-ocimene, linalool, (*E)*-4,8-dimethyl-1,3,7-nonatriene, Methyl salicylate, Indole, (*E*)- ß-Caryophyllene, (*E*)-Trans-ß-farnesene, and s-Cadinene (Figures 7 and 8).

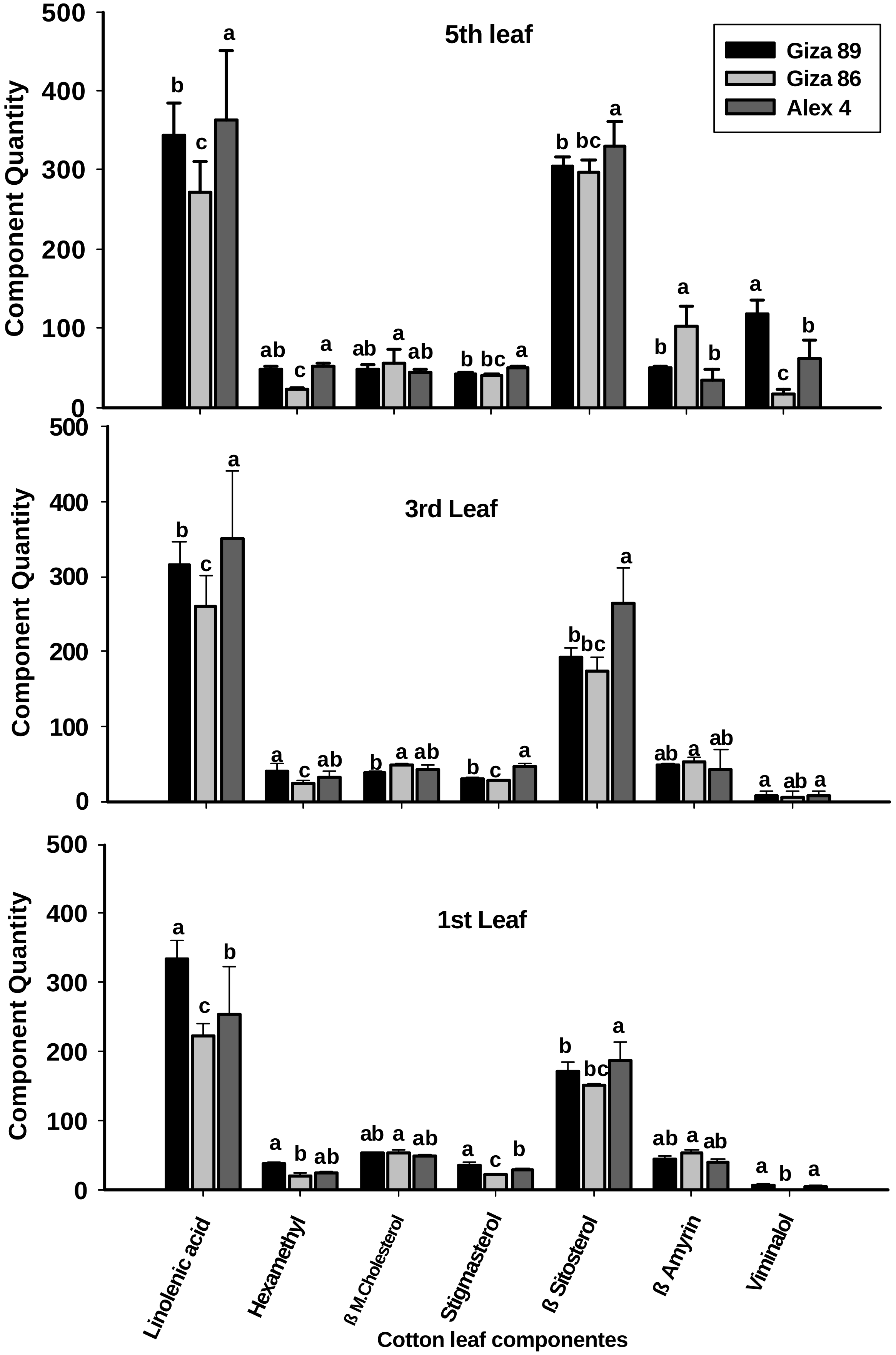


Fig (6). Chemical componentes of cotton leaves on 3 different cotton cultivars identified by GC-MS. Different letters indicate significant differences

There was no significant difference between volatiles emitted from damaged larvae and control plants in BT cultivar, because this cultivar was transgenic cotton against *Helicoverpa* and damaged plants occurred with *Helicoverpa* larvae (Fig. 8).

Hexanal, *(Z)*-3-hexenol, and hexyl acetate, all Lipoxygenase Pathway compounds, were detected in response to herbivore damage, but in a small response to MeJA treatment (Figures 7 and 8).

There was a relationship between emitted volatiles and parasitism rates of *Trichogramma* species on *Helicoverpa* eggs on cotton cultivars Giza 89 and Giza 86 and BT- and non-BT-cotton. Generally, the induced volatiles were higher in Giza 89 and BT-cotton plants compared to Giza 86 and non-BT-cotton, and the parasitism rates were also higher on Giza 89 and BT-cotton plants compared to Giza 86 and non-BT-cotton Table (2).

Table (2) Mean (±SE) parasitism rates of *Trichogramma* species on *Helicoverpa* eggs on different cultivars (volatiles emitted by larval damage and jasmonate application)

Cultivar	Treatment	Parasitization rate *	
		T. pretiosum	*T. minutum*
Giza 89	Control	83.9 ± 3.5 c	82.8 ± 2.8 cd
	Larval damage	94.4 ± 4.8 ab	89.4 ± 5.9 bc
	Jasmonate	98.3 ± 2.6 a	93.9 ± 2.1 ab
Giza 86	Control	80.0 ± 2.2 cd	75.6 ± 2.6 d
	Larval damage	92.2 ± 3.7 b	83.9 ± 5.4 c
	Jasmonate	96.1 ± 2.3 a	88.9 ± 1.8 bc
BT (DPL 422B/R)	Control	82.8 ± 3.1 c	82.2 ± 2.3 c
	Larval damage	84.4 ± 4.6 c	83.9 ± 6.4 c
	Jasmonate	97.8 ± 2.1 a	95.6 ± 1.9 ab
Non-BT (DPL 420R)	Control	81.7 ± 2.0 c	80.0 ± 2.3 c
	Larval damage	91.7 ± 3.9 b	90.0 ± 5.6 b
	Jasmonate	96.1 ± 2.3 a	94.4 ± 1.3 ab

* Different letters within and between columns indicate significant differences

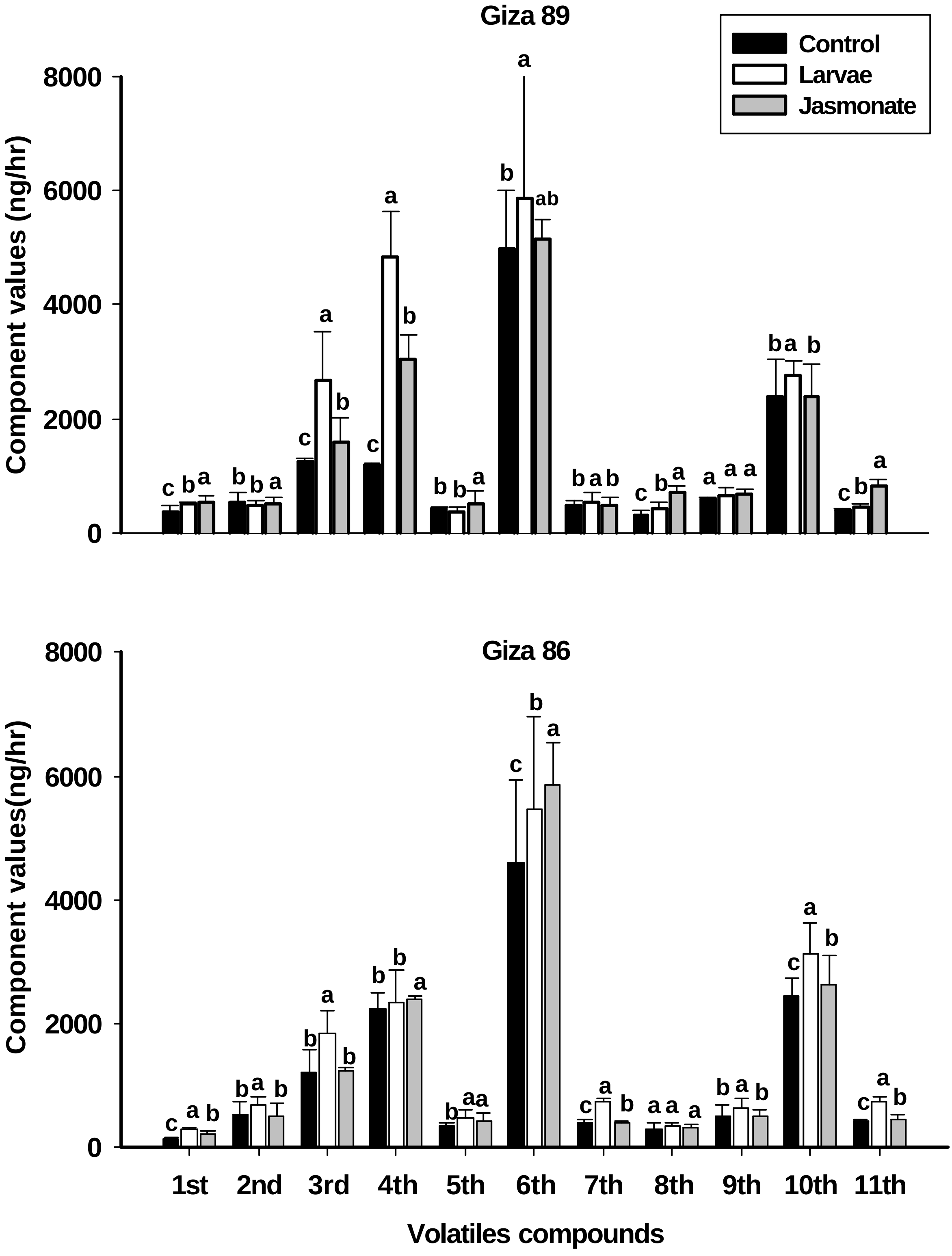


Fig. (7) Volatiles collected from undamaged cotton plants (control), plants damaged by *H. armigera* and plants treated with Jasmonate. Volatiles were collected for 24 hours and identified based on NIST. Each bar represents mean+/-SE 1: Hexanal-2ethyl; 2: ßMyrcene; 3: Cis-3-Hexenyl acetate; 4: ß Ocimene; 5: Linalool; 6:(E)4,8 Dimethyl-1,3,7 Nonatriene;7: methyl salicylate; 8: Indole; 9:(E)ß Caryophyllene; 10:(E) trans-ß-farnesene and 11: Cadinene. Different letters indicate significant differences

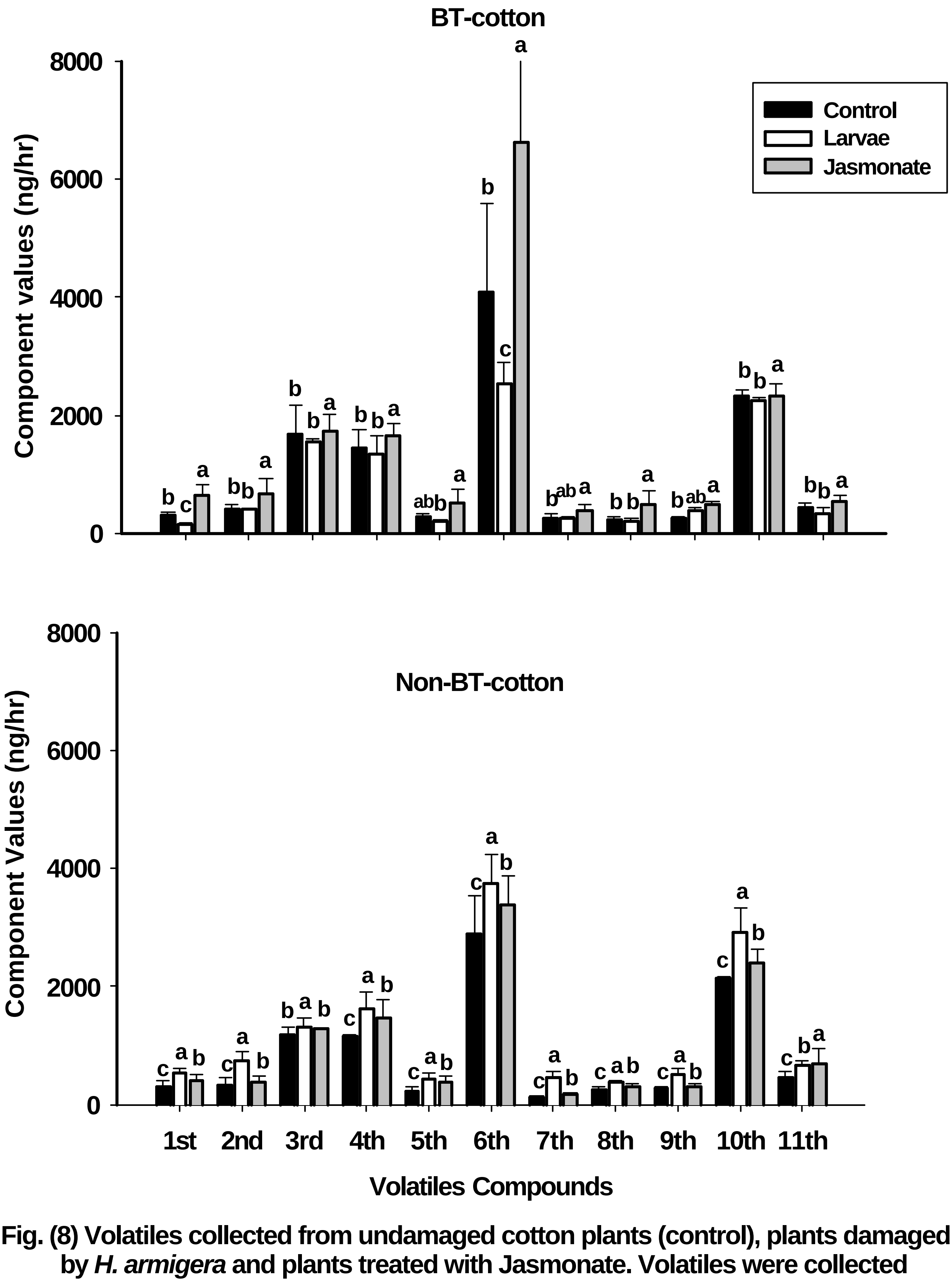


Fig. (8) Volatiles collected from undamaged cotton plants (control), plants damaged by *H. armigera* and plants treated with Jasmonate. Volatiles were collected for 24 hours and identifed based on NIST. Each bar represents mean+/-SE 1: Hexanal-2ethyl; 2: ßMyrcene; 3: Cis-3-Hexenyl acetate; 4: ß Ocimene; 5: Linalool; 6:(E)4,8 Dimethyl-1,3,7 Nonatriene;7: methyl salicylate; 8: Indole; 9:(E)ß Caryophyllene; 10:(E) trans-ß-farnesene and 11: Cadinene. Different letters indicate significant differences

Discussions

Parasitism rates of *Trichogramma* species on cotton leaves were the highest on the 1st and 2nd and 5th and 6th leaves, while they were less on the middle leaves. This result corresponds to Albes *et al.* (1980) and Berdegue *et al.* (1996). They reported that the broadly comparable structure of different leaf positions may have influence on plant–herbivore–parasitoid interactions. *T. pretiosum* was more efficient than *T. minutum* in finding their hosts. Cotton cultivars could be arranged for their attractiveness of *Trichogramma* as the followings: Alex 4, Giza 89 and Giza 86.

The level of parasitism was negatively correlated with the distance that the sentinel eggs located from the release point. There are several possible explanations. First, the wasps took a time to find eggs the farther away from release point. Secondly, once wasps encountered eggs, they spent a time handling their hosts before continuing to forage. Thirdly, a proportion of the released wasps might have disappeared, as has been proposed by Andow and Prokrym (1991) for *T. nubilale*. The decrease of parasitism with increasing in distances in this experiment implies that for inundative releases, the distance between the release points must be taken into consideration to maximize parasitism rates of *Trichogramma* species in biological control programs.

Trichogramma behaviour was affected by leaf cotton trichome and black glands densities. Parasitism rates were correlated negatively with leaf cotton trichome and positively with black glands. These results are consistent with Romeis and Shanower (1996). They reported that the trichome density may be inhibiting parasitoid searching behaviour. Based on the present results, leaf trichome may affect *Trichogramma* host location behaviour on *Helicoverpa* eggs, and black glands may attract *Trichogramma* to parasitize *Helicoverpa* eggs. These results correspond with Mohite and Uthamasamy (1998). They reported that rates of parasitism were negatively associated with trichome density, when they studied the interaction between eight wild species of *Gossypium* and a cultivated cotton cultivar MCU 9, resistant to *H. armigera,* and the pest's natural enemies. Theses results are also similar with Romeis *et al.* (1998). They mentioned that the trichome numbers the responsible for low parasitism rates of *Trichogramma* on *H. armigera* on pigeonpea plants. Leaf morphological traits should be one option for plant breeders to produce cultivars with high density of glands and low density of trichome which affect strongly parasitism rates of *Trichogramma* species in controlling *Helicoverpa* eggs on cotton.

Parasitism rates were higher on glanded plants than glandless plants. Plant surfaces contain epicuticular waxes, chemicals produced internally and leached into the leaf wax, and compounds actively exuded to the plant surface including trichome exudates (Eigenbrode and Espelie 1995). The chemicals responsible for *Trichogramma* species deterrence are unlikely to be present in the exudates secreted by the types of glandless, may glandless-plant structure affect *Trichogramma* host location behaviour and glanded plants may have special chemical volatiles to attract *Trichogramma* wasps.

The decision made by *Trichogramma* species to accepts or reject is made externally, during the examining phase. Criteria that deter attack are probably assessed at this time. Females were never observed to reject hosts once oviposition had commenced; Pak and de Jong (1987) noted the same phenomenon. The results suggest that *Trichogramma* females do not reject the host during examining, both types of plant hosts; glanded and glandless are accepted, but the difference between two plant hosts was appeared in parasitism rates of *Trichogramma*. This result was also consistent to Romeis *et al.* (1996). They mentioned that leaves mainly possess short and erect non-glandular trichomes which are more densely spaced on the lower than on the upper surface. The parasitoids prefer to walk on the leaf margins and on the major veins of the lower leaf surface. In contrast to the interveinal areas, veins and margins are covered with long non-glandular trichomes which are appressed to the surface (Romeis *et al.* 1996), making walking easier on these structures. A similar preference by *Trichogramma* species has been observed on maize (Suverkropp 1994).

Significant differences in phytosterols compositions were found between cotton cultivars and leaf positions. There was a relationship between phytosterol components and parasitism rates. Parasitism rates were positively related to Linolenic acid, Hexamethyl, Stigmasetrols, ß-Sitosterols and Viminalol and negatively to ß-Methyl Cholesterols and ß-Amyrin. These results correspond to Karban and Baldwin (1997). They reported that most phytophagous insects and their natural enemies make some sensory exploration of the leaf surface before sitting on wax layer of leaf surface. Waxes also produce compounds to attract the parasitoids or predators of the phytophagous insects.

In cotton, jasmonate mimicked the response of volatile emission induced by insects. In fact, a blend of volatiles released by cotton plants treated exogenously with jasmonate contained all compounds like herbivore damage. This result is consistent with Rodriguez-Saona *et al.* 2001). They reported that volatiles emitted from jasmonate application like larval damage, and serve as essential host-location cues for parasitic insects. Similar to report by Turlings *et al.* (1998) herbivore-induced volatile emission was highest as well as jasmonate. Contrary, Methyl jasmonate did not induce emissions of stored terpenes, a result that was expected since the release of stored terpenes is dependent on physical damage caused by herbivores (Paré and Tumlinson 1997).

Cotton was investigated for its releases of volatiles in response to caterpillar damage as well as to jasmonate treatments. In cotton it takes at least a day before induced volatiles can be detected. These results correspond to Loughrin *et al.* (1994) and Roese *et al.* (1996). They reported that induced terpenoids and indole in response to herbivory could be detected after one day; they are the result of a de-novo synthesis, observed also by (Paré and Tumlinson 1997). Like many other perennials, however, cotton also possesses constitutive defence chemicals that are stored in specialised glands (Elzen *et al.* 1985). Among the stored substances are a- and ß-pinene, a -caryophyllene, and a–humulene (Loughrin *et al.* 1994). Unlike the induced terpenoids, these compounds are not synthesised de-novo in response to herbivory and their emission is instantaneous upon plant damage and ceases rapidly when an attack stops.

The first volatiles released immediately after damage and induction by jasmonate are the ``green leafy'' odours. Some of the compounds [i.e.linalool and (3E)-4,8-dimethyl-1,3,7-nonatriene] that are commonly released by most herbivore-damaged plants might be more attractive to generalists than the less common compounds. However, parasitic wasps appear able to learn to use any odour that reliably guides them to hosts in a particular environment (Turlings *et al.* 1993; Vet *et al.* 1995). It is therefore likely that the entire blend of odours released by a specific plant can be used as a signal. Parasitoids and predators of herbivores could use these volatiles as cues to pinpoint the location where the herbivores were last feeding. This evolutionary approach takes variation in discrimination among herbivore-induced plant volatiles as a starting point rather than as an end point (Vet 1999). It considers the role of natural selection in shaping foraging decisions of carnivorous arthropods, while taking into account constraints of information availability as well as physiological, developmental constraints of the behavioural response.

The results indicated that jasmonate can activate indirect defences in cotton. Volatiles released from cotton after herbivore feeding and induced by jasmonate treatment provide cues for natural enemies to locate hosts. The results suggest that jasmonate may be used as an elicitor of volatiles to attract natural enemies in cotton cultivars. For instance, in other plant systems, indirect evidence has been presented that volatiles induced by jasmonate serve as important cues for natural enemies to locate potential host sites. Thus, it appears that jasmonate are potential agents that may be used to improve biological control in organic cotton production and also generally in organic agricultural production.

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Chapter 5

Effects of jasmonate induction on attractiveness of *Cotesia marginiventris* by olfactory cues on BT- and non-BT-cotton plants

ABSTRACT

Biocontrol organisms respond to olfactory cues with behaviour which can affect their efficacy as pest control agents. A four-armed olfactometer was used to investigate olfactory responses of *C. marginiventris* in laboratory experiments in Texas. Performance evaluations of the olfactometers have been qualitative and based on visual assessment by computer monitor. Attractiveness of *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae) females were conducted using plants induced by jasmonate and plants damaged by Beet armyworm on *Bacillus thuringiensis* (BT) and non-BT-cotton plants. Three parameters were studied; searching time, selecting time and numbers of visits for each arena. Induced-jasmonate-plants were more attractive than plants damaged by 2 larvae, and the least attractive were plants damaged by 1 larva. *Cotesia* females significantly selected more jasmonate arenas than larval damage arenas on BT-cotton plants, and the same trends were observed on induced-non BT-cotton plants. *C. marginiventris* females spent significantly more time in jasmonate zones; time spent on BT-cotton plants was longer than on non-BT-cotton plants. In non-jasmonate treatments, females spent more time on BT than on non-BT-cotton, the same trend was also observed in selecting time and numbers of visits.

In another experiment, BT- and non-BT-cotton plants were moved from a greenhouse to a non-BT-cotton field and either treated with jasmonate or untreated. This experiment was carried out simultaneously in a conventional cotton field to exclude any field-related factors on cotton pests and their natural enemies. Non-BT-cotton plants without jasmonate hosted the highest pest's numbers and the least natural enemy numbers. BT plants with jasmonate hosted the least number of cotton pests and the highest number of natural enemies. On the other hand, BT without jasmonate was similar with non-BT with jasmonate in hosting the number of natural enemies and cotton pests.

Keywords: *Cotesia,* Olfactometer, Jasmonate, Cotton, Beet armyworm

INTRODUCTION

Cotton transgenic plants express BT- toxins and provide high levels of resistance to a range of lepidopteran and coleopteran insect pests feeding on these plants (Roush 1996, 1997 a, b; Gould 1998; Schuler 1999). The potential impact of transgenic plants treated with jasmonate on natural enemies in general and on parasitoids in particular has so far received little attention (Gu and Dorn 2000).

C. marginiventris is a gregarious larval endoparasitoid that attacks many lepidopterous pest species. This parasitoid is known to respond to contact kairomones present in the by-products of the host such as silk, saliva, and exuviae with a strong response to host faeces and host plant feeding damage caused by host larvae (Loke & Ashly 1984; Dmoch *et al.* 1985; Turlings *et al.* 1989, 1990, 1991). The main source of attraction to damaged plants is a volatile, called volicitin. This volatile has been isolated, identified and synthesized from the oral secretion of beet armyworm (*Spodoptera exigua*) larvae (Potting *et al.* 1995; Alborn *et al.* 1997; Turlings *et al.* 2000). *C. marginiventris* plays an important role in the regulation of lepidopteran pest species and is being extensively used in biological control programs. It could therefore be presumed that this wasp innately responds to compounds that are common to most green plants, such as green leaf odors (Visser *et al.* 1979). During foraging experiences the female wasps appear to learn to respond to odor blends that are specific for a certain plant-host complex (Turlings *et al.* 1989, 1990, 1993).

In the process leading to successful parasitization, both plant and host-derived volatile chemicals are important in both host habitat and host location by parasitoid insects (Nordlund *et al.* 1988; Turlings *et al.* 1991; Vet and Dicke 1992). In host habitat location, volatile chemicals from the food plant of the host are released in relatively large amounts and provide long range, olfactory information to the parasitoid, whereas chemicals involved in host location, once the parasitoid reaches the microhabitat of its hosts, appear to operate short range and may originate from the host itself and from its by-products (Vinson 1976). The release of chemical compounds by the plant that stimulates the attraction of herbivore’s natural enemies are considered an indirect mechanism of plant defense (Price *et al.* 1980; Dicke *et al.* 1990; Takabayashi *et al.* 1995), whereas chemical compounds repellent to herbivores are considered a direct mechanism of plant defense or antixenotic resistance of the plant against herbivores (Storer and van Emden 1995; Petterson *et al.* 1996).

Biological control often involves the ability of one organism to locate, interact with, or avoid another organism. Since olfactory cues often control these interactions, an understanding of olfactory response is important in effective use of biocontrol agents. Olfactometry is commonly used in investigations of organism behaviour and responses to olfactory stimuli (Nettles 1980; Janssen *et al.* 1990; Martin *et al.* 1990; Pham-De`legue *et al.* 1991). Four-armed olfactory chambers, (Vet *et al.* 1983; Bakchine *et al.* 1990), provide a relatively unconfined central arena in which organisms can freely move and into which four olfactory treatments or controls can be introduced. The four-arm device chambers are designed to be symmetrical such that the geometries of all arms or ''channels'' are identical. With equal airflow rates through each channel, it is assumed that the balanced flow field will prevent mixing of air between channels (Vet *et al.* 1983; Giles *et al.* 1996).

Plants are known to produce jasmonic acid following herbivore damage, which results in increased production of compounds involved in resistance against herbivores (McAuslane *et al.* 1997). Application of jasmonic acid results in induced production of proteinase inhibitors and polyphenol oxidases and a decrease in the abundance of many common herbivores and an increase natural enemy's population in the field (Thaler 1999). Host habitat location by parasitic wasps has been studied in several tritrophic a system consisting of plants, insect's pests, and parasitic wasps under laboratory conditions using olfactometers (Vinson 1998, 1999; Fujiwara *et al.* 2000).

Manipulation of this attraction may have tremendous implications for biological control, therefore jasmonate were used in this study to test whether it will affect attractiveness of *Cotesia* species. Therefore, the objectives of this study were

- To evaluate the attractiveness of *Cotesia* females to four different odor sources, and preference of the wasp for a specific odor sources comparing the followings:
  - i) Between olfactometer responses of larval-damaged plants (Beet Armyworm), jasmonate and control on BT- and non-BT-cotton plants.
  - ii) Between BT- and non-BT-cotton plants treated with and without jasmonate.
- To study effects of jasmonate on cotton pests and natural enemies on plants transplanted from greenhouse to conventional cotton fields.

MATERIAL AND METHODS

Jasmonate

Jasmonic acid was synthesized from methyl jasmonate 95% (Aldrich Chemical Company). Four to five weeks old cotton plants were sprayed with jasmonate; each plant received about 2.5 µ jasmonate (Thaler 1999). After 3 days following applications, these plants were used to study their attractiveness to *C. marginiventris* females.

Cotton plants

Both BT- and non-BT-cotton plants; cultivar DPL 422 B/R (BT) and 420R (non-BT) (Deltapine company, Mississippi, USA) were grown in pots (8cm diameter) and kept under greenhouse conditions and watered daily. Plants with 4 to 5 true leaves were used for the olfactometer experiments. Four plants were used in four-arm-olfactometer cylinders and replicated 8 times.

For the field plants, cotton plants were grown in pots in the greenhouse, and the pots were replaced in a non-BT-cotton field to study the effect of greenhouse and field on cotton pests and their natural enemies. This experiment was carried out simultaneously in a conventional cotton field to exclude any field-related factors, and it had 12 replications in each treatment, (BT- and non-BT-cotton plants treated with and without jasmonate).

Beet Armyworm

S. exigua used in the experiments were obtained from a stock culture maintained in growth chambers (25°C, 60-70% RH and a 14: 10 (L: D)) at the biological control laboratory of Texas A&M. Second to third instars larvae were used to feed on cotton plants for 24 hrs for comparing this effect to effects of jasmonate (induced by jasmonate application) on BT- and non-BT-cotton after 3 days of application.

Parasitoids

A founder colony of *C. marginiventris* was obtained from USDA Western cotton laboratory, Phoenix, Arizona. These parasitoids were reared on artificial diet-fed beet armyworm larvae (Wiedenmann, 1992). After being stung by female *C. marginiventris*, beet armyworm larvae were maintained in growth chambers (25°C, 60-70% RH and a 14: 10 L: D). Parasitoid cocoons were collected after 10-12 days and maintained at 25°C in glass vials until adult emergence. Adults emerged were placed in small vials and sexed; 1-2 day old female were used in olfactometer experiments.

Olfactometer system

A four-arm olfactometer built of four Perspex crescents (900 arc, 27 cm diameter), similar to that of Vet *et al.* (1983) was used in these experiments. A flask filled with distilled water was used to maintain air humidity at 80-90% RH (Fig. 1). Temperature was kept at 22-25 °C. Airflow was set at 20 cm/sec in each of the four arms. An individual female *C. marginiventris* was inserted in the olfactometer arena and its activity monitored for 10 minutes using a computer connected to the olfactometer. The treated cotton plants were changed every 5 trails. Forty *Cotesia* females were used in each trail as replications.

Fig. (1) Olfactometer System

Data collection

A preliminary experiment was carried out to determine the best day, either the 1st, 3rd or 7th for *Cotesia* attractiveness after jasmonate application. The third day was chosen for the reason that *Cotesia* females responded better on this day.

The attractiveness of female wasps to the four different odor sources was measured using the following parameters: Searching time (time spent in searching to select the favorite arena), selecting time (time spent in selected arena) and number of visits (number of visits the selected arena). Four different odor sources in BT-cotton and non-BT-cotton experiments were observed as the followings:

A. Jasmonate
B. One larval-damage
C. Control.
D. Two larval-damage

For comparing between BT- and non-BT- plants treated with and without jasmonate, four odors were investigated BT with jasmonate (BT / JA), BT without jasmonate (BT / NJA), non-BT with jasmonate (NBT / JA) and non-BT without jasmonate (NBT / NJA).

Data analysis

Friedman's one way analysis of variance by ranks, based on searching time, selecting time, number of visits per odor area, was used to test for odor zone preference by *C. marginiventris* females in the olfactometer experiments (Zar 1999). Means were separated as warranted using the Student Newman-Keuls procedure for ranked data (Zar 1999).

RESULTS

I. Olfactometer experiments

A) Preliminary experiment

Cotesia females spent more time for selecting on the 3rd day compared to the 1st and 7th day (Fig. 2). They spent 63.5% of this time in the jasmonate arenas at 3rd day compared to 13.4 and 23.1% at 1st and 7th day, respectively. *Cotesia* females spent in the one larva zones 47.0% of the time at 3rd day compared to 18.0 and 35.0% at 1st and 7th day, respectively; while they spent 51.1% of this time at 3rd day compared to 20.8 and 28.1% at 1st and 7th day, respectively in the two larvae arenas

Cotesia females spent 53.3 % of this time in jasmonate arenas for searching (of rest 4 arenas), 68.8% for selecting time and 64.3% for number of visits. On other hand, *Cotesia* females spent about 26.1% (searching), 20.5% (selecting) and 23.2 % (number of visits) in the two larvae zones, while they spent the least time (17.6% for searching, 10.7% for selecting and 12.5% for number of visits) in the one larva zones. In the control zones, *Cotesia* females spent only 3.0% for searching and 0.0% for selecting and number of visits (Fig. 3).

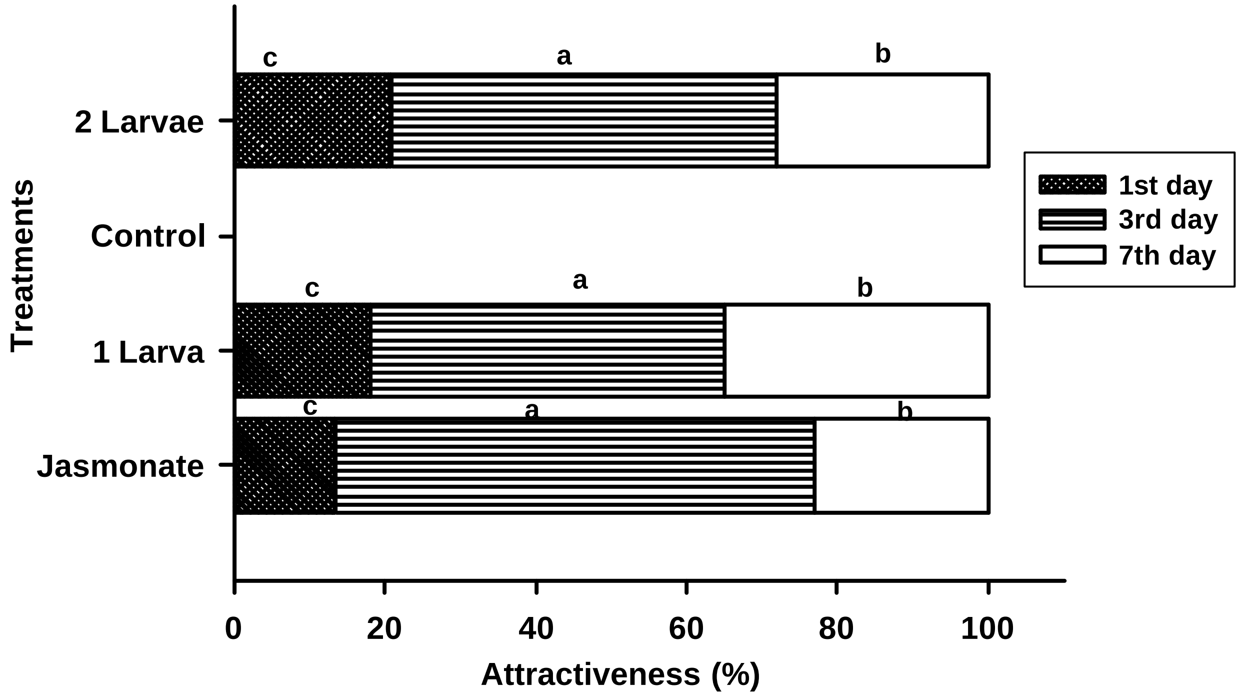

Fig. (2) Responses of *C. marginiventris* females to four odors in a preliminary experiment to select the best day after treatment. Different letters within a treatment indicate siginificant differences

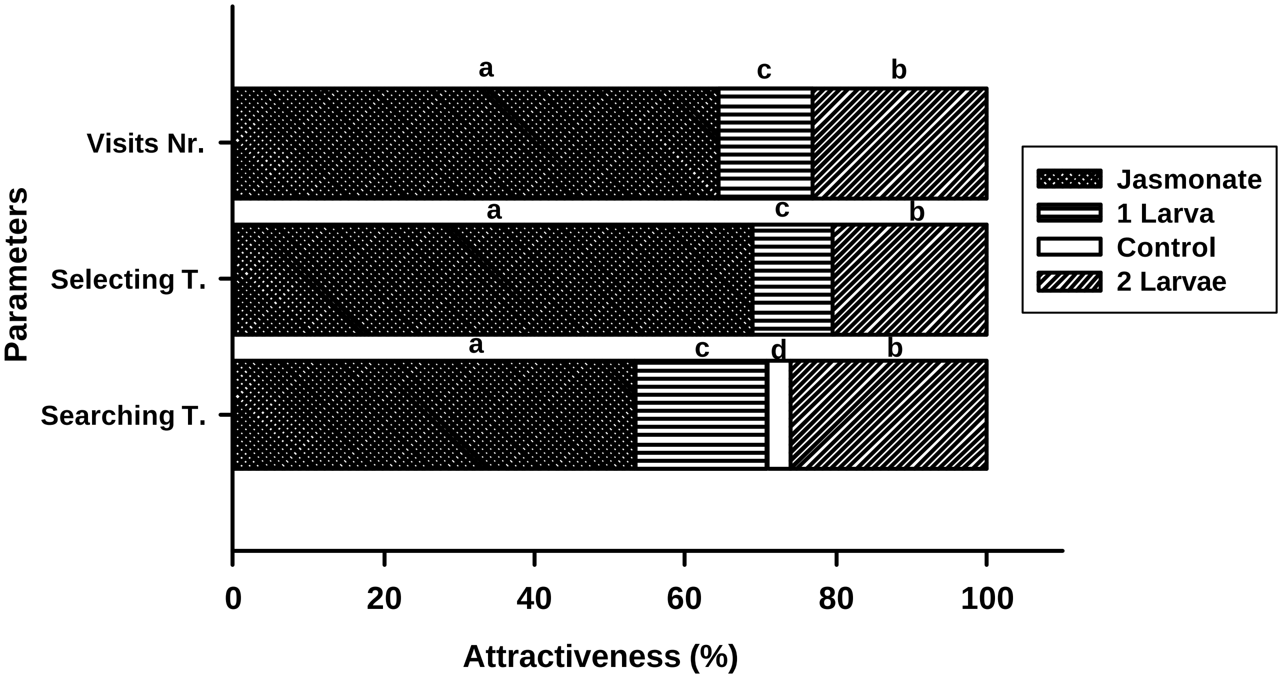

Fig. (3) Responses of *C. marginiventris* females to four odors in a preliminary experiment at different treatments. Different letters within a treatment indicate siginificant differences

B) Responses of *Cotesia* females to different odors on BT-cotton

C. marginiventris females spent significantly more time for searching in the jasmonate zones compared to larvae-damage zones, specially 2 larvae zones (Fig. 4). On the other hand, *Cotesia* females spent only 19.8 and 6.4% for searching in the 1 larval-damage and control zones, respectively. After several searching attempts in different zones, the females selected the favorite odors, and they spent different time depending on these favorite odors. *Cotesia* females spent time for selecting (77.7%) of the whole time in jasmonate zone compared to (17.5%) in the 2 larval-damage zone. Conversely, *Cotesia* females spent time for selecting 4.8% in the 1 larval-damage zone compared to and 0.0 % in control zone. Number of visits was 73.2% of the total numbers in the jasmonate zone compared to 21.4% in the 2-larval-damage zone, 5.4% in the1-larval-damage and 0.0% control (Fig. 4).

C) Responses of *Cotesia* females to different odors on non-BT-cotton plants

Cotesia females spent time for searching 44% of the whole time and 75% for selecting time and 69% for numbers of visits in jasmonate arenas. Conversely, *Cotesia* females spent time about 27.6% (searching), 16.4% (selecting) and 18.9 % (number of visits) in the 2 larval-damage zone, while they spent the least time (21.3% for searching, 8.5% for selecting and 11.6% for number of visits) in the 1 larva zone. In the control zone, *Cotesia* females spent only 6.3% time for searching and 0.0% for selecting and number of visits (Fig. 5).

D) Comparing between responses of *Cotesia* on induced and un-induced BT- and non-BT-cotton plants

C. marginiventris females spent significantly time for searching in jasmonate zones compared to non-jasmonate zones (Fig. 6). *Cotesia* females spent time for searching in BT-cotton arenas longer than in non-BT-cotton arenas (42.3 and 28.8% of the whole time). On the other hand, females spent time for searching 24.0 and 4.9% in the non-jasmonate arenas on BT- and non-BT plants, respectively. *Cotesia* females spent the longest selecting time (72.2%) in BT-cotton with jasmonate zones (BT/JA) compared to (20.5%) in the non-BT with jasmonate zones (NBT/JA). While *Cotesia* females spent time for selecting (7.3 %) in the BT- without jasmonate zones (BT/NJA) compared to (0.0 %) in the non-BT-cotton without jasmonate zones (NBT/NJA).

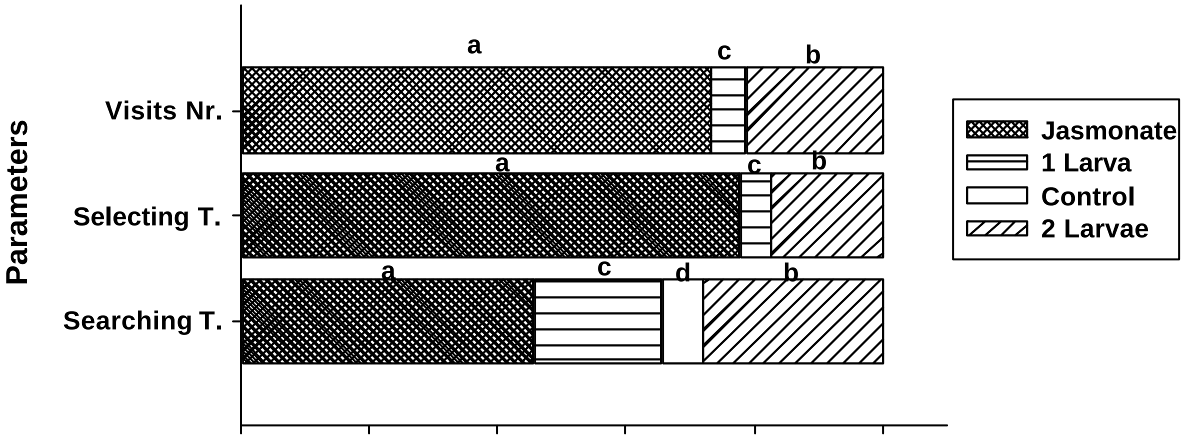

Fig. (4) ***C. marginiventris*** **females responses to four odors on BT-cotton plants; different letters indicate significant differences**

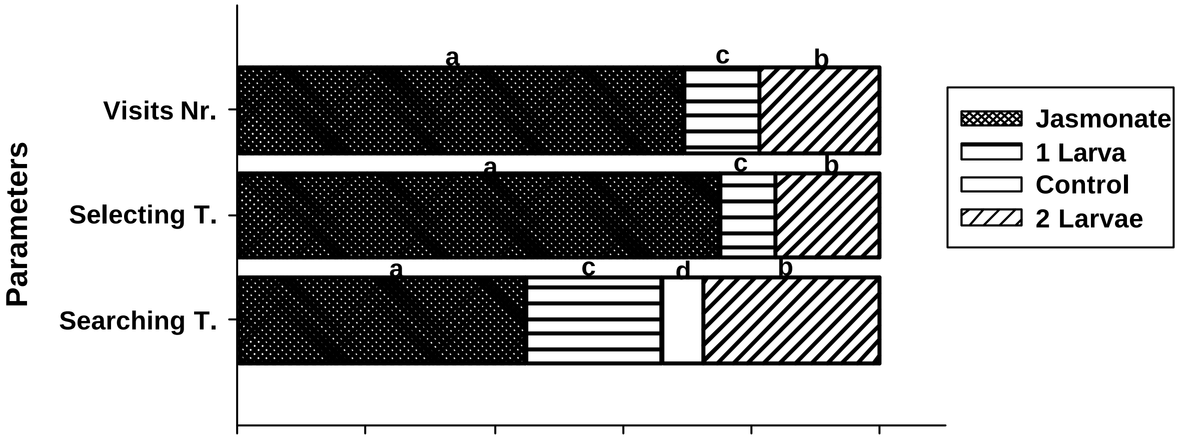

Fig. (5) ***C. marginiventris*** **females responses to four odors on non-BT-cotton plants; different letters indicate significant differences**

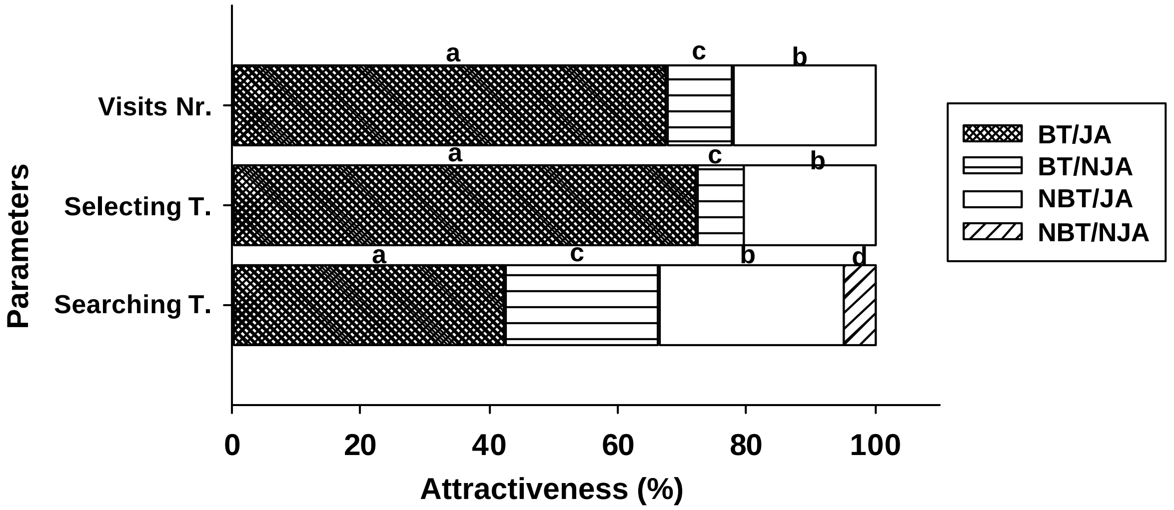

Fig. (6) ***C. marginiventris*** **females responses to four odors on BT compared to non-BT-cotton plants. Different letters indicate significant differences**

The same trend was observed in the number of visits; *Cotesia* females visited BT-cotton arenas more than non-BT-cotton arenas and they also visited jasmonate zones more than non-jasmonate zones (Fig. 6).

II. Effects of field conditions on cotton pests and natural enemies on cotton treated with and without jasmonate and transplanted to conventional cotton fields

Figure (7) shows a significant difference between cotton pests on BT- and non-BT- cotton. The induced plants received 0.42 ± 0.07 and 0.65 ± 0.07 fleahopper nymphs in BT- and non-BT- cotton, respectively, compared to 0.85 ± 0.1 and 1.1 ± 0.08 in control plants. Fleahopper adult numbers were (0.52 ± 0.07 and 0.5 ± 0.07) on treated BT and non-BT plants compared to (0.88 ± 0.1 and 1.2 ± 0.08) on control plants.

There was a significant difference between BT- and non-BT- cotton in hosting the insects; BT-cotton plants had higher natural enemy numbers than non-BT-cotton plants. Jasmonate effects on cotton pest –associated natural enemies differed between those natural enemies. In predatory spiders, differences between treatments were less (0.65 ± 0.07 and 0.46 ± 0.07 on induced BT- and non-BT-cotton plants, respectively) compared to 0.56 ± 0.07 and 0.33 ± 0.07 on un-induced plants (Fig. 7). Ant numbers showed the same trend. *Orius lavigatus* numbers were 1.4 ± 0.07 and 1.0 ± 0.08 on treated BT- and non-BT- plants compared to 0.7 ± 0.09 and 0.5 ± 0.07 on un-treated BT- and non-BT- plants. Sryphid larvae numbers were 0.4 ± 0.07 and 0.3 ± 0.06 on induced BT- and non-BT-cotton plants compared to 0.3 ± 0.07 and 0.15 ± 0.05 on un-induced plants. *Sycmnus* adults and Lacewing larvae numbers were 0.46 ± 0.07, 0.52 ± 0.07 and 0.25 ± 0.06, 0.38 ± 0.07 on induced BT and non-BT plants compared to 0.3 ± 0.07, 0.38 ± 0.07 and 0.15 ± 0.05, 0.19 ± 0.06 on un-induced plants. Coccinelld larvae and adult numbers were 0.73 ± 0.08, 0.5 ± 0.07 and 0.5 ± 0.07, 0.42 ± 0.07 on treated BT and non-BT plants compared to 0.46 ± 0.07, 0.38 ± 0.07 and 0.31 ± 0.07, 0.31 ± 0.07 on un-treated plants (Fig. 7).

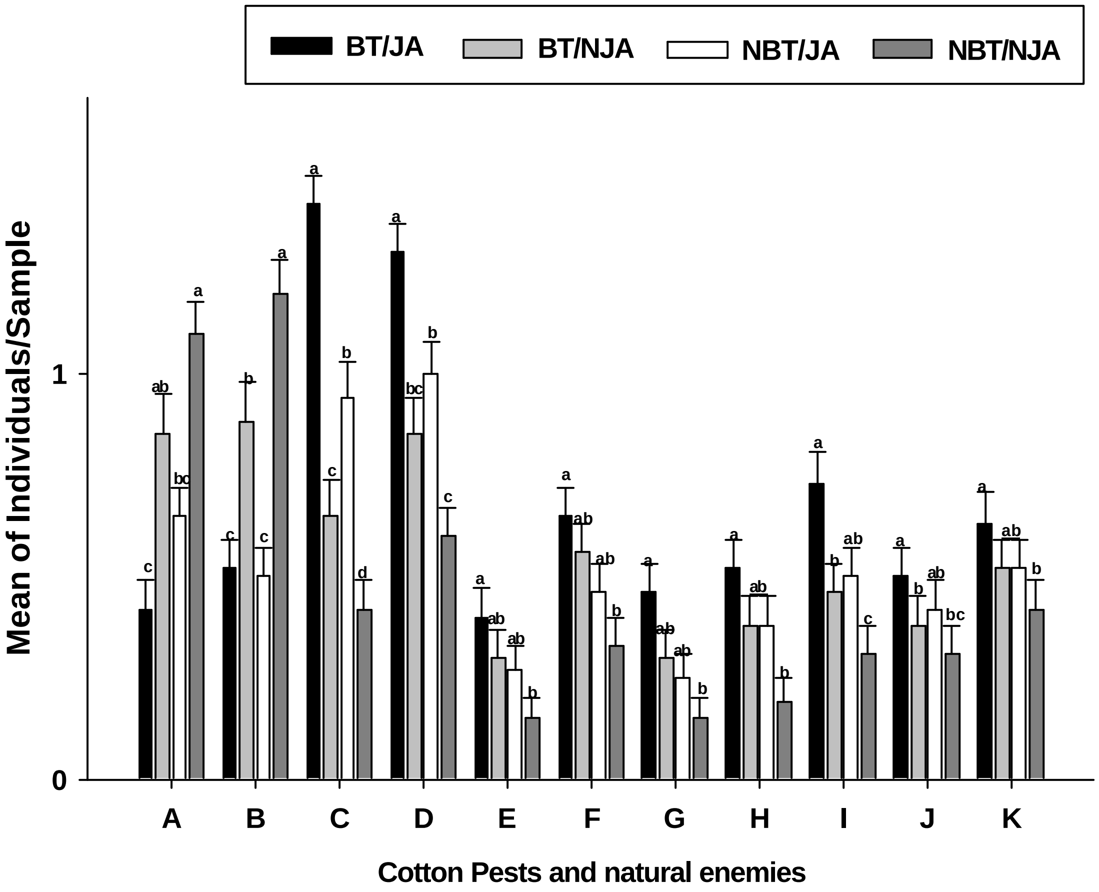

Fig. (7) Mean of cotton pests and natural enemies on cotton plants transplanted from greenhouse to cotton field: A) Fleahoppers nymphs, B) Fleahoppers adults, C) Orius nymphs, D) Orius adults, E)Sryphids larvae , F)predatory Spiders , G)Scymnus , H)Lacewing larvae, I)Coccinella larvae , J) Coccinella adults and K) Ants

DISSCUSSION

In the present study, results indicate that females of *C. marginiventris* are more attracted by odors emanating from jasmonate, followed by infested plants of Beet armyworm (*S. exigua*) on two different cultivars BT- and non-BT-cotton. These results are consistent with Nordlund *et al.* (1988). They mentioned that Allelochemicals released passively by cotton or induced by jasmonate or beet armyworm infestation may influence the success of *C. marginiventris* females as a biological control agent against *S. exigua*. Therefore, it might be possible to mediate the parasitoid's behaviour via plant synomones and increase its effectiveness in controlling the insect pest. By isolating and identifying factors from plants that are attractive to parasitoids, it may be possible to use this knowledge to, for example, increase the level of such chemicals through plant breeding and thereby increase parasitoid effectiveness as reported by other authors (Lewis and Nordlund 1985; Turlings *et al.* 1991, 2000). Analysis and testing of collected volatiles from plants, beet armyworm, and the plant–beet armyworm complexes are needed to determine the relative importance of these infochemicals in the host-finding process of *C. marginiventris* females.

Induced plant volatiles are used by natural enemies (parasitoids) to locate caterpillars by means of odors emitted by plants after jasmonate treatment or caterpillar attack. This result corresponds to Price *et al.* (1980) and Petterson *et al.* (1996). They studied the tritrophic system which comprises the parasitoid *C. marginiventris*, host caterpillars *Spodoptera*, or induction by jasmonate and cotton plants. It is expected that parasitoids (*Cotesia*) significantly reduce herbivory (*Spodoptera*) as mentioned by (Dicke *et al.* 1990; Takabayashi *et al.* 1995).

C. marginiventris females spent significantly more time in jasmonate zones compared to non-jasmonate zones, and also in BT-cotton arenas compared to in non-BT-cotton arenas. The same trend was observed in selecting time and numbers of visits. In the transplanted experiment; numbers of natural enemies were the highest in induced BT-cotton compared to the lowest numbers in un-induced non-BT-cotton. These results are consistent with El-Wakeil *et al.* (unpublished data). They found that jasmonate plots in cotton fields were more attractive to natural enemies compared to non-jasmonate plots, and also in BT-cotton plots compared to non-BT-cotton plots.

Biocontrol organisms respond to olfactory cues with behaviour which can affect their efficacy as pest control agents. Jasmonate induction of volatiles will be very useful tool for increasing attractiveness of parasitoids especially *Cotesia* to their hosts to maximize the utilization from biological control agents in organic cotton fields to maintain the environment clean.

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Chapter 6

Effects of jasmonate applications on pest and natural enemy recruitment in cotton fields

ABSTRACT

Worldwide, organic cotton production and processing is still at an experimental level. Although occupying a niche market, organic cotton production is steadily increasing, which increases the need for additional biological control research. Foliar jasmonic acid applications are known to change levels of polyphenol oxidase in plants; polyphenol oxidase is an oxidative enzyme implicated in resistance against several insect herbivores. Our experiments aimed at evaluating the effect of a jasmonic acid ("jasmonate") application as a means for attracting natural enemies, and thus reducing the impact of insect pests on different cotton cultivars, including BT cultivars.

Plants treated with jasmonate had 70% fewer insect pests of cotton seven days after application than control plants. The effects of jasmonate application were stronger with time: effects were minimal one and three days after application, and moderate to strong seven days after application. Cultivars expressing *Bacillus thuringiensis* (BT) (with or without jasmonate) hosted higher numbers of natural enemies than conventional cultivars (non-BT). However, plants sprayed with jasmonate and control plants showed similar parasitism and predation rates of sentinel beet armyworm eggs. Overall, the results suggested that jasmonate induces resistance in both BT and conventional cultivars (non-BT). Enzyme elicitors such as jasmonate may be valuable pest management tools, especially when there are high densities of herbivores that impact yield.

Keywords: jasmonate, BT-cotton, *Helicoverpa,* Spodoptera, Parasitoids, predators

INTRODUCTION

Conventional cotton uses more insecticides than any other single crop and epitomizes the worst effects of chemically dependent agriculture (Swezey and Broome 2000). Organic agriculture prohibits the use of toxic and persistent chemical pesticides and fertilizers, as well as genetically modified organisms (Alfoeldi 2001). Organic cotton is produced in organic agricultural systems that produce food and fibre according to clearly established standards. It seeks to build biologically diverse agricultural systems, replenish and maintain soil fertility, and promote a healthy environment (Stokstad 2002).

Jasmonic acid is found in many plant species and is involved in regulating diverse plant functions, including plant resistance and senescence (Creelman and Mullet 1997). Plants are known to produce jasmonic acid following herbivore damage, which results in increased production of compounds involved in resistance against herbivores (Constabel *et al.* 1995; Thaler *et al.* 1996; McAuslane *et al.* 1997). In tomatoes, application of jasmonic acid results in induced production of proteinase inhibitors and polyphenol oxidases and a decrease in the preference, performance, and abundance of many common herbivores in the field, including western flower thrips (*Frankliniella occidentalis* (Pergande), beet armyworm (*Spodoptera exigua* (Hübner), and cabbage looper (*Trichoplusia ni* (Hübner), among others (Thaler *et al.* 1996, 1999 a, b, c). These herbivore species and beet armyworm in particular, can be important pests of tomatoes (Lange and Bronson 1981).

As the interactions between transgenic plants and arthropod natural enemies may be advantageous, disadvantageous or neutral, it is important to investigate the potential side effects. Indeed, an evaluation of such side effects is a mandatory part of most environmental risk assessments for transgenic plant cultivars (Schuler *et al.* 1999). The effects of conventional microbial and synthetic insecticides on beneficial organisms, which provide a means of judging the acceptability of risks posed by transgenic plants, are reviewed elsewhere (Croft 1990). Current transgenic plants express Bt toxins and provide high levels of resistance to a range of lepidopteran and coleopteran insect pests feeding on these plants (Roush 1996, 1997 a, b; Gould 1998). The potential impact of transgenic plants on natural enemies in general and on parasitoids in particular has so far received little attention.

Natural enemies of herbivorous insects are known to utilize volatile infochemicals emitted by plants under herbivore attack during host location (Turlings *et al.* 1990, 1991; Dicke 1994; Mattiacci *et al.* 1994; Du *et al.* 1996; Paré and Tumlinson 1997; Turlings and Benrey 1998). Moreover, herbivore feeding may attract natural enemies and deter additional herbivore oviposition on plants (DeMoraes *et al.* 2001). Studies of the mechanisms of induction of plant synomones used by natural enemies have provided information on elicitors (e.g., volicitin), mediators of responses within plant tissues (e.g., jasmonic acid), induced volatiles, and the specificity of plant response (Turlings *et al.* 1993; Boland *et al.* 1995; Mattiacci *et al.*

1995; Alborn *et al.* 1997; Karban and Baldwin 1997; De Moraes *et al.* 1998; Du *et al.* 1998; Paré and Tumlinson 1998; Paré *et al.* 1998). Moreover, herbivore oviposition, in addition to herbivore feeding, can induce volatile emissions in plants that are attractive to egg parasitoids. Meiners and Hilker (1997) have shown that oviposition of the elm leaf beetle, *Xanthogaleruca luteola* Muller, induces leaves of the field elm *(Ulmus minor* (Miller) to emit volatiles that are attractive to the egg parasitoid *Oomyzus gallerucae* (D.).

Herbivore infested plants release volatiles that attract natural enemies and repel herbivores (De Moraes *et al.* 1998, 2001). Moreover, it has been suggested that the jasmonate pathway may regulate production of volatile compounds that attract foraging parasitoid wasps (Boland *et al.* 1995; Alborn *et al.* 1997; Ozawa *et al.* 2000). Several studies have shown that jasmonate treatment of plants fortifies their indirect defenses by attracting greater numbers of natural enemies under both field and laboratory conditions (Thaler *et al.* 1999 a; Gols *et al.* 1999).

The present study was designed to test whether treatment with jasmonate affected recruitment of natural enemies and herbivores by cotton plants at early and late growth stages in the field. Specifically, the objectives of this study were to assess the effects of jasmonate on: (i) recruitment of cotton aphid; *Aphis gossypii* (Glover)*,* cotton bollworm; *Helicoverpa zea* (Boddie), cotton fleahopper; *Pseudatomoscelis seriatus* (Reuter)*,* beet armyworm; *Spodoptera exigua* (Hübner), and their associated natural enemies at early and late growth stages of experimental plants, and; (ii) recruitment of parasitoids and predators of beet armyworm using sentinel individuals.

MATERIAL AND METHODS

Cotton plots

Three experimental dry-land cotton fields were planted on the Texas A&M University experimental farm near College Station, Texas. The first two fields, were used to evaluate late stage cotton, and were planted at 13 April, 2001 as part of yearly cotton cultivar tests conducted at Texas A&M University. Both fields were 40 rows (102 cm wide by 45.6 m long), and were separated from each other by ca. 60 m. Plots within each field were outlined by dividing each row into three 15.2 m segments and selecting two adjacent row segments yielding 60 plots. Three plots in each field were randomly selected and planted with a transgenic cotton cultivar expressing *Bacillus thuringiensis* (Berliner) (hereafter "BT") endotoxin (*B.*

thuringiensis subsp. *kurstaki* Cry1A(c) d-endotoxin, Deltapine Nucotn 33B) and three with a cultivar not expressing *B. thuringiensis* endotoxins (hereafter "non-BT") (Stoneville 474). The remaining 54 plots were planted with 18 different cotton cultivars. Thus, each cultivar was replicated six times in the early-planted field. Both fields were subjected to conventional management practices.

The third field, used to evaluate young cotton, was planted at 15 May 2001 also on the Texas A&M University experimental farm, and was located ca. 2 km from the first two fields. This field was 102 cm rows wide by 70 m long, and was divided into eight plots of 40 rows separated by 11 unplanted rows. Four alternate plots were planted with a commercial cultivar expressing *B. thuringiensis* endotoxins and resistance to glyphosate herbicide (hereafter BT) (DPL 422BR) and the remaining four with a cultivar's expressing glyphosate resistance (hereafter "non-BT") (DPL 420R). Two subplots within each of six plots (three BT and three non-BT plots) were outlined by selecting 12 m row segments. Thus, each cultivar was replicated six times in the late-planted field. The field was subjected to conventional management practices.

Beet Armyworm (BAW)

Newly collected BAW pupae were placed in container with paper towel. Once the moths emerged and laid eggs, the eggs were collected daily and transferred to small jar to hatch. The jars were kept in a controlled room (25±1C). The egg hatched within 48h, after larvae reached the 4th instar, they were transferred to Petri dishes with diet 15-20 larvae per Petri dish, and kept in the same conditions until pupation. After moths emergence, and oviposited, egg masses were used to conduct egg–attractive experiment. After larvae reached 1-2nd instars, they were taken for larvae attractiveness experiment in the field.

Treatments

Half of the plots/subplots in the early- and late-planted cotton fields were treated with jasmonate to assess its influence on herbivore and natural enemy recruitment by plants. Thus, four treatments were evaluated in both the early- and late-planted fields: (i) untreated non-BT cultivar; (ii) non-BT cultivar treated with jasmonate; (iii) untreated BT cultivar, and; (iv) BT cultivar treated with jasmonate. Treatments (i) and (iii) were treated with a control solution (see below). Thus, each treatment was replicated three times in both the early- and late-planted cotton fields.

Jasmonate (Jasmonic acid, Aldrich Chemical Company, Milwaukee, WI, 53233, USA) was prepared for application to cotton plants by mixing 3 ml in 3 ml of acetone and 8 l of water. The control solution consisted of 3 ml of acetone mixed in 8 l of water. The jasmonate concentration used was chosen on the basis of greenhouse studies and is considered below levels toxic to plants (Thaler *et al.,* 1996). Jasmonate was applied with a backpack sprayer to flowering cotton plants (40-45 leaves/plant) in the early-planted field, and each plant received ca. 11.8 mµ of jasmonate. Plants in the late-planted field were treated before flowering (20-25 leaves/plant), and each plant received ca. 8 mµ. In each case, neighboring plants were protected from the jasmonate or control treatments by covering with plastic during application.

Data recorded

Cotton pests and their associated natural enemies were monitored in advance of jasmonate applications to monitor baseline densities on 20 plants per plot. Insects were sampled again at the 1st, 3rd, 7th and 14th day after jasmonate applications. The results were calculated based on mean ± SE of sample per plant. Cotton pests and their natural enemies were surveyed by using beat Pucket. Aphids colonize the small cotton field only at the end of the experiment, and were monitored by counting them on the third bottom cotton leaf. Cotton pests included cotton aphid, cotton bollworm, cotton fleahopper, and beet armyworm, while natural enemies included *Orius* nymphs and adults, larvae of Syrphidae, spiders (Genera; *Phidippus* and *Salticus*), *Scymnus* adults, *Chrysoperla carnea* (Stephens) larvae, *Coccinella* larvae and adults, and fire ants *Solenopsis invicta* (Buren).

Sentinel beet armyworm eggs and larvae

Newly laid eggs and 2-3 d old (the first to second instars) larvae were used. Larvae and eggs were placed on cotton plants at 3rd and 10th day after treatment with jasmonate.

Five egg masses with known numbers of eggs (˜ 20 eggs each) were attached on the underside of BT- and non-BT-cotton leaves with TRAGANT glue (Aldrich Chemical Company, Milwaukee, WI, 53233, USA) in both jasmonate and non-jasmonate plots. Egg masses were recovered after 24 h and returned to the laboratory where the numbers of eggs missing or consumed (i.e. preyed) per egg mass were scored, and then incubated in small vials (25 ml) plugged with cotton in an incubator at 25 °C and 70-80%RH. Eggs were subsequently checked daily for eclosion, parasitism, and egg mortality.

Beet armyworm larvae were placed in vials (40 ml) with diet and fixed on bamboo stalks (˜ 5 mm diam.) and distributed in the cotton canopy of plots. After 24h, larvae were recovered and returned to the laboratory, transferred to small (20 ml) cups with diet, and incubated at 25 °C and 70-80% RH. Larvae were checked daily for pupation, parasitism, and larval mortality.

Statistical analyses

Herbivore and natural enemy counts were analyzed by analysis of variances (ANOVA) and mean of treatments were compared by the Bonferroni-test using Statistix 7.0. Percentage data (parasitism and predation rates) were arcsine square-root transformed prior to analyses.

RESULTS

1. Effect of jasmonate application on cotton pests population and their natural enemies in two cotton fields

A. Old cotton

a) Cotton pests

There was a significant difference (df= 1; F= 43.9; P< 0.002) between BT- and non-BT-cotton before jasmonate applications in overall densities of pests (Fig. 1). After jasmonate applications, these differences increased gradually with days. Cotton pest numbers differed significantly (df= 4; F=63.7; P< 0.0006) between sample days. Jasmonate applications exhibited a small effects at 1st and moderate effects at 3rd and 14th day, and the highest effects were at 7th day. Induced- plants hosted 1.4 ± 0.06 and 3.6 ± 0.11 fleahopper nymphs on BT- and non-BT-cotton, respectively, compared with 4.1 ± 0.1 and 7.4 ± 0.14 on un-induced plants at 7th day after application. Fleahopper adults were less abundant (1.5 ± 0.05) on induced plants compared to control plants (7.4 ± 0.13). The same trend was observed with Boll weevil larvae in induced plots (0.26 ± 0.04) compared to control plots (0.60 ± 0.05).

b) Natural enemies

There was a significant difference (df=1; F= 54.7; P< 0.001) between BT- and non-BT-cotton before jasmonate application. BT-cotton plants hosted higher–natural enemy’s numbers than non-BT-cotton plants. Natural enemy numbers differed significantly (df= 4; F=32.7; P< 0.0001) between sample days. Jasmonate effects

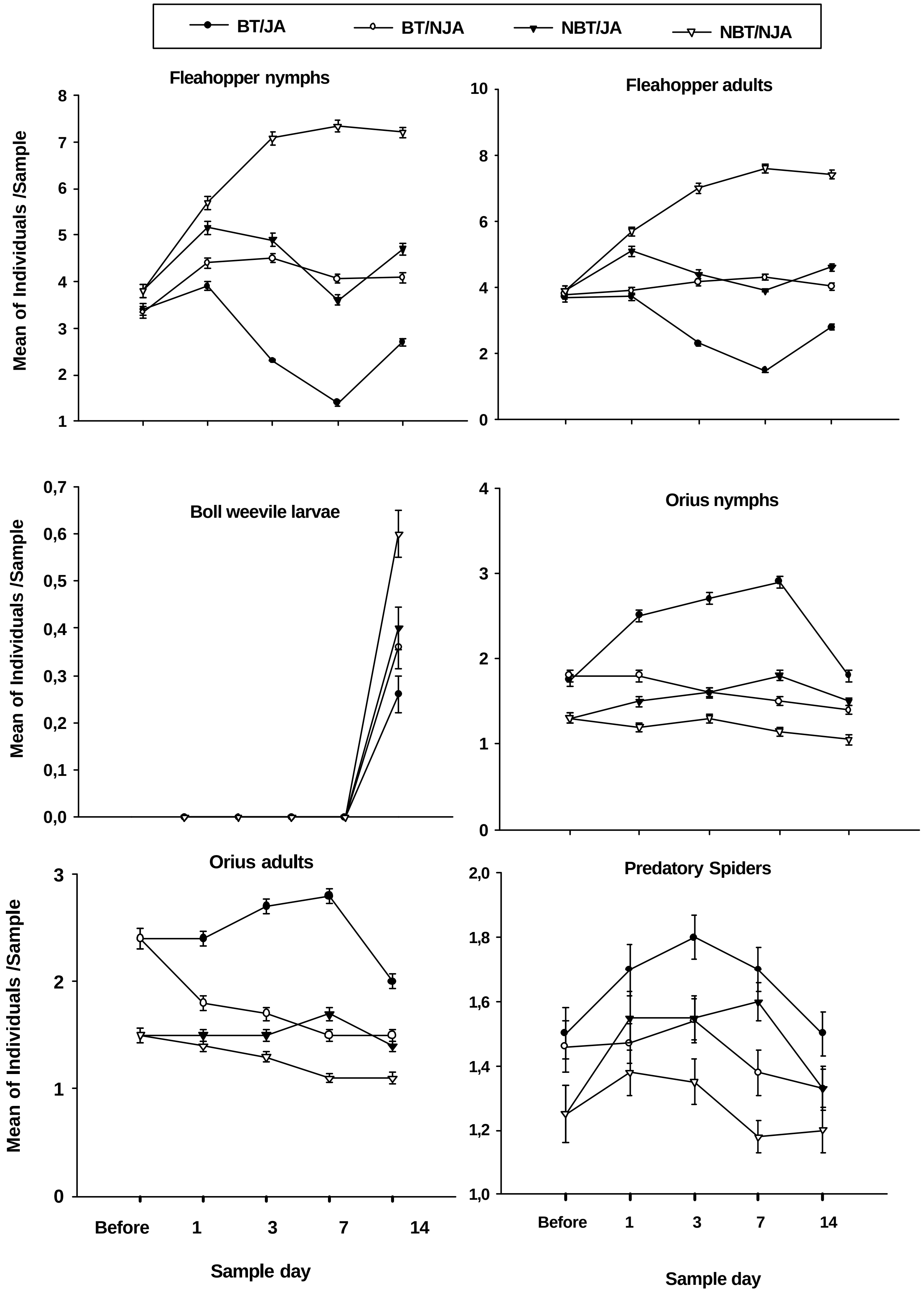

Fig. (1) Mean of cotton insects (sample/plant) in old cotton field in BT, non-BT and in JA and non-JA cotton plots

increased gradually with days to reach the highest numbers at 7th day-application. Mean of predatory spiders were 1.8 ± 0.07 and 1.6 ± 0.07 on BT- and non-BT-cotton treated with jasmonate, respectively at 3rd day, compared to 1.5 ± 0.07 and 1.3 ± 0.07 on BT- and non-BT-cotton un-treated with jasmonate (Fig. 1). *Orius lavigatus* reached maximum numbers (2.9 ± 0.07 and 1.8 ± 0.06) on treated BT- and non-BT-cotton plants at 7th day-application compared to (1.5 ± 0.05 and 1.1 ± 0.05) on non-treated BT- and non-BT-cotton. *O. lavigatus* adults were high abundance (2.8 ± 0.08) on induced plants compared to (1.6 ± 0.04) on un-induced plants (Fig. 1).

Mean of Sryphid larvae were 0.5 ± 0.05 and 0.4 ± 0.04 on induced BT- and non-BT-cotton plants compared to 0.28 ± 0.04 and 0.19 ± 0.04 on un-induced plants, respectively. Ants reached maximal numbers (2.0 ± 0.07 and 1.6 ± 0.06) on applied BT- and non-BT-cotton plants at 7th day compared to (1.47 ± 0.06 and1.19 ± 0.06) on un-applied plants (Fig. 2). Mean of Sycmnus adults and Lacewing larvae were 1.03 ± 0.03, 1.3 ± 0.04 and 0.7 ± 0.04, 0.9 ± 0.04 on treated BT- and non-BT-cotton plants, respectively at 7th day compared to 0.5 ± 0.04, 0.8 ± 0.04 and 0.3 ± 0.04 , 0.5 ± 0.05 on un-treated plants, respectively. Mean of Coccinellds larvae and adults reached 2.03 ± 0.07, 1.45 ± 0.05 and 1.5 ± 0.05, 1.1 ± 0.04 on induced BT- and non-BT-cotton plants, respectively compared to 1.3 ± 0.06, 1.1 ± 0.05 and 0.9 ± 0.05, 0.7 ± 0.05 on un-induced plants (Fig. 2).

B. Young cotton

a) Cotton pests

There was a significant difference (df=1; F= 78.4; P< 0.0009) between BT- and non-BT-cotton in cotton pests before jasmonate applications. Cotton pest numbers differed significantly (df= 4; F=37.2; P< 0.0004) between sample days. Effects of jasmonate on cotton pests increased with days; there were a little effect at 1st day and moderate at 3rd and 14th day, while the highest effects were at 7th day. Induced plants received 4.6 ± 1.06 and 25.6 ± 5.5 aphid nymphs in BT- and non-BT-cotton plants, respectively compared to 6.6 ± 1.3 and 32.5 ± 2.66 on un-induced plants at 14th day (aphids recorded on this day, conceivably from neighboring fields). Boll worm eggs were not seen before the 14th day and the eggs appeared only in non-BT-cotton (0.9 ± 0.1 and 1.4 ± 0.2) on treated and un-treated plants. Mean of fleahopper nymphs and adults were 1.6 ± 0.05 and 2.8 ± 0.08 on induced plants compared to 3.5 ± 0.1 and 4.7 ± 0.08) on controls (BT- and non-BT-cotton) (Fig. 3).

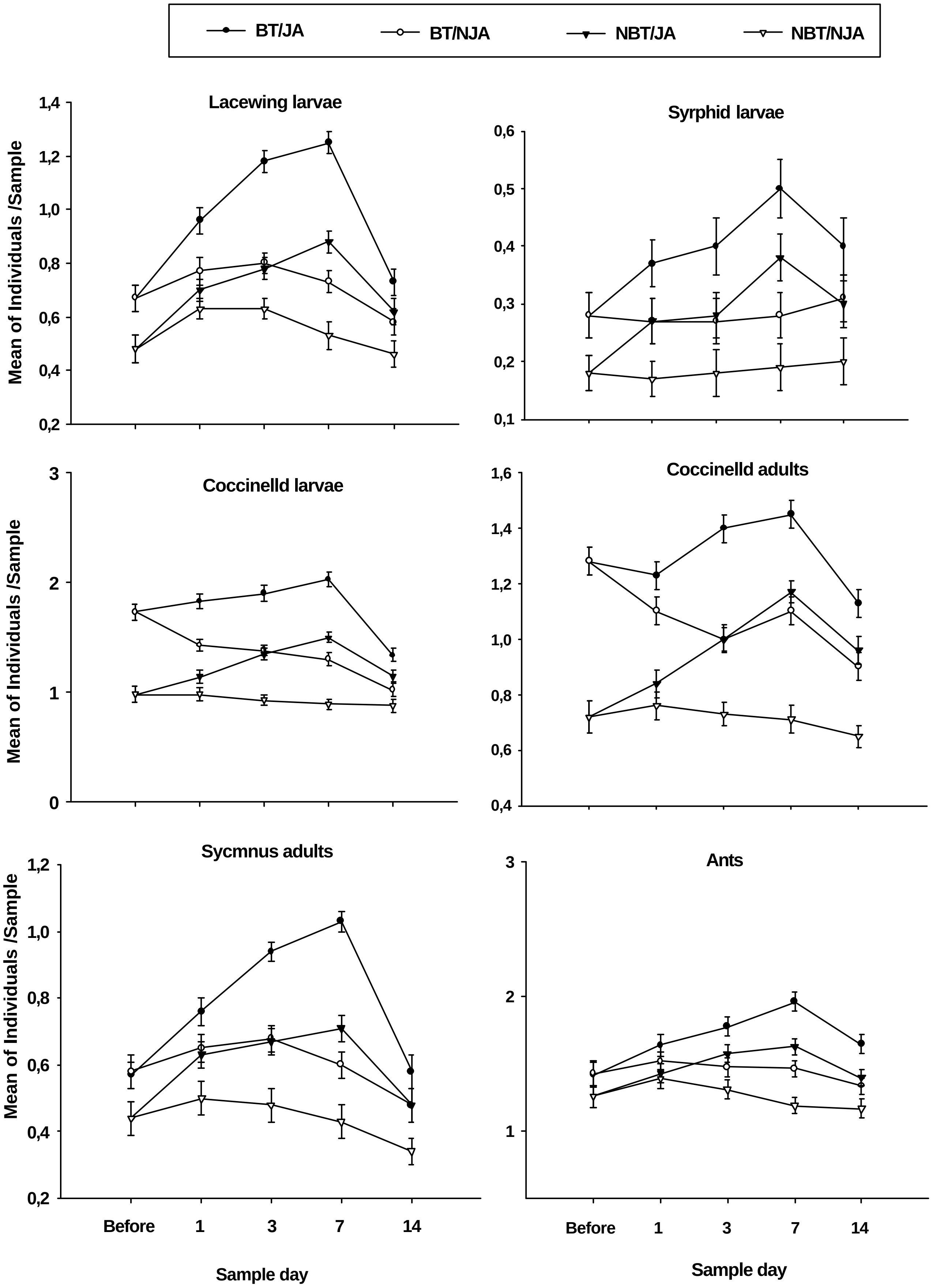

Fig. (2) Mean (+/- SE) numbers of cotton insects-natural enemies (sample/plant) in old cotton field in BT, non-BT and in JA and non-JA cotton plots

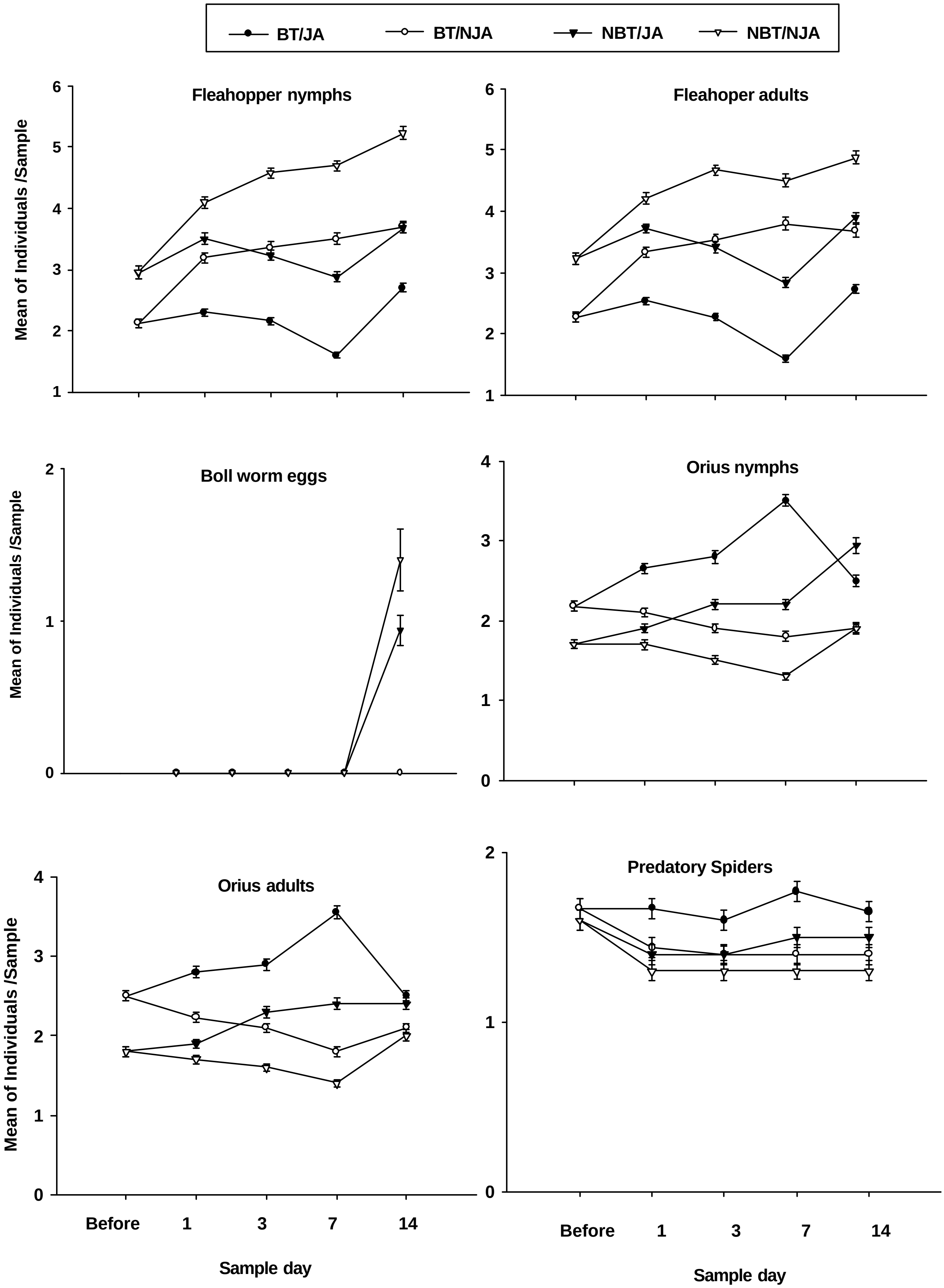

Fig. (3) Mean (+/- SE) numbers of cotton insects (sample/plant) in young cotton field in BT, non-BT and in JA and non-JA cotton plots

b) Natural enemies

There was a significant difference (df=1; F= 96.8; P< 0.0005) between BT- and non-BT-cotton before jasmonate application. BT-cotton plants hosted higher–natural enemy's numbers than non-BT-cotton plants. Natural enemy's numbers differed significantly (df= 4; F=4.7; P< 0.0003) between sample days. Jasmonate effects increased gradually with days to reach the highest numbers at 7th day-application. Mean of predatory spiders were 1.77 ± 0.06 and 1.5 ± 0.06 on induced BT- and non-BT-cotton plants at 7th day compared to 1.4 ± 0.06 and 1.3 ± 0.05 on un-induced plants (Fig. 3). *Orius lavigatus* numbers were the highest (3.5 ± 0.08 and 2.3 ± 0.06) on treated BT- and non-BT-cotton plants compared to (1.8 ± 0.06 and 1.35 ± 0.05) on un-treated plants at 7th day-application. *O. lavigatus* adults were high abundance (3.8 ± 0.09) on induced plants compared to (1.9 ± 0.06) on un-induced plants.

Mean of Sryphid larval numbers were 0.6 ± 0.05 and 0.4 ± 0.04 on induced BT- and non-BT-cotton plants compared to 0.35 ± 0.04 and 0.18 ± 0.04 on un-induced plants (Fig. 4). The ant reached their maximal numbers (3.5 ± 0.08 and 2.4 ± 0.08) on applied BT- and non-BT-cotton plants compared to (1.9 ± 0.06 and 1.6±0.07) on un-applied plants at the 7th day. Mean of *Sycmnus* adults and Lacewing larvae were 0.92±0.05 , 1.72 ± 0.05 and 0.8 ± 0.05, 1.4 ± 0.05 on induced BT- and non-BT-cotton plants compared to 0.6 ± 0.05, 1.1 ± 0.05 and 0.5 ± 0.05, 0.8 ± 0.05 on un-induced cotton plants at 7th day (Fig. 4). Coccinelld larvae and adults reached their maximal numbers (1.22 ± 0.04, 0.9 ± 0.04 and 1.0 ± 0.05, 0.8 ± 0.05) on treated BT- and non-BT-cotton plants for larvae and adults, respectively compared to (0.9 ± 0.05, 0.6 ± 0.05 and 0.7 ± 0.05, 0.5 ± 0.05) on un-treated plants. Numbers of *O. lavigatus*, ants and lacewings were higher in young cotton plots than in old cotton plots.

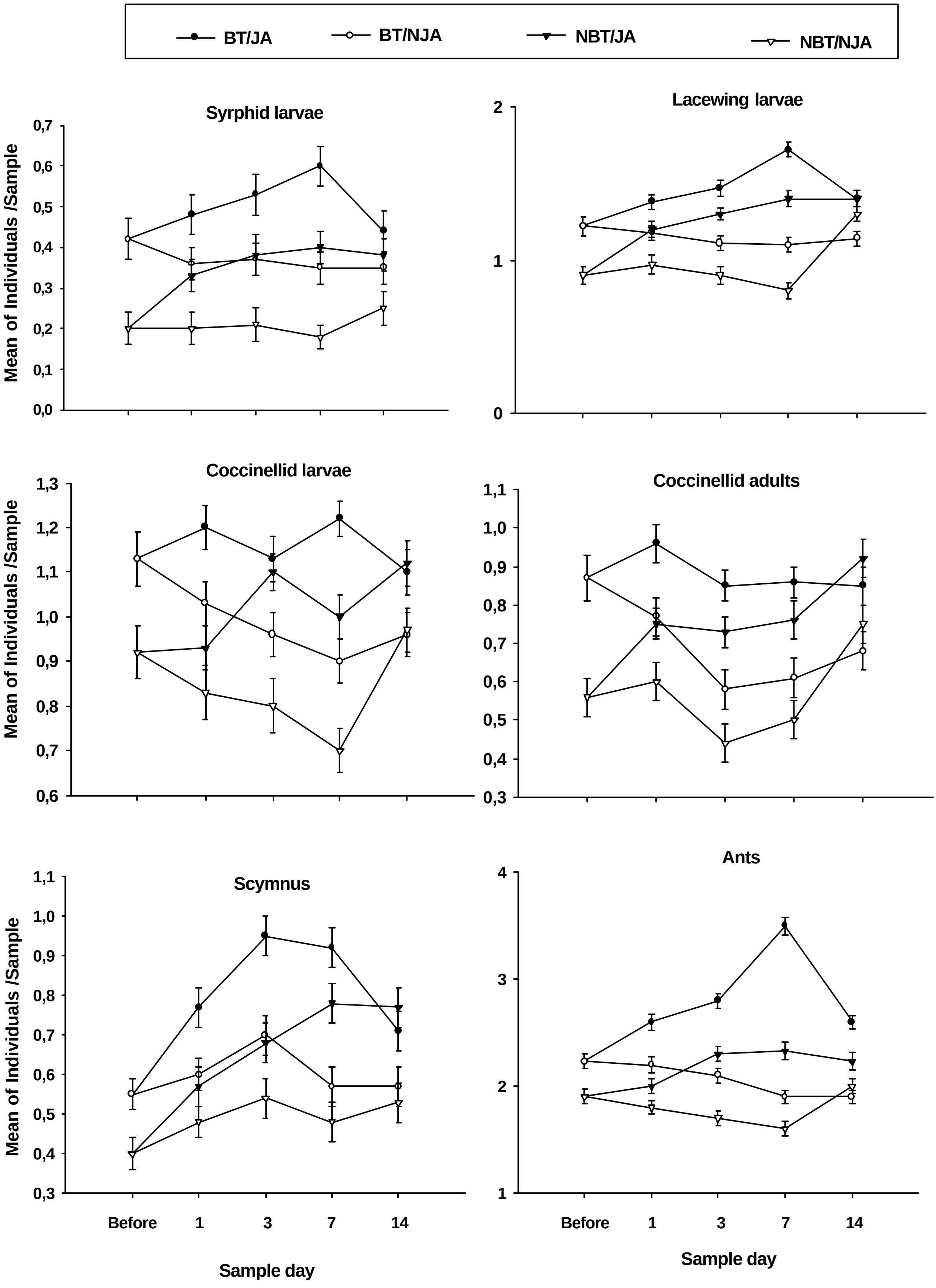

Fig. (4) Mean (+/- SE) numbers of cotton insects-natural enemies (sample/plant) in young cotton field in BT, non-BT and in JA and non-JA cotton plots

2. Influence of jasmonate application on parasitoids and predators of sentinel Beet armyworm eggs and larvae

A. Old cotton

Egg predation

The results showed that egg predation rates were 64.4 ± 4.1, 50.9 ± 5.2, 58.2 ± 3.8 and 54.0 ± 4.2 in BT with and without jasmonate plots and in non-BT with and without jasmonate plots at 3rd day, respectively. On other hand at 10th day, predation rates were 71.1 ± 3.8, 63.2 ± 4.1, 60.0 ± 4.6 and 57.1% ± 4.7 for the same treatments (Fig. 5). The results indicated that more predators were attracted to eggs at 10th day than 3rd day. Predation rates significantly differed (df=1; F= 5.1; P< 0.02) between in induced and un-induced cotton plots.

Egg parasitism

Parasitism rates were 21.1 ± 3.5 and 16.0 ± 3.5 % in jasmonate plots compared to 11.2 ± 2.6 and 5.1 ± 1.9% in control plots at 3rd day. While the parasitism rates increased at 10th day reached (28.6 ± 3.4 and 23.1 ± 2.8%) in induced BT- and non-BT-cotton plots compared to (11.5 ± 3.3 and 7.5 ± 2.4%) in un-induced plots (Fig. 5). Parasitism rates significantly differed (df= 1; F= 15.8; P< 0.0005) between treated and un-treated cotton plots. The induced-jasmonate plots attracted the egg parasitoid (*Trichogramma pretiosum*) of Beet armyworm eggs, more than control plots.

B. Young cotton

Egg predation

Egg predation rates were 58.3±6.1 and 49.6 ±6.4% in treated BT- and non-BT-cotton plots compared to 53.4 ± 4.8 and 45.8 ± 3.6% in un-treated plots at 3rd day. Conversely, predation rates were 60.0 ± 4.8 and 54.1 ± 3.8% in induced BT- and non-BT-cotton plots at 10th day compared to 48.0 ± 5.5 and 34.7 ± 4.4% in un-induced plots. Predation rates increased with days in induced plots; they were higher at 10th day than 3rd day (Fig. 5). Predation rates significantly differed (df= 1; F= 3.8; P< 0.04) between induced and un-induced cotton plots.

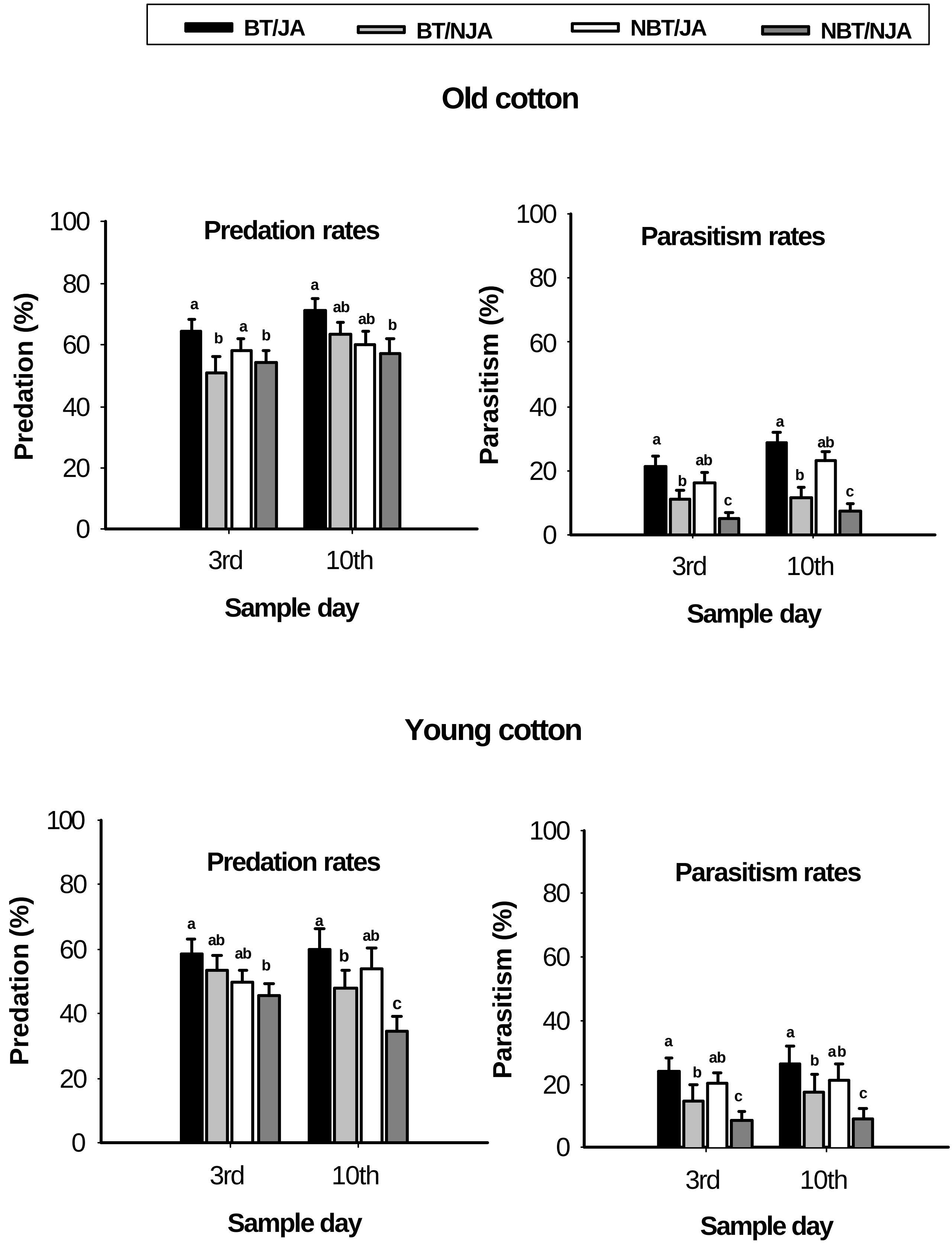

Fig. (5) Predation and parasitism rates of sentinel Beet Armyworm eggs in old and young cotton in BT and Non-BT cotton in JA and Non-JA plots at 3rd and 10th day after Jasmonate application

Egg parasitism

Parasitism rates were 23.8 ± 4.3 and 20.3 ± 3.3 % in induced BT- and non-BT-cotton plots compared to 14.9 ± 4.7 and 8.7 ± 2.5% in un-induced plots at 3rd day. Conversely, parasitism rates increased at 10th day; they were 26.4 ± 5.7 and 21.2 ± 5.0 % in treated BT- and non-BT-cotton plots compared to 17.7 ± 5.2 and 9.3 ± 3.1% in un-treated plots (Fig. 5). Parasitism rates were significantly different (df= 1; F= 15.8; P< 0.05) between treated and un-treated cotton plots. Parasitism rates and attractiveness of the egg parasitoids (*T. pretiosum*) to Beet armyworm eggs increased in induced plots compared to un-induced plots.

Larvae predation and parasitism

Distributed beet armyworm larvae in BT-and non-BT-cotton in old and young cotton fields did not attract any larval parasitoids. Twenty percent of these larvae were missed in cotton fields.

DISCUSSIONS

The results indicated that there was a significant difference in overall densities of pests between BT- and non-BT-cotton before jasmonate applications. After applications, these differences increased gradually with days. Cotton plants hosted the least pest numbers at 7th day in induced plots compared to the highest numbers in un-induced plots. These results are consistently with Felton *et al.* (1989) and Duffey and Stout (1996) and Gols *et al.* (1999). They reported that chemicals induced after jasmonate applications are well characterized and identified by GC. These compounds affected cotton herbivores and their natural enemies in cotton fields. This result is also similar with other authors in other crop plants, including cotton, tomato and potato (Blechert *et al.* 1995; Karban and Baldwin 1997; Thaler 1999 c).

BT- and non-BT-cotton plants differed significantly in hosting natural enemies before applying the jasmonate. BT-cotton plants have hosted higher–natural enemy's numbers than non-BT-cotton plants. Jasmonate effects increased gradually with days to reach the highest numbers at 7th day-application. Natural enemy's numbers were higher in induced plots than in un-induced plots. These results are consistent with (Thaler 1999 b). She reported that the positive effects of jasmonate increased attraction of natural enemies to induced plants compared to control plants.

Jasmonate effects increased the attractiveness of natural enemies of cotton herbivores. This result is corresponding with Constabel *et al.* (1995) and Duffey and

Stout (1996) and Karban and Baldwin (1997). They stated that cotton plants could be induced by jasmonate and that this induction has positive effects of natural enemies and negative effects on the abundance of many cotton insects. By understanding the chemical mechanisms of induction, we can predict which herbivores will be affected by induced responses. This prediction is important if we want to use induced resistance in agriculture especially in organic cotton farms.

Induced-BT-cotton plots hosted the highest number of predators, but their preys were the least numbers. Natural enemies that have difficulty-finding hosts may benefit from the increased attractiveness of induced plants. For natural enemies for whom host finding is not a limiting step, the positive effects of increased host finding will be outweighed by the negative effects of feeding on poor quality hosts from induced plants. For example, Coccinellds were found in a high abundance on induced plants, but syrphid fly larvae were less affected by induced plots. These results correspond with (Thaler 1999 a). She stated that the wasp *Hyposoter exiguae* was found in higher abundance on induced plants, but aphelinid parasitoids and syrphid fly larvae (both aphid feeders) were not affected by induced resistance. This could be because Coccinellids feed on low density hosts (eggs and caterpillars) whereas the syrphid fly larvae feed on high density hosts (aphids). The high attractiveness of natural enemies in induced- BT- plots could pose a problem in finding food for those natural enemies. Therefore, I recommend using jasmonate only in conventional cotton cultivars to increase their resistance to cotton pests and attract more natural enemies.

The induced-jasmonate plots attracted the egg parasitoids and predators of sentinel Beet armyworm eggs more than control plots. These results are similar with Paré and Tumlinson (1998). They mentioned that the induction by jasmonate attract both parasitic and predatory insects more than un-induced plants. The results also corresponded with Alborn *et al*. (1997). They reported that Volicitin induces the plants to emit volatile compounds that attract parasitic wasps and other natural enemies of the insect’s eggs. Jasmonic acid renders intact cotton leaves attractive to parasitoids. The finding that induction with jasmonate leads to the formation of attractants for predators and parasitoids agrees with other studies (Gols *et al*. 1999; Thaler 1999 c).

Since jasmonic acid is a natural plant hormone and it intact cotton leaves reduce cotton herbivores and attractive to parasitoids and predators. The results indicated that jasmonate with their highly effects should be used in biological control program to maintain organic cotton production. The results lead to potential practical application that cotton Hectare will need 250-300 ml jasmonate (spray using back sprayer). This amount will be less when the farmers use the big sprayers or aircraft in spraying large cotton farms. Jasmonate is highly recommended for cotton farmers to maintain and increase organic cotton production and save chemical control costs.

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GENERAL DISCUSSIONS

Cotton (*Gossypium* spp.) crop in major cultivated areas in Egypt is subjected to infestation by a wide range of pests. Among insects, the major ones being *H. armigera* complex causes the boll rots leading to low quality lint production (Fitt, 1989). Management of these pests heavily depends exclusively on insecticides. Due to significant assured success based on chemicals, research on natural enemies of pests and its field application received a back seat. Quest to harvest more and large scale farm mechanization led to adoption of modern farming system which undoubtedly heavily relies on, organic cotton farms. Egypt being diverse, this new type of cultivation has led to minimize pesticides application. The development of a strategy that may conserve and maximize the abundance as well as effectiveness of natural enemies will be crucial in the management of cotton pests (Smith 1996).

The main aim of my study is to maintain cotton production organically. The following questions played a central role: (1) Could the efficiency of *Trichogramma* suppress *Helicoverpa* in the laboratory, greenhouse and further in cotton fields? (2) Could the efficacy of *Chrysoperla* control *Helicoverpa* in the laboratory and greenhouse? (3) Does *Chrysoperla* prey on *Helicoverpa* eggs parasitized by *Trichogramma* spp.? (4) How *Trichogramma* species find their hosts, and plant characteristics affecting host location behaviour? (5) Does jasmonic acid affect cotton insects and their natural enemies, and what are the benefits of jasmonate in biocontrol programs in organic cotton production? Each subject is discussed in a general context and topics for future research are suggested.

Efficiency of *Trichogramma*

Parasitism rates of *Trichogramma minutum* and *T. pretiosum* were significantly different on *Helicoverpa* eggs; the latter parasitoid species was more efficient. These results correspond to Gross *et al.* (1984) and Lopez and Morrison (1985). Contrary to these results Greenberg *et al*. (1998 a, b) reported that parasitism rates of *T. minutum* were higher than *T. pretiosum* on beet armyworm. Temperatures regimes had a significant influence on parasitism rates and life history parameters in *T. pretiosum* and *T. minutum*. These results are consistent with Navarro and Marcano (1997). They evaluated the effect of different temperatures on biological characters of *T. pretiosum* and *T. caiaposi.* Longevity is negatively correlated to increasing temperatures. The same trend has been reported by Harrison *et al.* (1985). *T. pretiosum* is recommended for the control of *Helicoverpa armigera* for its high efficiency. *Trichogramma* species should be reared at a suitable temperature and hosts to gain the highest parasitization rates and life history parameters ensuring high efficient *Trichogramma* specimens and many generations per year.

Parasitism rates of *Trichogramma* species on *Helicoverpa* eggs significantly differed between cotton cultivars, density of cotton plants and release rates. Greenberg *et al.* (1998 a, b) demonstrated the ability of *T. pretiosum* and *T. minutum* to parasitize and develop on noctuids eggs. The results suggest a potential for *Trichogramma* species to be used in field augmentation efforts. However, additional studies are needed to evaluate the potential impact of *Trichogramma* in organic cotton fields.

Efficacy of *Chrysoperla*

The predation behaviour of *C. carnea* showed that the consumption rate reached a peak in the third larval instar on *H. armigera* eggs and larvae. This feeding behaviour was consistent with results on other host species (Sengonca and Grooterhorst 1985; Obrycki *et al.* 1989; Hegde and Lingappa 1998). The third instars consumed more than the first two instars. These results are similar with Obrycki *et al.* (1989). They used noctuids eggs and larvae as preys, and they found that third instar of *C. carnea* larvae consumed more than the first two instars. *Chrysoperla* larvae took the longest period to complete their life cycle at 20°C compared to 25° and 30°C, and the first and second instars needed a shorter time, while the third instar took a longer time. According to New (1984) the 3rd instars *C. carnea* live longer than the first two instars.

The results indicate that the 3rd instars of *C. carnea* are sufficient to control *Helicoverpa* eggs in the greenhouse. Predation rates of C*. carnea* increased gradually, reaching a maximum at the 2nd day. These results correspond with the results of Klingen *et al.* (1996). They reported that the daily predation rate of *C. carnea* on eggs and larvae of *M. brassicae* increased during the two first instars and reached a peak in the third larval instars. *C. carnea* larvae are a promising predator of *Helicoverpa* eggs and larvae, specially the third larval instars for their high predaceous efficiency. Therefore, there is a potential for using *C. carnea* for controlling *H. armigera* in organic cotton, and additional studies are also needed to evaluate the potential efficiency of *C. carnea* in field production to get a good and clean product and protect our skin from bad residues of pesticides in cotton fibers.

Combination *Chrysoperla* and *Trichogramma*

Preying time of *Chrysoperla* was positively related with predation rates and negatively with parasitism rates. Parasitism rates were higher in *T. pretiosum* than in *T. minutum*. The differences found between *Trichogramma* species in the proportion of *H. armigera* eggs parasitized are due to differences in host suitability for *Trichogramma* species after combining with *C. carnea* larvae as reported by Silva and Stouthamer (1998). They reported that variability in parasitism rate is due to host suitability not to host acceptance. Age of parasitized eggs affected predation rates of *Chrysoperla.* These findings are consistent with Pintureau *et al.* (1999). They reported that the dark substance in parasitized eggs reduce the predation risks. There was a negative relationship between parasitized eggs and predation of *Chrysoperla*. *Chrysoperla* larvae could not prey on parasitized *Helicoverpa* eggs compared to un-parasitized eggs as mentioned by Ciociola *et al.* (1998). They reported that eggs parasitized by *T. pretiosum* were not preyed compared to un-parasitized eggs. The results indicated that preying *C. carnea* for non-parasitized eggs compared to parasitized eggs showing a complementary action in the control of *Helicoverpa* combining with *Trichogramma* species.

Combining *Trichogramma* species and *C. carnea* for controlling *H. armigera* eggs resulted in higher mortality rates than *Trichogramma* species alone. These results are consistent with Ciociola *et al.* (1998). They reported that the percentage of *H. armigera* eggs that hatched in natural enemies release plots was significantly reduced compared to control plots. The results suggest a potential for using *T. pretiosum* and *T. minutum* in combination with *Chrysoperla* for controlling *H. armigera* in organic cotton fields. However, other environmental factors should be studied to evaluate their effects on potential impact of combining organisms in organic cotton, because these factors may play a role in determine this combination to control *H. armigera*.

Host plant and *Trichogramma* behaviour

Parasitism rates of *Trichogramma* species on cotton leaves were the highest on the 1st and 2nd and 5th and 6th leaves, while they were less on the middle leaves. This result corresponds to Albes *et al.* (1980) and Berdegue *et al.* (1996). They reported that the broadly comparable structure of different leaf positions may have influence on plant–herbivore–parasitoid interactions. The level of parasitism was negatively correlated with the distance that the sentinel eggs located from the release point. The same direction was observed by Andow and Prokrym (1991) for *T. nubilale*. The decrease of parasitism with increasing in distances in this experiment implies that for inundative releases, the distance between the release points must be taken into consideration to maximize parasitism rates of *Trichogramma* species in biological control programs.

Trichogramma behaviour was affected by leaf cotton trichome and black glands densities. Parasitism rates were correlated negatively with leaf cotton trichome and positively with black glands. These results are consistent with Romeis and Shanower (1996) and Romeis *et al.* (1998). They reported that the trichome density may be inhibiting parasitoid searching behaviour. Based on the present results, leaf trichome may affect *Trichogramma* host location behaviour on *Helicoverpa* eggs, and black glands may attract *Trichogramma* to parasitize *Helicoverpa* eggs. Parasitism rates were higher on glanded plants than glandless plants. Plant surfaces contain epicuticular waxes, chemicals produced internally and leached into the leaf wax, and compounds actively exuded to the plant surface including trichome exudates (Eigenbrode and Espelie 1995). Leaf morphological traits should be one option for plant breeders to produce cultivars with high density of glands and low density of trichome which affect strongly parasitism rates of *Trichogramma* species in controlling *Helicoverpa* eggs on cotton.

Significant differences in phytosterols compositions were found between cotton cultivars and leaf positions. Parasitism rates were positively related to Linolenic acid, Hexamethyl, Stigmasetrols, ß-Sitosterols and Viminalol and negatively to ß-Methyl Cholesterols and ß-Amyrin. These results correspond to Karban and Baldwin (1997). They reported that most phytophagous insects and their natural enemies make some sensory exploration of the leaf surface before sitting on wax layer of leaf surface. Waxes also produce compounds to attract the parasitoids or predators of the phytophagous insects.

In cotton, jasmonate mimicked the response of volatile emission induced by insects. In fact, a blend of volatiles released by cotton plants treated exogenously with jasmonate contained all compounds like herbivore damage. This result is consistent with Rodriguez-Saona *et al.* (2001). They reported that volatiles emitted from jasmonate application like larval damage, and serve as essential host-location cues for parasitic insects. Similar to report by Turlings *et al.* (1998) herbivore-induced volatile emission was highest as well as jasmonate. Contrary, Methyl jasmonate did not induce emissions of stored terpenes, a result that was expected since the release of stored terpenes is dependent on physical damage caused by herbivores (Paré and Tumlinson 1997).

The results indicated that jasmonate can activate indirect defences in cotton. Volatiles released from cotton after herbivore feeding and induced by jasmonate treatment provide cues for natural enemies to locate hosts. The results suggest that jasmonate may be used as an elicitor of volatiles to attract natural enemies in cotton cultivars. For instance, in other plant systems, indirect evidence has been presented that volatiles induced by jasmonate serve as important cues for natural enemies to locate potential host sites. Thus, it appears that jasmonate are potential agents that may be used to improve biological control in organic cotton production and also generally in organic agricultural production.

Olfactometer studies

The results indicate that females of *C. marginiventris* are more attracted by odors emanating from jasmonate, followed by infested plants of Beet armyworm (*S. exigua*) on two different cultivars BT-and non-BT-cotton. These results are consistent with Nordlund *et al.* (1988). They mentioned that Allelochemicals released passively by cotton or induced by jasmonate or beet armyworm infestation may influence the success of *C. marginiventris* females as a biological control agent against *S. exigua*. Therefore, it might be possible to mediate the parasitoid’s behaviour via plant synomones and increase its effectiveness in controlling the insect pest.

Induced plant volatiles are used by natural enemies (parasitoids) to locate caterpillars by means of odors emitted by plants after jasmonate treatment or caterpillar attack. This result corresponds to Petterson *et al.* (1996). They studied the tritrophic system which comprises the parasitoid *C. marginiventris*, host caterpillars *Spodoptera*, or induction by jasmonate and cotton plants. It is expected that parasitoids (*Cotesia*) significantly reduce herbivory (*Spodoptera*) as mentioned by (Takabayashi *et al.* 1995). *C. marginiventris* females spent significantly more time in jasmonate zones compared to non-jasmonate zones, and also in BT-cotton arenas compared to in non-BT-cotton arenas. The same trend was observed in selecting time and numbers of visits. These results are consistent with El-Wakeil *et al.* (unpublished data). They found that jasmonate plots in cotton fields were more attractive to natural enemies compared to non-jasmonate plots, and also in BT-cotton plots compared to non-BT-cotton plots.

Biocontrol organisms respond to olfactory cues with behaviour which can affect their efficacy as pest control agents. Jasmonate induction of volatiles will be very useful tool for increasing attractiveness of parasitoids especially *Cotesia* to their hosts to maximize the utilization from biological control agents in organic cotton fields to maintain the environment clean. Further experiments are needed to test effects of olfactory cues emitted by jasmonate induction and larval damage by *H. armigera* on behaviour of *Trichogramma* wasps.

Jasmonate in BT-cotton

There was a significant difference in overall densities of pests between BT- and non-BT-cotton before jasmonate applications. These differences increased gradually with days after applications. Cotton plants hosted the least pest numbers at 7th day in induced plots compared to the highest numbers in un-induced plots. These results are consistently with Alborn *et al*. (1997) and Gols *et al.* (1999). They reported that chemicals induced after jasmonate applications affected negatively the herbivores and positively the natural enemies. BT- and non-BT-cotton plants differed significantly in hosting natural enemies before applying the jasmonate. BT-cotton plants have hosted higher–natural enemy's numbers than non-BT-cotton plants. Natural enemy's numbers were higher in induced plots than in un-induced plots. These results are consistent with (Thaler 1999 a). She reported that the positive effects of jasmonate increased attraction of natural enemies to induced plants compared to control plants.

Induced-BT-cotton plots hosted the highest number of predators, but their preys were the least numbers, therefore natural enemies faced difficulty in finding their hosts. Similar results observed on Coccinellids and syrphid fly on induced tomato plants by (Thaler 1999 b). The high attractiveness of natural enemies in induced- BT-cotton plots could create a problem in finding insect hosts for those natural enemies. Therefore, I recommend using jasmonate only in conventional cotton cultivars to increase their resistance to cotton pests and attract more natural enemies.

The induced-jasmonate plots attracted the egg parasitoids and predators of sentinel Beet armyworm eggs more than control plots. These results are similar with Paré and Tumlinson (1998). They mentioned that the induction by jasmonate attract both parasitic and predatory insects more than un-induced plants. The results indicated that jasmonate with their highly effects should be used in biological control program to maintain organic cotton production. The results lead to potential practical application that cotton Hectare will need 250-300 ml jasmonate. Jasmonate is highly recommended for cotton farmers to maintain and increase organic cotton production and save chemical control costs.

Finally, these natural controls are important and need to be conserved and considered while making pest management related decisions. Implementation of Integrated Pest Management (IPM) or ecological based pest management relies on the knowledge that, stability in biological systems relies on feedback between organisms. For example, as the number of one organism increases, the number of predators and parasites that attack that the organism also proportionally increases and provides density dependant relationship. In the stable system, the amplitude of these oscillations is minimal. This means that potentially damaging species usually are never abundant enough to become actual pests. All these biological control agents and methods should be good options in controlling cotton pests biologically and maintaining the cotton production organically to save control costs and keep the environment clean. Further studies are required to apply all these methods in cotton farms on large scales in commercial organic cotton production farms in Egypt.

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SUMMARY

Cotton is one of the most important economic crops in Egypt. Egypt started organic cotton production in 1991 in order to meet the demands for organically grown cotton in countries of the Western hemisphere, especially Europe. Cotton is subjected to the infestation of a wide range of insect pests, including the lepidopteran pest *Helicoverpa armigera*, one of the most damaging pests in cotton. However, control measures other than insecticides in general and biological control measures in particular are not yet properly elaborated for most species. Therefore, this study aimed at testing the effectiveness of biological control measures in organic cotton production by

1. Testing the efficacy of two egg parasitoid species, *Trichogramma pretiosum* and *T. minutum*, to parasitize *Helicoverpa armigera* under laboratory and greenhouse conditions.
2. Evaluating the predation efficacy of lacewing larvae on *Helicoverpa* eggs and larvae.
3. Testing the efficacy of a combined release of predators and parasitoids.
4. Measuring chemical and physical traits of cotton cultivars related to parasitoid searching efficacy.
5. Evaluating the effect of a jasmonate application on the behaviour of natural enemies in laboratory and field experiments.

1) Parasitism rates of *Trichogramma* species were significantly influenced by temperature, *T. pretiosum* being more effective than *T. minutum*. However, adult lifespan was slightly higher in *T. minutum* compared to *T. pretiosum*. Longevity was influenced by the host species, being higher when reared on *Helicoverpa* compared to *Sitotroga* eggs. Cotton cultivars significantly influenced the parasitism rates, indicating differences in host location or host handling capabilities by the searching parasitoid species.

2) Predation rates of *C. carnea* increased during the two first instars and reached a peak in the third one. The third instars consumed about 80% of *Helicoverpa* eggs and larvae. The developmental time for *C. carnea* fed on eggs was shorter than those fed on larvae. Position of eggs on the cotton plants significantly influenced predation efficacy; eggs on the 5th leaf were consumed more often than those on the 1st leaf. Cotton cultivars had no significant influence on the predation rate.

3) Preying time (i.e. time *Chrysoperla* larvae had access to the eggs in the vials) of *Chrysoperla* both affected predation and parasitism rates; predation rates increased with increasing preying time. Age of eggs parasitized by *Trichogramma* species affected predation rates; predation rates decreased with aging parasitized eggs, both in parasitized *Helicoverpa* or *Sitotroga* eggs. However, a combination of *Trichogramma* species and *C. carnea* for controlling *H. armigera* eggs resulted in higher mortality rates than in *Trichogramma* alone. In most of experiments *Chrysoperla* did not prey on parasitized eggs.

4) *T. minutum* and *T. pretiosum* differed significantly in searching efficacy on vertically distributed egg batches. Parasitization rates were higher on the upper and lower leaves compared to the middle leaves of different cotton cultivars. Furthermore, parasitization rates were negatively correlated to distance between releasing site of *Trichogramma* species and egg batches on the cotton plants. Morphological traits of the cotton plants, i.e. presence of glands or trichome densities, significantly influenced *Trichogramma* behaviour. Parasitization rates on cultivars with glanded leaves and reduced trichome density were higher than on cultivar with non-glanded leaves and high trichome density. Results from GC-MS analysis revealed that volatiles and the phytosterols composition of the leaves differed significantly between cotton cultivars.

5) Females of the parasitoid species *Cotesia marginiventris* were attracted to plants when treated with jasmonate or damaged by Beet armyworm larvae. Induced-jasmonate-plants were more attractive than plants damaged by 2 Beet armyworm larvae; the least attractive were plants damaged by 1 larva. *Cotesia* females significantly preferred jasmonate treated arenas compared to larval damaged arenas both on BT-cotton and non-BT-cotton plants. In experiments without jasmonate treatments, females spent more time for searching and selecting and number of visits were higher on BT compared to non-BT-cotton.

6) In a field experiment plants treated with jasmonate had 70% fewer insect cotton pest’s species seven days after application than control plants. The effects of a single jasmonate application increased with time: effects were small on the first and third day after application and moderate to strong at the seventh day after application. BT cotton cultivars (with or without jasmonate treatment) have hosted higher numbers of natural enemies than conventional cultivars. However, sentinel beet armyworm eggs

exhibited similar parasitism and predation rates on plants treated with jasmonate compared to control plants.

The results of these experiments are discussed with regard to the demands for biological control strategies in organically grown cotton. Large scale field experiments are required to evaluate whether these measures will work under field conditions as well.

ZUSAMMENFASSUNG

Baumwolle ist eine der ökonomisch wichtigsten Nutzpflanzen in Ägypten. Im Jahr 1991 begann in Ägypten die organische Produktion, um die Nachfrage nach organisch gewachsener Baumwolle in den Ländern der westlichen Hemisphäre, besonders Europas, zu befriedigen. Baumwolle wird von einem grossen Spektrum von Insekten befallen. Die größten Schäden werden dabei von *Helicoverpa armigera*, einer Schmetterlingsart, verursacht. Biologische Maßnahmen für die Kontrolle der Schädlingsarten sind bisher jedoch in den meisten Fällen noch nicht etabliert. Daher wurden dieser Studie mit dem Ziel durchgeführt, die Wirksamkeit verschiedener biologischen Kontrollmaßnahmen gegenüber *H. armigera* in einer organischen Baumwollproduktion zu überprüfen. Folgende Fragestellungen standen im Mittelpunkt:

1. Die Wirksamkeit der Parasitisierung von *Helicoverpa armigera* durch zwei Ei-Parasitoide, *Trichogramma pretiosum* und *T. minutum* unter Labor- und Gewächshausbedingungen.
2. Die Effizienz räuberischer Florfliegen Eier und Larven von *Helicoverpa* zu fressen.
3. Die Auswirkung einer kombinierten Freilassung von Florfliegen und Parasitoiden.
4. Der Einfluss der chemischen und morphologischen Merkmale verschiedener Baumwollsorten auf das Suchverhalten und die Parasitierungsrate der *Trichogramma*-Arten.
5. Die Auswirkung einer Behandlung der Pflanzen mit Jasmonat auf das Verhalten der natürlichen Feinde von *H. armigera* in Labor- und Freilandexperimenten.

1) Die Parasitisierungsrate der *Trichogramma* Arten wurde signifikant durch die Temperatur beeinflusst, wobei *T. pretiosum* effektiver war als *T. minutum*. Im Gegensatz dazu lebten adulte *T. minutum* im Durchschnitt etwas länger als adulte *T. pretiosum*. Die Langlebigkeit hing von der Wirtsart ab. Aus Eiern von *Helicoverpa* schlüpfende Parasitoide lebten länger als aus Eiern von *Sitotroga* schlüpfende Parasitoide. Die Parasitierungsrate wurde außerdem stark von der Baumwollsorte verändert, welche die Wirtsfindung und -behandlung suchender Parasitoide zu beeinflussen.

2) Die Prädationsrate von *C. carnea* stieg während der ersten zwei Larvenstadien an und erreichte ein Maximum im dritten Larvenstadium. Die dritten Larvenstadien frassen ungefähr 80% der Eier und Larven von *Helicoverpa*. Die Entwicklungszeit von mit *Helicoverpa*-Eiern gefütterten *C. carnea* war kürzer als bei einer Fütterung mit Larven. Die Position der Eier auf den Baumwollepflanzen beeinflusste die Wirksamkeit der Räuber erheblich; Eier auf dem fünften Blatt wurden häufiger als solche auf dem erste Blatt konsumiert. Die Baumwollsorten hatten keinen signifikanten Einfluss auf die Frassrate der Prädatoren.

3) Die Zeit zwischen dem Ende der Parasitisierung und dem Beginn der Prädation beeinflusste sowohl die Frass- als auch die Parasitisierungsrate. Je älter die parasitisierten Eier, desto weniger wurden sie noch vom Räuber akzeptiert, unabhängig davon, ob es sich beim Wirt um Eier von *Helicoverpa* oder *Sitotroga* handelte. Trotzdem ergab eine Kombination von *Trichogramma* spp. mit *C. carnea* eine bessere Kontrolle von *H. armigera* als *Trichogramma* alleine, weil die Mortalität der Eier erhöht war. *Chrysoperla* frass jedoch fast keine parasitisierten Eier.

4) *T. minutum* und *T. pretiosum* unterschieden sich erheblich in ihrem Erfolg, vertikal verteilte Eier zu finden. Die Parasitisierungsraten waren auf den oberen und unteren Blattetagen verschiedener Baumwollsorten höher als auf den mittleren Blättern. Außerdem waren die Parasitisierungsraten negativ mit der Distanz zwischen dem Ort der Freisetzung der Parasitoide und dem Ort der Eierposition korreliert. Morphologische Merkmale der Baumwollpflanzen, d.h. Vorhandensein von Drüsen oder die Dichte von Trichomen, beeinflussten das Verhalten von *Trichogramma* signifikant. Die Parasitisierungsrate auf Sorten mit Blattdrüsen und verringerter Trichomdichte war signifikant höher als auf Sorten ohne Blattdrüsen und hoher Trichomdichte. Eine Analyse der volatilen Substanzen mittels GC-MS zeigte, dass sich die Baumwollsorten erheblich sowohl bei diesen Substanzen als auch in der Zusammensetzung ihrer Phytosterole unterschieden.

5) Weibchen der Parasitoidenart *Cotesia marginiventris* wurden verstärkt von Pflanzen angezogen, wenn diese mit Jasmonat behandelt oder von Larven des Beet Armyworm beschädigt waren. Jasmonat-induzierte Pflanzen waren signifikant attraktiver als durch Pflanzen, die durch 2 Beet Armyworm Larven geschädigt worden waren; am wenigsten attraktiv waren Pflanzen, die von 1 Larve befressen wurden. Weibchen von *Cotesia* bevorzugten die Jasmonat behandelten Zonen im

Vergleich zu von Larven beschädigten Zonen auf BT-Baumwoll- sowie Nicht-BT-Baumwollpflanzen. In Experimenten ohne Jasmonat- Behandlung besuchten die Weibchen Bt-Pflanzen häufiger als Nicht-Bt-Pflanzen und verbrachten dort mehr Zeit mit dem Suchen und Auswählen von Wirten.

6) In einem Freilandexperiment fanden sich auf den mit Jasmonat behandelten Pflanzen sieben Tage nach der Anwendung 70% weniger Arten schädlicher Insekten als auf den Kontrollpflanzen. Der Effekt einer einzelnen Jasmonat-Behandlung erhöhte sich mit Zeit; er war am ersten und dritten Tag nach der Anwendung klein und wurde am siebenten Tag nach der Behandlung mittel bis stark. BT-Baumwollsorten (mit oder ohne Jasmonat-Behandlung) hatten die höheren Anzahlen von natürlichen Feinden als konventionelle Sorten. Ausgebrachte Beet Armyworm Eier wiesen jedoch ähnliche Parasitisierungs- und Prädationsraten auf bei Jasmonat behandelten oder unbehandelten Pflanzen.

Die Resultate dieser Experimente werden hinsichtlich der Nachfrage nach biologischen Kontrollstrategien in organisch angebauter Baumwolle diskutiert. Großflächige Feldexperimente sind nötig, um abschätzen zu können, ob diese Maßnahmen auch im Freiland zur Anwendung kommen können.

CURRICULUM VITAE

Personal data

Name: Nabil Mohamed Emara El-Wakeil
Date of birth: 05/ 08/ 1969
Place of birth: Tanta, Egypt
Family status: Married and have 2 boys, Assem and Omar

Education

1976-1981: Ragy Aiz AlArab Primary school, Tanta, Egypt
1982-1984: El-Gaafaria Preparatory school, Tanta, Egypt
1985-1987: El-Gaafaria Secondary school Tanta, Egypt
1988-1991: Student at the Faculty of Agriculture, El-Moenfia University
1991: B.Sc in Agriculture Sciences
1993-1997 Master Degree (M. Sc.) in Economic Entomology at the Faculty of Agriculture, Cairo University
June-Sept.1999: Goethe-Institute, Bremen, Germany.
1999-2003: Ph. D. student at the Department of Entomology, Institute of Plant Pathology and Plant Protection, George-August University,

Professional experiences

1993-1997: Assistance Researcher at the Pests and Plant protection Dept., National Research Centre, Dokki, Cairo, Egypt.
1997 present: Researcher Associate at the same department
June- August 2001: Field work on cotton at the Experimental farm of Texas A&M University, Texas, USA

Appendix: The experimental work in Texas A&M University, USA, summer 2001

El-Wakeil spraying Jasmonate on cotton

cotton plants covered to avoid Jasmonate

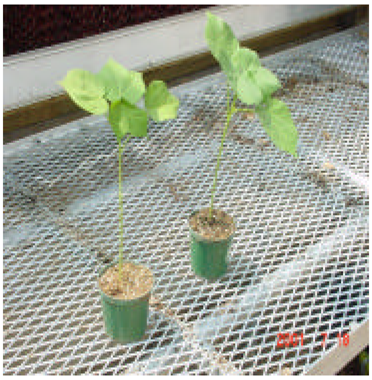
BT & Non-BT Cotton Fields in Texas

El-Wakeil uses Beat Packet to count pest insects

Lacewing larva preys on Beet Army Worm

Ants prey on BAW eggs

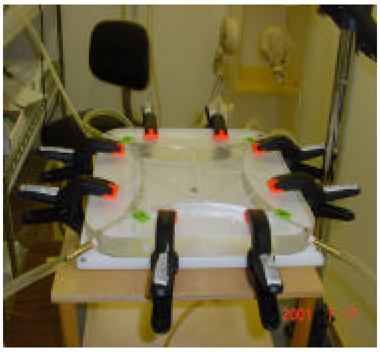
Beet Armyworm eggs& larvae on cotton



Olfactometer plants size

BAW Larval damage:1 & 2 larvae of cotton

Cotton plants in Olfactometer system



Olfactometer arena

Olfactometer Arena & Camera

Olfactometer camera

El-Wakeil optimize the Olfactometer

El-Wakeil record his Olfactometer data

Stautory Declaration

I herewith declare that I composed my thesis submitted independently without having used any other sources or means that stated therein.

Date: 07-02-2003 Signature: ***Nabil Emara***