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THE TRICHOGRAMMA MANUAL

A Guide to the Use of Trichogramma for Biological Control with Special Reference to Augmentative Releases for Control of Bollworm and Budworm in Cotton

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Cover: Black and white photograph courtesy of USDA Agricultural Research Service. Color photograph courtesy of H. Negri de Oliveira, Dept. de Entomologia–ESALQ/USP, Brazil.

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Introduction

It's an idea that has captured the imagination of entomologists, farmers, growers and entrepreneurs for more than 100 years. Rear the beneficial "mini-wasp" *Trichogramma* and release them by the thousands in fields, orchards and forests. Once liberated, the tiny parasites would seek out and destroy eggs of the most feared caterpillar pests, such as sugarcane borers, codling moths, cotton bollworms, corn borers, spruce budworms and many others. The result would be a living, biological "insecticide" that strikes only the target pest with no risk to other natural enemies, human health or the environment.

More than a thousand scientific papers have been published on *Trichogramma* and its use as a biological control agent, making it one of the most researched natural enemies in the world. In the U.S., research on *Trichogramma* continues and several *Trichogramma* species are mass reared and sold by a number of commercial companies. However, there are still questions about the effectiveness and practical application of *Trichogramma* in many crop production systems, and releasing *Trichogramma* plays a very minor role in insect pest management in the U.S.

Growers and pest management advisors must carefully evaluate Trichogramma to determine if and how this approach can best fit into their integrated pest management programs. Although the use of *Trichogramma* may at first seem simple, effective pest control is determined by many factors, including: the species of *Trichogramma* used; the quality and fitness of the parasite product; the numbers released and the timing of the release; the release method; and often complex interactions between the parasite, the target pest, the crop and environmental conditions.

The release of *Trichogramma* for the control of caterpillar pests in cotton has been promoted by commercial interests and was evaluated in a multi-year research project by the the USDA-Agricultural Research Service in the mid-1980s. The dramatic decline in insecticide use following eradication of the boll weevil in the southeast has brought renewed interest in biological control of cotton pests. Consultants and Cooperative Extension Service IPM specialists and agents are often called upon to help evaluate the effectiveness of *Trichogramma* releases. To assist in this effort, the second part of this publication details field and laboratory protocols for measuring the pest control value of augmentative releases of *Trichogramma* for control of the cotton bollworm, *Helicoverpa zea*, and the tobacco budworm, *Heliothis virescens*.

It is hoped that this publication will serve as a resource to pest management advisors, consultants, Cooperative Extension Service agents and specialists, and others seeking information on biological control using *Trichogramma*.

Trichogramma

Trichogramma are extremely tiny wasps in the family *Trichogrammatidae*. While it is uncommon for an insect's scientific name, especially one so long and unusual as *Trichogramma*, to also become its common name, the commercial development of this natural enemy and the fact that it attacks so many important caterpillar pests has earned it a place in the popular vocabulary of many pest management advisors and producers.

Trichogramma wasps occur naturally in almost every terrestrial habitat, and some aquatic habitats as well. They parasitize insect eggs, especially eggs of moths and butterflies. Some of the most important caterpillar pests of field crops, forests, and fruit and nut trees are attacked by *Trichogramma* wasps. However, in most crop production systems, the number of caterpillar eggs

destroyed by native populations of *Trichogramma* is not sufficient to prevent the pest from reaching damaging levels.

Recognizing the potential of *Trichogramma* species as biological control agents, entomologists in the early 1900s began to mass rear *Trichogramma* for insect control. Although a small commercial production of *Trichogramma* eventually developed in the U.S., insect control research and commercial efforts focused on the development of chemical pesticides following the discovery of DDT (73). This was not the case in the Soviet Union and China, both of which developed programs to control several crop pests with *Trichogramma*. In these countries, insectaries were less expensive and less sophisticated than production facilities for synthetic insecticides, and could be located on farms where labor was inexpensive and readily available. Also, control standards were not as stringent, and releasing *Trichogramma* was often better than no control at all (35).

Today, *Trichogramma* species are the most widely used insect natural enemy in the world (45), partly because they are easy to mass rear and they attack many important crop insect pests. Nine species of *Trichogramma* are reared in private or government owned insectaries around the world and released annually on an estimated 80 million acres of agricultural crops and forests in 30 countries (45, 61). The countries of the former Soviet Union lead in *Trichogramma* production, followed by China and Mexico.

Trichogramma are released to control some 28 different caterpillar pests attacking corn, rice, sugarcane, cotton, vegetables, sugar beets, fruit trees and pine and spruce trees. Most releases are to control corn borers, sugarcane borers and cotton bollworm. Although widely used, a recent review of these programs worldwide concluded that "because of considerable variability in success of releases and little evidence of consistently successful application of *Trichogramma*, the usefulness of these parasitioids is currently being debated" (24).

Secondary pest outbreaks, pesticide resistance, more stringent pesticide regulation, and concern about human health and environmental quality have renewed the interest in Integrated Pest Management programs that emphasize biological control (5, 8). The commercially successful use of *Trichogramma* to control the European corn borer in Europe has demonstrated the potential of this approach. Researchers in the U.S. are currently evaluating *Trichogramma* for the control of codling moth in apples and almonds, leafrollers in apples, European corn borer in corn, and bollworm/budworm in cotton.

Taxonomy and Identification

The genus *Trichogramma* is one of 80 genera in the family *Trichogrammatidae*. All members of this family are parasites of insect eggs. *Trichogrammatidae* includes the smallest of insects, ranging in size from 0.2 to 1.5 mm. Within the genus *Trichogramma*, there are 145 described species worldwide; 30 species have been identified from North America and an estimated 20 to 30 species remain to be described. The species most commonly collected from crops and orchards are *atopovirilia, brevicapillum, deion, exiguum, fuentesi, minutum, nubilale, platneri, pretiosum,* and *thalense* (61).

Trichogramma are difficult to identify because they are so small and have generally uniform morphological characters. Also, certain physical characteristics such as body color and the number and length of body hairs can vary with body size, season, rearing temperature and the host on which the adult was reared. Because of these difficulties and the lack of type specimens, species names in the literature in North America prior to 1968 were used

incorrectly and inconsistently and are therefore unreliable (66). Further studies have shown that with the exception of the common species *T. pretiosum*, *T. minutum* and *T. platneri*, identifications of North American Trichogramma species published before 1980 are also largely unreliable (61).

A major advance in the systematics of Trichogramma was the discovery that characteristics of male genitalia can be used to identify species. This is the primary means of identification today, but body color, wing venation and features of the antennae serve as supporting characteristics. Females can not be identified with the same level of confidence, so collections submitted for identification must include males. In addition to physical characteristics, studies of reproductive compatibility and mode of reproduction also have been especially valuable in identifying species. Additional studies of reproductive and molecular characteristics are underway to better understand the systematics of Trichogramma (66).

Taxonomic support is necessary to identify native and introduced species and to ensure mass cultures are not contaminated by undesired species. A key to the New World species of Trichogramma published in 1973 is useful (55), but important revisions have been made (65).

Biology and Life Cycle

Trichogramma wasps primarily parasitize eggs of moths and butterflies (Lepidoptera). However, certain species of Trichogramma also parasitize eggs of beetles (Coleoptera), flies (Diptera), true bugs (Heteroptera), other wasps (Hymenoptera), and lacewings and their relatives (Neuroptera).

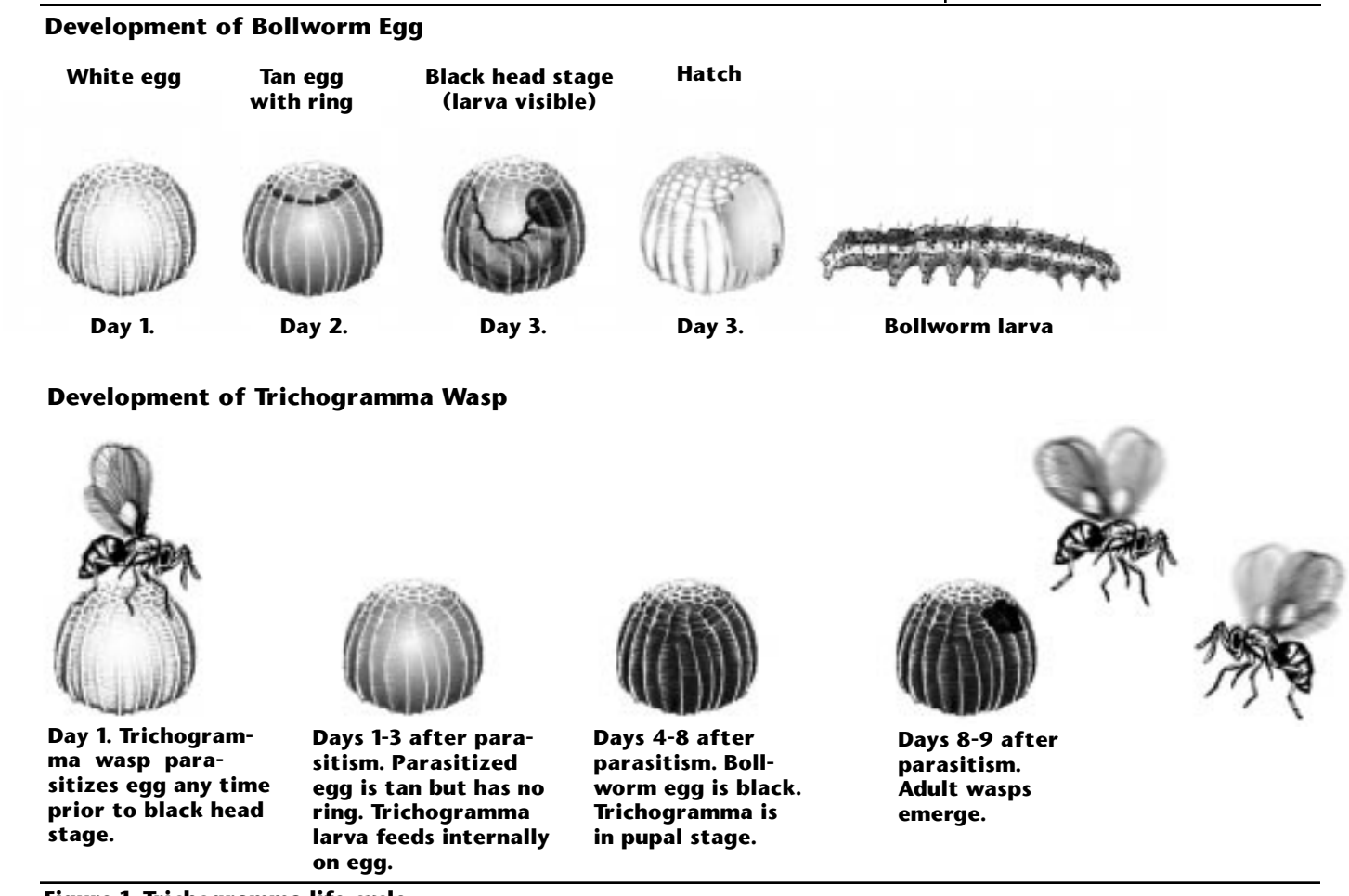


Figure 1. Trichogramma life cycle.

The adult female wasp uses chemical and visual clues to locate a bollworm egg. The chemical clues, called kairomones, are on the moth scales left near the egg by the female moth during oviposition (58). Some of these same chemicals are also bollworm sex pheromones. Egg shape and color also may be visual clues to the wasp (68).

Once a female finds a bollworm egg, she drills a hole through the chorion (egg shell) and inserts two to three eggs into the bollworm egg. The internal pressure of the bollworm egg forces a small drop of yolk out of the oviposition hole. Females feed on this yolk, which increases their longevity. Under laboratory conditions a female parasitizes from one to ten bollworm eggs per day or from ten to 190 during her life. Large females parasitize more eggs than smaller females. Females provided honey and young bollworm eggs to feed on live an average of 11 days, while females receiving only honey live 3 days (68). Another study found the average adult life span was 24 days (78).

Bollworm eggs in the early stages of development are more suitable for parasite development. Older bollworm eggs, especially those in which the head capsule of the larva is visible, are not usually parasitized and if they are, parasite survival is much lower (68).

The yolk and embryo of the parasitized bollworm egg are digested before the Trichogramma egg hatches. A venom injected by the female at the time of oviposition is believed to cause this predigestion of the egg's contents. Eggs hatch in about 24 hours and the parasite larvae develop very quickly. Two *T. pretiosum* larvae can consume the digested contents of a young budworm egg within 10 hours of hatching (77). Larvae develop through three instars. During the 3rd instar (3 to 4 days after the host egg was parasitized) dark melanin granules are deposited on the inner surface of the egg chorion, causing the bollworm egg to turn black. Larvae then transform to the inactive pupal stage. After about 4.5 days, the adult wasps emerge from the pupae and escape the bollworm egg by chewing a circular hole in the egg shell (Fig. 1). The black layer inside the chorion and the exit hole are evidence of parasitism by Trichogramma. The life cycle from egg to adult requires about 9 days, but varies from 8 days when mid-summer temperatures are high (90 degrees F) to as many as 17 days at 60 degrees F. Adults are most active at 75 to 85 degrees F.

An average of two *Trichogramma pretiosum* adults will emerge from a single bollworm egg. A single bollworm egg can yield wasps of the same or opposite sex. Trichogramma adults emerge from host eggs in the early morning. Males emerge first and remain at the host egg to mate with emerging females if they are present. Mated females produce male and female offspring. Unmated females produce only males. Females begin egg laying within a few hours of emergence.

Trichogramma overwinter as immature forms in host eggs. Some species enter a state of diapuase which allows them to tolerate long periods of subfreezing temperatures. Other species, such as *T. pretiosum*, slow their rate of development and may be active as adults during warm days as early as January and February in Texas. The lack of host eggs in the early spring may be a critical factor in determining the number of Trichogramma that are later present to move into field crops (49).

Trichogramma pretiosum

T. pretiosum is the most widely distributed Trichogramma species in North America. It parasitizes a large number of butterflies and moths in a variety of habitats. It has been reared from 18 genera of Lepidoptera representing nine families. Because it attacks important pest species such as bollworms and budworms in cotton and tomatoes, corn earworms in corn and armyworms (Spodoptera) and loopers (Trichoplusia) in vegetables and other crops, it has been the focus of many field and laboratory studies. *T. pretiosum* is found throughout the U.S. from Maryland to California and south through Mexico to South America north of the equator. Its widespread distribution may be due in part to commercial sales as a biological control agent (64). The impact of mass releases of *T. pretiosum* on non-target species such as butterflies has only recently been investigated.

Using Trichogramma in a Biological Control Program

Introduction of New Species

At least four species of Trichogramma have been imported to the U.S. and released for the control of crop pests. In 1968, *T. evanescens* was introduced from Europe into southern California and Missouri for control of imported cabbage worm and cabbage looper on cabbage. A species from Russia, *T. euproctidis*, was imported and released in cotton in Georgia in 1975 (31). In 1993, *Trichogrammatoidea bactrae* was introduced from Australia into California and Arizona for control of the pink bollworm in cotton (56). The establishment of these three introduced species has not been documented. During 1993-96, *T. ostriniae* was imported from China and released in New York for control of European corn borer in sweet corn (66).

Augmentation

Augmentation is the periodic release of a natural enemy that does not occur naturally in sufficient numbers to keep a pest below damaging levels. Agumentation can be carried out by inundative releases or inoculative releases. The inundative approach is achieved by flooding the crop with multiple releases of insectary-reared natural enemies. The released insects control pests present at the time, but there is little expectation that later generations will persist at sufficient levels to provide control. This approach requires a large number of the natural enemies at the precise time when pest eggs are present and crop and weather conditions are conducive to the release. Correct timing requires good coordination between the rearing facility and field staff.

Inoculative releases involve one or several releases to establish populations of the natural enemy before pest densities have begun to increase. The natural enemy reproduces on the target pest or an alternate host and its population increases to levels sufficient to control the target pest later in the season. In China, inoculative releases of Trichogramma in gardens in the spring produce populations of wasps which later in the season move into adjacent fields to control cotton pests (45).

Conservation

Conservation as a biological control method includes crop management practices that protect and encourage natural enemies and increase their impact on pests. Examples include using only selective insecticides and planting strip crops in and around fields to provide food and habitat for natural enemies. Insecticides such as Bt (formulations of *Bacillus thuringiensis*) and some insect growth regulators have very little or no impact on Trichogramma and can be used in IPM programs with Trichogramma. Interplanting rye grass in seed corn production fields lowered soil temperatures which otherwise would be lethal to released Trichogramma distributed in cardboard capsules deposited on the soil (63). Trichogramma species commonly parasitize bollworm (corn earworm) in corn and sorghum, and these crops may serve as an important source of adults which disperse into cotton (59).

Trichogramma in Cotton

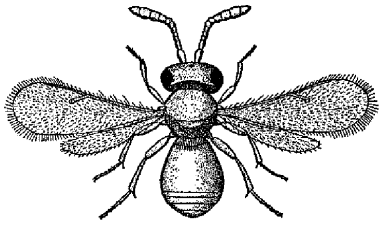


Figure 2. Telenomus heliothidis (Quaintance and Brues, USDA).

Naturally Occurring Trichogramma

Early cotton entomologists noted that Trichogramma parasites commonly attacked bollworm eggs in cotton. Parasitism rates reported in 1903 and 1945 ranged from 5 to 35 percent in Texas cotton, presumably in the absence of insecticides (17, 67). In Arkansas, parasitism of bollworm and budworm eggs in untreated cotton is typically 20 percent (44). In Louisiana, early season parasitism reached 60 to 80 percent but sharply declined once insecticide treatments began (30). In the Gulf Coastal region of Texas, natural parasitism of bollworm and budworm eggs in cotton increased from about 20 percent in early June to 65 percent by late July (70). In the U.S., *T. pretiosum* and *T. exiguum* are the most common species reported from cotton (see Table 1).

Augmentation of Trichogramma

Several U.S. companies rear and market *T. pretiosum* for control of cotton pests (29). However, small plot and large field evaluations have shown that although egg parasitism rates are sometimes increased, a reduction in bollworm or budworm larvae numbers is rarely achieved (Table 2). Because of inconsistent performance, agumentation of Trichogramma in cotton has not been adopted in the U.S.

The largest U.S. research effort to develop a Trichogramma augmentation program for cotton was conducted by USDA-ARS scientists beginning in 1981 (37, 38). This program was developed in response to the increasing difficulty and expense of controlling bollworms and insecticide-resistant budworms. Studies had shown that bollworm and tobacco budworm often reached pest status when populations of predators and parasites were reduced by insecticides. Augmenting these natural enemy populations was seen as a potential alternative to continued reliance on chemical insecticides. However, methods of mass producing predatory insects and spiders were not then available. Although field studies indicated parasitism by naturally occurring Trichogramma was of minor importance, Trichogramma could be mass produced. Also, small plot studies had shown that mass releases of Trichogramma could increase egg parasitism. (Table 2). Thus, Trichogramma was chosen for evaluation as an alternative to insecticides for bollworm and budworm control in cotton (37).

Other egg parasites of the bollworm.

Telenomus heliothidis (Scelionidae) and *Encarsia* species (Aphelinidae) are also occasionally reared from bollworm/budworm eggs in cotton. The Telenomus wasp is a shiny, smooth, black wasp slightly larger than an adult Trichogramma. It has been found parasitizing bollworms in cotton from South Carolina through Georgia and west to Texas (67). The Encarsia wasp has only been reported attacking bollworm eggs in west Texas.

Table 1. Species composition of Trichogramma collected from bollworm and budworm eggs in cotton.

Location	T. pretiosum	T. exiguum	T. thalense	T. minutum	Citation
Arkansas	30%	70%	—	—	(28)
North Carolina	40%	58%	—	1%	(28, 78)
Central Texas	78%	21%	—	<1%	(48)
Coastal Texas[1]	79%	13%	—	—	(70)
Texas[2]	80%	14%	6%	—	(47)

[1]9 percent of the eggs were parasitized by Telenomus.

[2]An aphelinid was common at one location in far west Texas.

Table 2. Summary of published research evaluating augmentative releases of Trichogramma for bollworm/budworm control in cotton.

Loc/Year	Release rate as no./acre x 1,000	No. of releases and release interval	No. host eggs/acre x 1,000	% egg parasitism		Evidence of Control	Citation
				Release	Check		
TX/1970	200	3 @ 2-3 days	—	58	11		(36, 37)
TX/1971	19-388	5 @ 2-3 days	3-11	33-81	5-7	60-80% reduction in larvae	(75)
TX/1973	50-100	—	—	24-73	1-7[a]		(37)
TX/1979	45-72	4 @ 2-3 days	6.5	55-84	17-81	21% reduction in larvae	(33)
LA/1978	5-15	3 @ 7 days	5-28	5-59	11-41	No increase in egg parasitism	(30)
TX/1979	45	5 @ 4-5 days	17-98	15-90	15-90	Fewer larvae in release on two of eight sample dates. Considerable damage occurred in release and check.	(1)
TX/1977	71	8@ 2-3 days	0.2-6.5	27-73	1-2		(32)
MS/1980	19	—	—	42-80	32-72	Inadequate larvae suppression	(37)
NC/1983	124	7 @ 2-3 days	6-57	40-60	5-30	Yields in release fields greater than in the check fields but less than in fields treated with insecticides. Larvae in release fields exceeded threshold.	(50, 38)
TX/1995-6	100	6 @ 3-4 days	5-84	24	20	No consistent reduction in densities of small larvae or fruit damage relative to check.	(41)
NC/1996-96	44 (females)	9@ 3-4 days	5-60	75-82	40-44	No reduction in 5th instar larvae in release fields. No yield differences between release and no-release plots.	(78)

[a]=pre-release, no check fields reported.

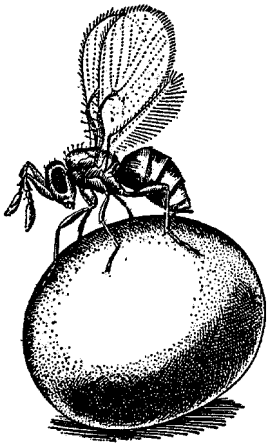


Figure 3. Female Trichogramma ovipositing on a moth egg (USDA, Bureau of Entomology and Plant Quarantine).

To support the release program, ARS scientists refined mass rearing methods to improve efficiency and reduce production costs. Equipment for releasing loose host eggs from aircraft was devised. Methods were also developed to temporarily halt development by refrigeration so that Trichogramma could be stored for short periods of time. This ensured that adult wasps would emerge from host eggs within hours after release in the field. Rapid adult emergence was necessary to reduce mortality caused by high soil temperatures, since host eggs applied by airplane were often deposited on the soil (9, 10, 54).

Extensive field trials were conducted in Arkansas and North Carolina during 1981-83. In North Carolina, seven aerial releases of *T. pretiosum* at 2- to 3-day intervals were made at a rate of 124,000 emerged adults/acre per release. Bollworm and tobacco budworm infestations exceeded the established economic thresholds despite relatively high levels of egg parasitism (40 to 60 percent) and predatory insects. Fields in which Trichogramma were released yielded significantly less than fields in which insecticides were applied for bollworm and tobacco budworm control. In one year, however, fields receiving Trichogramma releases yielded significantly more than fields in which no insecticide or wasps were applied (38).

The study concluded that it was not feasible to manage bollworms and budworms in cotton by augmenting Trichogramma populations using the technology then available. Constraints to the use of Trichogramma included the toxic effects from insecticides applied for boll weevil and plant bugs, the lack of a reliable means of predicting oviposition periods and egg density, the need for frequent applications due to the short life span of adult Trichogramma, and the inability to maintain high levels (80 percent) of egg parasitism (38). Following this pilot project, USDA-ARS research activities were limited to the development of artificial diets for mass rearing. In 1996, the project was terminated.

In contrast to the U.S., augmentation of Trichogramma for control of bollworms and related cotton pests is commonly practiced in China, the former USSR, Mexico, Columbia and Peru (45). Before its dissolution, the USSR maintained many large, government supported industrial operations to produce host eggs and Trichogramma, which were applied to numerous field and tree crops for a variety of pests (21).

Many studies have evaluated inundative releases of Trichogramma in cotton but few have studied inoculative releases (30). Several studies indicate Trichogramma does not recycle well in cotton. Insects and spiders that feed on parasitized eggs and kill the immature Trichogramma keep Trichogramma populations from increasing in cotton (1, 2, 32, 46). Also, Trichogramma adults may leave the field when host egg densities are low, which is often the case early in the season when inoculative releases would likely be made (23).

Eggs of the pink bollworm, cotton leaf worm, and cabbage and soybean loopers are parasitized by Trichogramma species in cotton. Eggs of the beet armyworm are seldom parasitized by Trichogramma in the field, presumably because they are protected by the dense mat of moth scales which covers the egg mass. However, Trichogramma will parasitize and kill beet armyworm eggs under laboratory conditions.

Augmentative releases of *T. bactrae* were evaluated in small plot field trials in Arizona for control of pink bollworm. Early-season parasitism was high, but the level of control was insufficient to protect the crop from damage later in the season. Moth migration from untreated areas suggested results might be more promising if releases had been made over a larger area (57).

Constraints to Using Trichogramma in Cotton

Predatory insects and spiders often maintain densities of bollworms and budworms below economically damaging levels, unless these predators are suppressed by broad-spectrum insecticides (20). Densities of bollworm and budworm eggs fluctuate and can rapidly increase as moths emerging from other crops (i.e., corn) move into cotton. It may then be impossible for existing natural enemy populations to maintain infestations below economic levels. This could be an opportunity to augment Trichogramma. However, as Trichogramma must be released prior to egg hatch, one would have to accurately predict moth occurrence and egg density to plan parasite releases (38). In field studies in North Carolina, densities of bollworm and budworm eggs between egg laying cycles were too low to maintain reproduction of Trichogramma in cotton. Also, low egg densities early in the season failed to support Trichogramma reproduction (38). Other studies have suggested that parasitism rates of Trichogramma are high only when host densities are high (23). Thus, an inundative approach is likely to have greater success than an inoculative approach in cotton.

Bollworm and budworm eggs hatch in about 3 days. Assuming adult parasites live about 4 days in the field and that there is little in-field reproduction, releases must be made at 2- to 3-day intervals to keep searching adults present continuously (38). This requirement increases product and application costs. To extend the release interval, Trichogramma of two different ages can be released (6, 78).

Adult Trichogramma are quickly killed by broad-spectrum insecticides applied to cotton. Some insecticide residues can remain toxic to adults for many days. ULV parathion drifting from treated fields up to a mile downwind has been shown to be highly toxic to adult Trichogramma (11, 75). The use of broad spectrum insecticides to control plant bugs, boll weevils and other pests in cotton is a significant constraint to the use of Trichogramma, and may account for the scarcity of Trichogramma in commercial fields.

Competition with predators for eggs and the predation of parasitized eggs by predatory bugs (*Orius, Geocoris, Nabis*), lacewing larvae, spiders and other predators also may be important factors in the poor performance of Trichogramma in cotton (1, 2, 30, 32, 53). Parasitism by released *T. pretiosum* was sustained at high rates (55 to 84 percent) in research studies in Texas only when egg predators were absent (13, 32, 46). Studies in Texas (74) and North Carolina (78) agree that many bollworm eggs are destroyed by predators. Under these conditions, releasing Trichogramma adds little additional mortality. This effect is termed compensatory or replaceable mortality as parasitism is replacing predation as the cause of death with no net gain in total egg mortality.

Predators feeding on parasitized eggs also kill the developing parasite. Egg predation by the insidious flower bug, *Orius insidiosus*, reduced egg parasitism in field cages, and parasitism by *T. pretiosum* was greatest in the absence of *Orius* (46). In Texas, life table studies found more than 90 percent of the bollworms died in the egg stage in unsprayed cotton. Egg predation was considered a major cause of death. Under these conditions, few Trichogramma would survive to the adult stage. In fact, parasitized eggs are more likely to be fed upon by a predator because they remain in the field 2.5 times longer than nonparasitized eggs (38).

Density-dependent mortality during the larval stage can also minimize the effects of increased egg parasitism. For example, if egg parasitism is low, a large number of eggs will hatch. There will then be numerous small larvae

Ecological constraints to releasing Trichogramma in cotton.

1. Parasitism represents replaceable mortality because of competition with predators for eggs.
2. Wasp mortality caused by egg predators.
3. Potential density dependent predation of young larvae.
4. Difficulty in maintaining field populations of Trichogramma.

and the subsequent impact of density dependent mortality on the larval stage will be greater. If egg parasitism is high, the resulting density of small larvae is low and the proportion killed by density dependent mortality factors declines. The result is no net reduction in numbers of later instar larvae, which are often the most damaging stage, even though a greater percentage of the eggs was killed by parasites. Studies in North Carolina concluded that because of density-dependent larval mortality factors, the egg stage may not be the appropriate target for biological control of bollworms and budworms in cotton (78). In contrast, the absence of density dependent mortality during the larval stage of the European corn borer makes this pest especially suited to control by Trichogramma (4).

Trichogramma in Corn

The egg stages of many caterpillar pests of corn, including the European corn borer, corn earworm, and southwestern corn borer, are attacked by various species of Trichogramma. In several European countries, augmentative releases of Trichogramma are as effective as insecticides in controlling the European corn borer. In 1993, about 67,000 acres of corn were treated with Trichogramma in France, Germany and Switzerland (24). The research and development program which led to this commercial success is a model for developing augmentation programs for other crop and pest systems.

Components of the successful program to augment Trichogramma for control of European corn borer.

1. Most effective species determined.
2. To maintain parasite quality, rearing procedures include semi-field conditions and renewal of the colony after six generations.
3. Development of effective release and distribution methods for field application.
4. Strong relationship between rates of egg parasitism and reduction in densities of borer larvae, leading to commercially accepted levels of control.

In Switzerland, Trichogramma were commercially released for European corn borer control in corn beginning in 1978. After several years of success, field performance dropped dramatically (below 75 percent egg parasitism), and a research program was initiated to improve the production system and field delivery methods (6, 7). More than 15 species and strains of native and exotic Trichogramma were evaluated in field and laboratory tests to determine those with a high preference for European corn borer egg masses. *Trichogramma brassicae*, a species native to Moldavia, was selected for commercial production although studies showed *T. ostrinia*, from China, was a suitable alternative. Rearing techniques with stringent quality control procedures were developed to ensure the production of effective parasites. The stock population of Trichogramma is maintained under semi-field conditions in an insectary or greenhouse planted to corn plants infested with European corn borer egg masses. Parasitized egg masses are collected to obtain adults for mass rearing on the Mediterranean flour moth, *Ephestia kuehniella*. After six generations, the production colony is terminated and a new culture initiated, again using wasps from the stock colony. Rearing for longer periods on the flour moth results in a decrease in field performance. This process selects for Trichogramma with the ability to fly and to locate and parasitize corn borer egg masses on corn, and it helps reduce the loss of these important characteristics caused by the mass rearing of multiple generations on an alternate host.

Trichogramma brassicae pupae can be programmed to enter a condition of arrested development called diapause. Once in diapause, wasp pupae can be stored for up to 9 months so that the large demand for Trichogramma during the summer can be met (6).

Cardboard capsules containing host eggs with developing Trichogramma are applied to the corn field and can be distributed by airplane. Capsules either fall to the ground or are caught in the corn plant. The capsules protect the Trichogramma from predators and weather extremes until the adults emerge from the host egg and escape through tiny holes in the capsules. Released Trichogramma are at different developmental stages so that adults emerge from the capsules over several days. This increases the time interval between applications.

Two releases each at a rate of 185,000 pupae per acre are made beginning at the first moth flight as determined by black light traps. European corn borer eggs hatch after about 5 to 6 days and the egg-laying period continues for 4 to 7 weeks. In-field reproduction of released parasites is believed to be important in providing residual control of eggs deposited after the second release. Field evaluations in Germany have shown releases result in a 70 to 93 percent reduction in corn borer larvae relative to untreated fields (6).

Efforts to transfer this technology for European corn borer from Europe to the U.S. led to field trials in several Midwestern states in 1994-97 (3, 4). Releases used *T. brassicae* and the same capsule technology developed in Europe. Releases often reduced both densities of larvae and stalk damage to low levels equal to that achieved by insecticides. There was also a strong relationship between rates of egg parasitism and reduction in densities of larvae. However, variability between release rates and rates of egg parasitism caused inconsistent control (4). This variability was attributed to differences in the quality of released parasites and in environmental factors such as plant structure, weather and alternative hosts (3).

Trichogramma are not being used commercially to control European corn borer in the U.S. A recent economic analysis of the potential use of Trichogramma in U.S. corn production (3) concluded that even with substantial improvements in efficacy and a reduction in costs, Trichogramma would not likely be used in field corn. The value of fresh market sweet corn is much greater and quality standards are more stringent, so using Trichogramma in this crop had greater economic benefit than no control, but fell far short of the benefits of control with insecticides. However, it was concluded that Trichogramma could be competitive with insecticides with modest improvements in effectiveness and reduction in costs. A premium for sweet corn grown without insecticides could also offset the additional costs of Trichogramma. The most likely use of Trichogramma was in seed corn production. Insecticides applied for European corn borer control in seed production fields must have low toxicity to humans as corn is detasseled by hand. Although detasseling is completed in about 10 days, it often corresponds to the optimum time for applying insecticides for European corn borer control. The insecticide carbaryl is commonly used to balance efficacy with human

First description of Trichogramma in the U.S. and their subsequent loss.

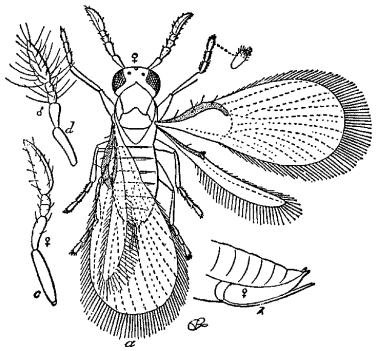


Figure 4. Trichogramma pretiosum (C.V. Riley, USDA).

Charles V. Riley, first Chief of the U.S. Bureau of Entomology, was the first to describe a Trichogramma species in North America. While serving as state entomologist for Missouri, Riley collected eggs of the Viceroy butterfly from willow near Kirkwood, Missouri. He described the tiny wasps that emerged from the eggs as *T. minutum* in a 1871 publication. The original specimens, termed type specimens, are a very valuable reference to taxonomists. Unfortunately, Riley's original specimens of *T. minutum* were later destroyed. In 1879, Riley described a second species, *T. pretiosum*, reared from eggs of the cotton leafworm, *Alabama argillacea*, collected from cotton near Selma, Alabama. Unfortunately, the original specimens of *T. pretiosum* were also later lost. Without type specimens, taxonomist must rely solely on the written description of the species. For decades, the loss of these type specimens and the similarity in appearance of Trichogramma species resulted in the misidentification of the most common species of Trichogramma. To re-establish the basis for these species, entomologists returned to Kirkland, Missouri in 1970 and again collected eggs of the Viceroy butterfly from willow. The Trichogramma emerging from the eggs matched Riley's original description of *T. minutum* and were preserved. Entomologists also returned to Selma, Alabama in 1972 to obtain *T. pretiosum* from eggs of the cotton leaf worm. These eggs could not be found in cotton, but Trichogramma emerging from bollworm eggs, *Helicoverpa zea*, collected from cotton matched Riley's description of *T. pretiosum*. These specimens, termed neotypes, now serve as the reference for these species and are preserved in the United States National Museum (65).

safety. Using the assumptions listed by Andow (3), releasing Trichogramma was nearly competitive with insecticides in seed corn production. A 40 percent reduction in cost and an increase in efficacy from 60 percent to 80 percent could lead to widespread use of Trichogramma in the seed corn industry. Trichogramma also can be integrated with compatible insecticides such as Bt for control of European corn borer (52).

Other caterpillar pests of corn, such as the southwestern corn borer and corn earworm, are attacked by native species of Trichogramma in the U.S. and throughout the world. Some companies sell Trichogramma for control of these pests, but the research to support this use is lacking. In the Texas High Plains, *T. pretiosum*, *T. deion* and *T. thalense* were recovered from southwestern corn borer eggs. Parasitism rates in insecticide-free fields during a 3-year study averaged less than 5 percent with a peak of 40 percent (43). Parasitism of corn earworm in corn in California averaged 38 percent, yet 99 percent of the ears were infested with an average of three larvae (59).

Use of Trichogramma in Other Crops and Commodities in the U.S.

Augmentation of Trichogramma has recently been promoted for pest control in cotton, corn, apple, spruce and avocado production (61). However, a recent survey found that very few state Cooperative Extension Services currently provide recommendations for controlling any insect pest with Trichogramma.

In California, parasitism of tomato fruit worms (bollworms and budworms) by native *T. pretiosum* in tomatoes is considered in the treatment thresholds for treating these pests with insecticides (26). Augmentation of *T. pretiosum* is an effective control tactic in Mexico and is part of the IPM program for fresh market tomatoes (81).

In California, two avocado pests, the omnivorous looper *Sabulodes aegrotata* and the moth *Amorbia cuneana*, can be managed by releasing *T. platneri* in every fourth avocado tree (61). Large field studies in Canada have shown that two releases each of 30 million *Trichogramma minutum* per acre resulted in 60 to 80 percent egg parasitism of spruce budworm, *Choristoneura fumiferana*, in white spruce stands (61). However, Trichogramma releases have not been adopted as a control tactic for spruce budworm in Canada (73).

Developing a Biological Control Program Using Trichogramma

Selecting the "Best" Trichogramma species

Different species and strains of Trichogramma typically prefer different host eggs and crop habitats and have different searching abilities and tolerance to weather conditions. Efficacy is improved by selecting the most effective and adapted species or strain for the specific crop/pest situation. Local species and strains collected from the target area and host often are the first choice for evaluation. However, exotic species and strains may be more effective and should be evaluated.

If a species of interest is available commercially, it should be evaluated along with native species. However, unless the supplier maintains high quality control standards both genetic and product quality can deteriorate, leading to poor field performance (51, 82).

Cultures of Trichogramma are begun from single isolated females to ensure species purity. Host eggs are collected from the field, placed individually in gelatin capsules, and held until adults emerge. A minute streak of pure, diluted honey should be put in each capsule just before adults emerge; capsules should be examined daily for adults. A colony is initiated from a single host egg that yields both male and female adults. The species is identified once each colony contains a number of adults. Single colonies of the desired species are then combined to broaden genetic diversity. A minimum of 500 to 1,500 individuals may be required to initiate a culture without loss of genetic diversity (21,73).

Standard laboratory trials are conducted to determine the ability of the candidate species to parasitize and develop in the target host egg, the species' preference for the target host egg, and total egg mortality caused by parasitism and adult feeding (82). Other important attributes include fecundity, development rate, sex ratio, and longevity. These traits indicate probable field performance and are important in mass-rearing programs. Species that show a high degree of host preference are then evaluated for searching ability by releasing adults into cages containing host eggs on the crop plant. Cage experiments are conducted under different temperatures and humidities and different ratios of parasites to host eggs. The species or strain(s) parasitizing or killing the greatest number of eggs are considered to have a high potential for success in field releases (24).

Mass Rearing Trichogramma for Commercial Release

Rearing Trichogramma requires first rearing an insect, typically a species of moth, to produce eggs in which the wasps will develop. The Angoumois grain moth, *Sitotroga cerealella*, and the Mediterranean flour moth, *Ephestia kuehniella*, are easily and inexpensively reared on wheat or other grains and are commonly used to rear Trichogramma (54). Studies to date indicate that there is no difference in field performance between Trichogramma reared on *Sitotroga* and those reared on *Ephestia* (73). To lower production costs, research is underway to develop an artificial egg. China has led in this area and commercially produces one species of Trichogramma on an artificial diet composed of insect blood (73). Further research should lead to major reductions in production costs (15, 21).

Poor quality of mass reared Trichogramma can result in control failures (4, 6, 14, 62). The artificial conditions of mass rearing can select for genetic changes that reduce the effectiveness of the Trichogramma in the field. Such rearing conditions include rearing multiple generations on unnatural host eggs, the absence of plants, crowding and interference, rapid generation time, and failure to rejuvenate genetic stock (6, 21).

Except for obvious problems such as lack of adult emergence or wing deformities, growers and pest advisors cannot detect poor quality Trichogramma prior to release. Commercial suppliers are responsible for maintaining desirable characteristics necessary for good performance in the field. Production colonies should be periodically (i.e., every six generations) replaced with individuals from a stock culture maintained on the natural or target host (6,21). Suppliers also should assess the percent host egg parasitization, adult emergence, and the sex ratio of emerged adults to be sure they are within acceptable standards. Standards for established cultures on *Sitotroga* are 80 ± 5 percent egg parasitization, 90 ± 5 percent adult emergence, and a sex ratio of 1.2 to 1.5 females per male (14, 21). The Association of Natural Biocontrol Producers and the International Organization of Biological Control

Farming Trichogramma: a short history.

The idea of "farming" Trichogramma for control of caterpillar pests was discussed in detail in 1895 at a meeting of the London Entomological and Natural History Society (16). By 1916, Russian entomologists were rearing and releasing small numbers of Trichogramma for codling moth control. In 1926, apparently unaware of the work in Europe, Stanley Flanders, an entomologist employed by the Saticoy Walnut Growers Association in California, and Harry Smith, an entomologist at the University of California, decided to investigate the possibility of mass rearing and releasing Trichogramma for control of codling moth, a recent pest of walnuts. Trichogramma was viewed as a desirable alternative to the use of arsenical insecticides, as residues of arsenic on apples were a current health concern. Flanders discovered that Trichogramma could be mass reared on eggs of the Angoumois grain moth, *Sitotroga cerealella*, which was reared on grains such as corn and later wheat. His methods quickly became the standard for rearing Trichogramma in the U.S. and other countries. Commercial companies soon began claiming successful control of many pests (although there was no scientific evidence). Concerned about overselling biological control, Smith and Flanders published an article titled "Is Trichogramma Becoming a Fad" in which they stated "Unless a conservative attitude is maintained, biological control can easily become quackery. Some of the work with Trichogramma seems to be tending in this direction" (69, 71). The ethical considerations necessary to ensure commercial augmentation continues to develop into a highly professional industry are still being discussed today (27).

Subcommittee on Quality Control are developing quality control standards for Trichogramma and other natural enemies (7).

Methods for Releasing Trichogramma

Trichogramma are typically shipped and released as pupae inside the host egg. Parasitized pupae are distributed in the field just prior to emergence of the adult wasps, although in some Latin American countries wasps are released after emergence. Parasitized host eggs can be mixed with a carrier and broadcast, or glued to cards or the inside of paper capsules which are then dropped onto or attached to the crop. Broadcasting host eggs is relatively simple but host eggs that fall on the ground may be subjected to lethal temperatures or drown in flooded areas. Also, adults emerging from loose eggs or egg capsules deposited on the ground must walk or fly to the plant to locate host eggs.

It is desirable for adults to emerge quickly so they escape predation and avoid high temperatures. Adult emergence can be synchronized to occur shortly after release by refrigerating host eggs at 16.7 degrees C in total darkness beginning on the eighth day of development. After at least 6 days and up to 10 days of refrigeration, adults will emerge within 4 hours once pupae inside host eggs are exposed to light and at least 27 degrees C. This reduced temperature regime results in about 73 percent of the adults emerging within 4 hours of field release. However, chilling and warming can interfere with development (54, 72, 75).

Both manual and mechanized release methods using ground and aerial applicators have been developed (45). Aerial release methods using refrigeration units were developed in the U.S. (9, 10). A liquid spray system (Biosprayer®) is available for ground application (42).

Evaluating Releases of Trichogramma

Field releases of Trichogramma are evaluated by measuring egg parasitism, larval densities, crop damage and economic return relative to similar fields treated with insecticides or not treated. The release method and release rate and frequency should be the same as used in commercial applications. Fields or plots should be as far apart as possible (at least 100 feet apart in corn and cotton) to reduce movement of adults between treatments (4, 24, 57).

Egg parasitism measures pest mortality directly and is determined by collecting eggs from the field and holding them for evidence of parasitism (eggs turning black or the emergence of a wasp). When eggs of the target species are uncommon or difficult to locate, egg parasitism can be estimated by placing sentinel eggs, either of the target species or other acceptable species, in the field. Sentinel eggs can be killed by UV light to prevent hatch yet still remain suitable for parasitism by Trichogramma (19). The density and distribution of sentinel eggs on the plant and in the field should be similar to that of wild eggs. Unnatural clumping of sentinel eggs increases parasitization (23). Also, sentinel eggs should not be washed because moth scales left by the female on and around the eggs contain chemical cues (karimones) that arrest Trichogramma (7).

Trichogramma wasps also kill host eggs by feeding on them. The host egg is stung and the adult feeds on the drop of liquid appearing at the site of the sting, but no egg is laid. The host egg dies, leaving no evidence of parasitism. In some species of Trichogramma, host feeding contributes significantly to pest control (82). For this reason, egg hatch should be recorded in addition to egg parasitism. Typically, field collected or sentinel eggs are held for hatch

and then scored as hatched or dead due to parasitization, predation, infertility (undeveloped) or undetermined causes.

Parasitism also can be estimated by determining the ratio of parasitized (black) and unparasitized eggs in a field sample and comparing it to the actual parasitism rate as determined by holding the eggs. This relationship was described for bollworm attacking tomato in California and is used to rapidly estimate egg parasitism and adjust treatment thresholds (26).

The length of time host eggs are in the field exposed to parasites determines the likelihood of their being parasitized. Thus, egg development at the time of collection should be recorded so that exposure time can be determined. Eggs of bollworms and budworms are pearly white when first deposited. After 15 to 18 hours, a reddish or tan ring becomes visible around the top of the egg. About 10 hours before hatch, the black head capsule of the larva is visible (67). Once an egg is parasitized, the band, if present, disappears and the egg becomes tan in color for 3 to 4 days before turning black. If only white eggs are collected, parasitism would be underestimated since eggs were not exposed a full 2 days. Since unparasitized eggs are in the field for 3 days and parasitized eggs are there for about 8 or 9 days, the chances of finding a parasitized egg are three times greater based on relative numbers alone. Thus, eggs that are black at the time of sampling should be excluded from the sample to avoid overestimating parasitism. Excluding white and black eggs and using only tan eggs provides the most accurate estimate of actual parasitism (50).

A parasitism rate of 80 percent is desirable according to theoretical studies and population models (40). Field parasitism of 75 percent or more is the acceptable level for European corn borer (6).

The densities of larvae must also be assessed because increased egg parasitism and mortality may not reduce densities of damaging larvae. For some pests an increase in egg parasitism by Trichogramma may represent compensatory or replaceable mortality rather than additive mortality (78). Comparisons of crop damage, yield and quality are important in assessing the economic return on augmenting Trichogramma. Other methods of evaluating releases may include trapping adult wasps to study movement and dispersion. Sticky traps, yellow pan traps and low malaise traps have been used to capture Trichogramma adults (22, 38).

When evaluating Trichogramma releases it is important to remember the indirect benefits. Unlike many insecticides, Trichogramma have very little impact on other natural enemies which may be valuable in holding the target pest and secondary pests in check. Also, augmentation programs do not pose risks to field workers or leave toxic residues on produce (45, 81).

Environmental Factors that Affect Field Performance

The ability of released Trichogramma to locate host eggs depends in part on the distribution pattern of the Trichogramma in the field, the release technique, the size and structure of the crop, and the location of the host eggs (7). The distribution pattern should bring Trichogramma as close as possible to host eggs to reduce searching time by the wasp.

Weather conditions such as low temperatures and rain can reduce searching and parasitism (78). Wasps are easily transported by wind and may be blown out of release fields. In corn, 40 to 60 percent of released Trichogramma daily move out of the field this way (6). In contrast, most *T. minutum* and *pretiosum* wasps in cotton remained in the release field (18, 34).

Host plant characteristics can influence the parasitism rate. The density and pattern of trichomes (minute, stout hairs on leaves) can slow the searching rate of Trichogramma and reduce parasitism (79). Dense canopies allow Trichogramma to disperse by walking and by short jumps between plants. Nectar feeding increases adult longevity, so the absence of nectaries in cotton reduces parasitism (80). Parasitism can decline as leaf surface area increases, creating a greater area for wasps to search. However, studies in cotton relating leaf surface area to parasitism rates have been contradictory (2, 78).

Integrating Trichogramma with Other Pest Management Tactics

Trichogramma are very sensitive to the broad spectrum insecticides commonly used in cotton. Parathion drifting from treated cotton fields was found to kill Trichogramma adults up to a mile away (12, 76). In Arkansas, a single application of thiodicarb (0.25 pounds active ingredient /acre) or cyhalothrin (0.033 pounds active ingredient /acre) significantly reduced field parasitism levels for at least 8 days after application. Cyhalothrin reduced adult emergence from treated host eggs by 47 percent, while thiodicarb had no effect on adult emergence. While the host egg provided some protection to developing Trichogramma, residues of these treatments on treated cotton leaves killed more than 90 percent of the emerging adults for 5 days after application. Application of *Bacillus thuringiensis* (Bt) insecticide had no impact on adult emergence or survival (44).

Fresh market tomato production is often plagued by tomato pinworm, tomato fruitworms (bollworm and budworm) and beet armyworms. The costs of multiple insecticide applications and insecticide resistance threatened the multi-million dollar fresh market industry in Mexico about 1990. Previous research had shown that *T. pretiosum* was effective in tomato for bollworm/budworm control, but not with insecticides used to control tomato pinworm and beet armyworm. An IPM program was developed using mass releases of *T. pretiosum*, microbial pesticides, abamectin and mating disruption. This program was more profitable than the conventional insecticide program, reduced the potential for development of insecticide resistance, was less toxic to field workers and non-target organisms, and reduced fruit contamination (81).

Future Prospects for Trichogramma

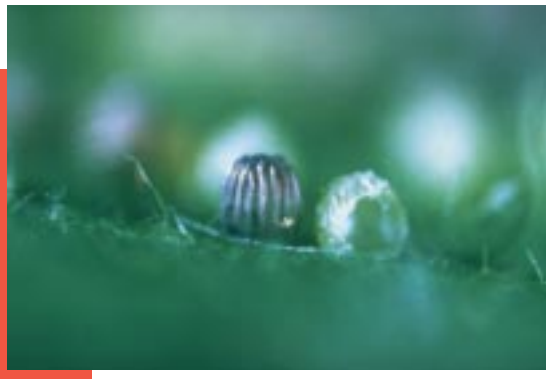
Controlling specific caterpillar pests with augmentative releases of Trichogramma is technically feasible in some field and forest crops. However, adoption has been slow because of variable levels of pest control in the field, high production costs, lack of application technology, and incompatibility with insecticides applied for other pests. In some pest and crop systems, targeting the egg stage may not be appropriate because of compensatory and density dependent mortality factors (78). These are the major challenges to researchers in developing augmentation programs (3, 39):

1. Develop mass rearing methods and quality controls that consistently yield high quality parasites. The variable and often unmeasured genetic changes that Trichogramma undergo in mass rearing need to be understood and managed to preserve the behavioral and biological characteristics necessary for high efficiency in the field.

2. Understand and quantify the relationship between numbers of parasites released and their impact on the pest population and on the level of crop protection, as well as the influence of the crop and environment on the action of the natural enemy.
3. Ensure that the most suitable species or biotype is selected for augmentative use.
4. Determine the optimal size of the release area and the dispersal of the predator or parasite.
5. Develop pest management systems that eliminate or limit insecticide interference with natural enemies.
6. Develop efficient methods of producing Trichogramma and define environmental parameters and specifications for storage, shipment and field release.

The successful use of augmentative releases of Trichogramma in IPM programs will depend on a sound and thorough research program, favorable economics, commercial investment, and the development of an Extension program to transfer this technology to crop consultants and growers.

Bollworm egg with ring. (W. Sterling)



Bollworm egg parasitized by Trichogramma (left) and egg shell remaining after bollworm caterpillar has hatched (right). (J. K. Clark)

Adult Trichogramma wasp mounted on microscope slide. (W. Sterling)

Egg tray for holding leaf disks with eggs. Leaf punch for collecting eggs from leaves is shown at bottom. (A. Knutson)

Trichogramma pretiosum parasitizing an egg of the cotton bollworm, *Helicoverpa zea*. (H. Negri de Oliveira)

Trichogramma pretiosum parasitizing an egg of the Mediterranean flour moth, *Ephestia kuehniella*. (H. Negri de Oliveira)

Protocols for Evaluating Augmentative Releases of Trichogramma in Cotton

Protocol No. 1
Experimental Treatments and Design

Objective: Establish replicated field plots in a commercial field of cotton to evaluate the release of Trichogramma for bollworm control by comparing two treatments.

Treatments: **Trichogramma release.** Release rates should be reported as the number of females per acre as only females attack the pest egg. Trichogramma will be released at a rate of 100,000 pupae per acre. Adult emergence is typically about 80 percent, yielding 80,000 adults per acre. At a sex ratio of 60 percent, the release would yield 48,000 female Trichogramma per acre. The insectary should provide expected adult emergence and sex ratio. This can also be confirmed by using the quality control protocol No. 5. Releases will be made twice a week. Releases will begin 1 to 2 weeks prior to expected egg lay and continue for about 4 to 8 weeks or until eggs are no longer present.

Check. No Trichogramma will be released in this treatment.

Field Selection: The most important factor in field selection is the grower's assurance that it will not be treated with insecticides once Trichogramma releases begin. Review the "bail-out" procedure below with the producer to address concerns about crop damage from bollworms. Keep in close contact with the grower regarding infestation levels so informed decisions can be made about the need to apply an insecticide. To reduce the effects of insecticide drift, fields should be distant from other fields that will be sprayed .

Experimental Design: Each treatment will be replicated four times within the study field of about 40 acres. The study field is divided into four quadrants, each with a minimum of 9 to 10 acres. Within each quadrant, establish a "Release" plot and a "Check" plot. Each plot should be 0.5 acre, or 150 feet wide (45 40-inch rows) X 145 feet long. Plots will be separated by a minimum of 300 feet to reduce movement of released wasps between plots and by a minimum of 50 feet from the field margin. The distance between plots should be greater where larger fields are available.

Bail-Out Provision: Because these studies will be conducted in commercial cotton fields, insecticides will be applied for bollworm/budworms if sampling shows the released Trichogramma fail to maintain pest densities below economic levels and excessive losses are imminent.

Protocol No. 2

Sampling Plants to Determine Bollworm Density and Damage and Collecting Bollworm Eggs

Objective: Evaluate treatments by comparing the following measurements in the Release and Check plots:

1. Percent egg parasitism.
2. Densities of boll/budworm larvae per plant or per acre.
3. Percent worm damaged fruit and total sound fruit per plant or per acre.

Frequency: Sample once a week on Monday for bollworm eggs, larvae, other predators, and fruit damage. Sample three times a week, on Mondays,Wednesdays and Fridays, to determine egg densities and to collect bollworm eggs.

1. Sampling for Bollworm Eggs, Larvae and Damage (once a week)

Materials: The following materials should be taken to the field: field plot plan; Plant Sampling Form; egg trays; marking pen; leaf disc cutter; cooler and blue ice; carpenter's bag.

Procedure: A stratified random sampling program will be used. Leave five rows buffer on each side of the plot and sample in the center. Select four sample sites equa-distant along a looping path while walking through the center of each 0.5-acre plot. Sample three adjacent plants at each of the four sample sites, for a total of 12 plants per plot (total of 48 per treatment). The first of these three plants must be selected at random and the next two consecutive plants in the row are sample plants number two and three. To ensure the plant selection is a random process, stop at the sample site, select a plant and number it "0." Obtain a number at random by reading the number on a die pulled from your pocket. Using that number, count from the "0" plant to arrive at the first sample plant. The next two plants in the row are sample plants numbers two and three. This process is repeated four times in each quadrant and will total 48 sample plants per treatment.

Carefully examine the plant "terminal" (the upper 6 to 8 inches) for bollworm eggs and small larvae feeding in the terminal buds. Also search stems and squares for eggs. Pull open the bracts around squares and open or pull off blooms to uncover bollworms. Record bollworm larvae by size: small < 1/8 inch; medium 1/8-1/4 inch, or large > 1/4 inch. Record all bollworm eggs and larvae on the Plant Sampling Form. Collect all bollworm eggs as described below.

Record bollworm damage for each sample plant as: a) number of worm damaged squares; b) total number of squares (damaged and sound); c) number of worm damaged bolls; d) total number of bolls (damaged and sound). Blooms should be counted with bolls.

Once three plants at a location are examined for bollworm eggs and larvae, sample three nearby, but undisturbed, plants for predators using a beat bucket. A beat bucket is a white, 5-gallon white plastic pail. The sample plant is quickly bent into the pail and vigorously shaken and beaten against the side of the bucket eight to ten times within 2 to 3 seconds. This action dislodges predators, which fall to the bottom of the bucket. Remove the plant and record on the field sampling data sheet the number of spiders and predatory insects (including Orius, big-eyed bugs, lacewing larvae and lady beetle adults

and larvae) collected in the bucket. As with sampling for bollworms, sample 12 additional plants for predators in each plot.

2. Recording and Collecting Bollworm Eggs (three times a week)

Materials: Eight eggs trays, Plant Sampling Form, permanent marker, leaf disc cutter, cooler. Carry the egg tray without the glass, along with the marker and leaf disc cutter, in a carpenter's bag tied around the waist.

Procedure: Search only for bollworm eggs on 12 plants per plot as described above. Record egg density on the Plant Sampling form. Place all eggs in an egg tray using the leaf disc cutter described under Equipment and Supplies. Only tan eggs will be used later but to ensure accuracy of aging eggs and to save time in the field, all eggs should be collected. Upon returning from each plot, attach the glass cover over the egg tray. Use a permanent marker to record the treatment and plot number and collection date on the back of the egg tray. Use a different egg tray for each plot. Thus, you will need eight trays for each sample date. Place trays in a cooler with ice until they are returned to the lab. The developmental stage of each egg must be recorded on the same day it was collected as described in Protocol 4.

Once all 12 plants have been sampled in a plot, search for and collect additional bollworm eggs on other plants as time permits. Do not record these eggs on the Plant Sampling Form as they will not be used to determine egg density. The additional eggs will be used to determine percent parasitism. A total of 30 minutes sampling and in searching for eggs is recommended for each plot (eight plots will require at least 4 hours).

Weekly Activity Summary

Activity	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
Sample, collect bollworm eggs only	X		X		X
Release Trichogramma upon arrival		X			X
Sample plots; record bollworm, worm damage, beneficial insects	X				
Examine collected eggs; record fate; preserve wasps		X		X	
Determine wasp emergence and sex ratio from quality control samples			X	X	

Protocol No. 3
Receiving and Releasing Trichogramma

Objective: Prepare shipment for field release and collect sample of shipment to determine percent wasp emergence and sex ratio.

Frequency: Twice a week, every shipment.

Procedure: Shipments of Trichogramma will be received from the insectary by overnight delivery on Tuesdays and Fridays and should be released the same day. If necessary, host eggs can be kept overnight in a cool (about 60 degrees F), dark area and released the following day. Upon opening the container, look closely to see if adult wasps have already emerged. If large numbers of adults are present, contact the supplier. Also, the ice pack should still be cool.

Releasing host eggs on cards by hand: Each shipment will contain two "cards," each divided into 30-inch "squares." The squares should be cut with scissors along the perforations. Each card should contain about 100,000 wasp pupae, so each of the 30 squares should represent about 3,300 wasps. Each 1/2-acre plot will need 15 squares for a release rate of 50,000 per 1/2-acre (100,000 per acre) on each release date. A total of 4 X 15 or 60 squares will be needed for all four plots. Cut each square in half to yield 30 half squares per plot and place these 30 halves into a container. Repeat for a total of four containers.

Next, use a paper punch to punch out a disk from the center of one half square from each of the four containers. Place each disk individually in a tightly sealed vial and process these samples as in Protocol 5.

Place the containers with half squares in a covered cooler (no ice); keep it out of direct sunlight and transport it to the field. Place the squares on 30 different plants spaced uniformly across each 1/2-acre release plot. Use a small stapler to fasten each section to the underside of a cotton leaf in the terminal, with the host eggs facing out. Handle the sections carefully to avoid knocking host eggs from the cards.

For each Trichogramma release, record the release date, time, number per acre, crop growth stage, and weather conditions as listed on "Record of Releasing Trichogramma" data form.

Releasing host eggs by ground applicator: Host eggs will arrive as loose eggs (not glued to cards). The insectary will package the eggs in quantities based upon weight per your request. Since the number of host pupae per unit weight varies with parasite development and moisture content, the number of host eggs per gram should be periodically confirmed.

An analytical scale can be used to weigh out 0.01 gram of loose host eggs. Count the number of parasitized moth eggs in the 0.01-gram sample. As an example, 0.01 gram of *Ephestia* eggs parasitized by Trichogramma will contain about 350 to 450 host eggs. Repeat this measurement for at least ten 0.01-gram samples to obtain an average. The weight of host eggs necessary for the target release rate can then be determined. Four subsamples (ca. 0.01 grams each) of loose host eggs should be placed in a tightly sealed vial and held for adult emergence as described in Protocol 5.

Protocol No. 4
Handling Bollworm Eggs to Determine Parasitism

Objective: Determine parasitism of bollworm eggs collected from field plots.

Frequency: Three times each week (Monday, Wednesday, Friday).

Methods: Each egg in the egg trays should be examined with a microscope, or a good quality 10 X magnifier, as soon as possible (the same day) after collection from the field to determine the stage of development. Once they are taken from the field eggs should be held in a cooler to slow development until they are aged.

The stage (age) of each egg should be recorded on the "Fate of Bollworm/Bollworm Eggs" form as white, ringed, black-head or parasitized (black). Any parasitized eggs should be removed from the leaf disk and placed in a gelatin capsule for later identification of the wasp as described in Protocol 6. Use a fine brush moistened in water to gently lift the black egg off the leaf disk and place it in the gelatin capsule. Label the capsule with the collection date and treatment name (release or check plot).

Hold the eggs at about 75 degrees F and 70 to 80 percent relative humidity. Four to 5 days later, examine each egg again and record its fate as hatched (larva present and chorion clear), parasitized (egg black or adult parasites and emergence hole in chorion), or dead from unknown causes (eggs discolored, collapsed or white indicating sterility). Again, parasitized eggs should be removed from the leaf disk and placed in a gelatin capsule for later identification of the wasp. Although uncommon, eggs also can turn black due to parasitism from Telenomus wasps. Telenomus adults are uniformly shiny black and therefore easily distinguished from Trichogramma adults.

Calculating Percent Parasitism: Eggs that were in the "white," "black-head" or "parasitized" stage on the day of collection are removed from the data (see rationale below). Percent parasitism is then calculated as:

$$\text{Percent parasitism} = \frac{\text{No. of eggs parasitized}}{\text{No. of eggs in the ring stage at the time of collection}}$$

Trichogramma may cause some egg death which does not result in the bollworm egg turning black or yielding adults wasps. For this reason, percent egg survival may also be an important measure of the impact of Trichogramma.

$$\text{Percent egg survival} = \frac{\text{No. of eggs hatching}}{\text{No. of eggs in the ring stage at the time of collection}}$$

Rationale: Bollworm eggs change color as they develop. Newly oviposited eggs are white. After 15 to 18 hours a reddish-brown ring develops around the top of the egg (67). This band is composed of uric acid crystals, a waste product of the developing bollworm embryo. About 10 hours before hatch (eclosion) the black head capsule of the larva is visible.

Older eggs, which have been in the field longer, have been exposed to parasitism by Trichogramma for a longer period of time and are more likely to be parasitized than those recently laid. Thus, knowing the age of the egg is important in accurately measuring egg parasitism.

White eggs have only been in the field for at most 15 hours, while ringed (tan) eggs have been exposed to parasites for at least 2 days. Thus, the pres-

ence of white eggs in the egg sample will underestimate percent parasitism as they have not been available to parasites as long as have ringed eggs.

Once an egg is parasitized, the brown band, if present, disappears and the egg becomes tan in color for 3 to 4 days. The parasitized egg then turns black and remains black for about 5 more days before the wasp(s) emerges (Fig. 1). An unparasitized egg remains in the field for about 3 days while a parasitized egg remains for 8 or 9 days. Because of this, black eggs accumulate in the field and are more likely to be collected than unparasitized eggs. In practice, this does not usually occur because black eggs are much more difficult for most people to see. However, to avoid the bias caused by the greater number of black eggs, black eggs in the initial sample should not be used to determine percent parasitism. Black eggs should be held separately in gelatin capsules to collect adult wasps for identification.

Considering only eggs that have the brown or reddish ring at the time of collection should provide the best estimate of egg mortality due to parasitism; this method is sufficient for comparing parasitism in release fields to non-release fields.

Another approach to measuring parasitism calculates densities of parasitized and unparasitized eggs using the "area under the curve method." Field temperature data are used to determine developmental rates and residence times for parasites and bollworm eggs.

Protocol No. 5
Determining the Quality and Quantity of Released Trichogramma Wasps

Objective: Estimate release rates of adult female Trichogramma by measuring adult emergence, sex ratio and wing condition from a sample of host eggs.

Frequency: Once a week.

1. Measuring Adult Emergence

The following methods determine the number of adult Trichogramma that emerge from a standard unit of host eggs. A record of adult emergence can be used to estimate field release rates and indicate the need to take corrective action if emergence rates or sex ratio fall below expected averages.

A. Host Eggs Glued to Cards

Use a paper punch to punch out a disk of eggs from four cards. The disk should be punched from the bottom of the card so the eggs are not crushed by the surface of the punch.

Place each disk in a separate screw-top glass vial. Label vials with the release date and field number. Place them in a lighted area at room temperature, about 75 degrees F, but out of direct sunlight. The relative humidity should be 75 to 80 percent. After 7 to 8 days, place vials in the freezer for 4 to 5 hours to kill any wasps still alive. The number of Trichogramma can then be counted with a dissecting scope. To avoid losing the tiny adults and to aid in getting an accurate count, the following steps are suggested:

Use a very fine brush to mark a thin line of dilute white glue (50 percent water) along a microscope slide. Carefully tap the Trichogramma adults from the vial onto the slide. Gently tap the slide until all the adults have become fixed in the line of glue. Check the sides of the vial carefully to be sure all adults have been removed from the vial. Use a small brush to remove those adhering to the sides.

Measure the diameter of the disk and divide by two to get the radius. The area of the disk is then given by the equation: Area = 3.14 X radius squared. Knowing the area of the disk, one can calculate the number of adult wasps emerging per unit area of card. This value can be compared to the expected number of wasps per card as stated by the commercial supplier. Shipments that yield fewer adults per disk than average indicate problems in rearing, shipping or handling host eggs and parasites.

B. Loose Host Eggs

Parasitized *Sitotroga* eggs received from the supplier may contain from 50,000 to 100,000 host eggs per gram. Only one Trichogramma adult typically emerges from a Sitotroga egg because these host eggs are small. If 90 percent of the host eggs are parasitized, a 0.1-gram subsample could contain 9,000 parasitized host eggs, far too many to count. An analytical balance is necessary to weigh 0.001- to 0.002-gram samples of loose eggs. These samples can be held in screw-top vials as described above and should yield about 50 to 100 adults. The number of adults emerging per unit weight can be compared to the expected number per gram as certified by the commercial supplier.

Background: Actual release rates of Trichogramma depend on the number of host eggs parasitized and the percent of parasitized eggs that yield one or more adult parasites. Trichogramma are commonly reared on eggs of the Angoumois grain moth, *Sitotroga cerealella*, and shipped from the insectary as pupae inside of the *Sitotroga* egg. Once the eggs are parasitized by Trichogramma, there is a constant weight loss until the parasites enter the pupal stage. The actual number of parasitized host eggs per unit weight will vary according to the number of unparasitized host eggs and the amount of foreign material such as dead eggs, moth scales, dust, etc. in the sample of loose eggs.

2. Measuring Sex Ratio and Wing Condition

Procedure: Details necessary to sex and determine wing condition are best seen by examining live or recently killed adults with a dissecting microscope. If the adults are dead and dried, use a fine brush to place them in a watch glass with 20 percent ethanol. Leave the insects overnight to soften and expand. This makes it much easier to see antennal hairs necessary to determine sex, and to note any wing deformities. Examine each wasp with the scope and determine if antennal hairs are abundant and long (male) or sparse and short (female). You may need to position the wasp with an insect pin to make the antennae visible. If antennae are missing or difficult to see, skip that individual. Examine about 50 individuals and record their sex and the number that lack wings or have very small or stubby wings (a condition called brachyptery) on the Quality Control data form.

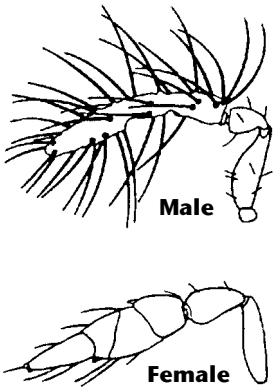


Figure *5*. Antennae of male Trichogramma have many long hairs. Antennae of females have a few short hairs.

Background: A high proportion of females in the released Trichogramma is important as only the females parasitize host eggs. The insectary standard is at least 50 percent females. Adult female Trichogramma have only a few, short hairs on their antennae, while males have a large number of long hairs (22). Trichogramma adults may have wing deformities because of unfavorable rearing or shipping conditions or prolonged cold storage. Wings may be absent or very small or stubby. If a large percentage of adults has malformed wings, parasitism will be reduced because of poor searching ability.

Protocol No. 6
Curating Adult Trichogramma for Identification

Objective: Mount adult Trichogramma on microscope slides as a permanent specimens for species identification. Species collected from bollworm eggs can then be compared to the species of Trichogramma released in the study.

Procedure: Trichogramma adults must be mounted on glass slides because identification relies upon microscopic examination of male genitalia and other morphological characters. The following methods were provided by Dr. John Pinto, University of California at Riverside.

The best results are obtained when live adults are placed into Hoyer's solution as described in 2. "Mounting Procedures." If the specimen is dried or preserved in alcohol, begin with 1. "Specimen Preparation."

1. Specimen Preparation

1. Dried or alcohol preserved specimens should be placed in 10% KOH solution for 6 to 8 hours to clear bodies enough to view genital capsules in detail (internal structures).
2. When an adequate level of clearing has been achieved, the specimens can be transferred to 10% ETOH with a minute amount of dish detergent (5 to 8 drops in 500 ml of solution) for 1 hour.
3. Next, transfer specimens to 20% ETOH for 1 hour.
4. Specimens can now be mounted directly into Hoyer's medium from the 20% ETOH.

2. Mounting Procedure

1. With a small (size 00000) brush, place a small amount of Hoyer's mounting solution onto a glass slide. With a bent minuten probe, lift the specimen from the ETOH and place it in the drop of Hoyer's. Using the same probe, position the Trichogramma specimen with antennae, legs and wings as spread out as possible (this helps keep the specimen from rolling during placement of the cover-slip). Try to position the specimen so that the genital capsule remains in a dorsal-ventral plane (see photo p. 23).
2. Let the drop of Hoyer's dry for about 23 to 35 minutes before placing the coverslip on the specimen.
3. After 25 to 35 minutes, place a small, fresh drop of Hoyer's on top of the specimen and place a round coverslip on top. As the coverslip settles on the specimen and the Hoyer's moves to the outer edges, try to slightly compress the abdomen and keep the genital capsule as dorsal-ventral as possible.
4. Place the slide in a drying oven (100 to 110 degrees F) for about 10 to 14 days. For long-term storage, the slide coverslip should be sealed with a suitable sealant. (Glyptal paint from electronic supply stores works well.)

Additional Information

1. If very dark or black Trichogramma are collected, treatment in the 10% KOH may need to be extended (up to 24 hours total).
2. Round coverslips are easiest to use than square or rectangular coverslips and require less mounting media.
3. The amount of Hoyer's needed before placing the coverslip on the specimen will vary depending upon the size of the coverslip being used.
4. A probe made from the wooden dowels of cotton swabs and a stainless steel minuten pin (bent at the very tip) is a most usable tool.

Equipment and Supplies

These protocols were designed to use commonly available materials. Some materials are purchased (suppliers are listed) while others are made from readily available materials. Use this checklist for field sampling and for equipping a laboratory.

Field:

1. Plant sampling forms, clipboard, pencil
2. Map of field locations and plot numbers
3. Egg trays and disk cutter for collecting bollworm eggs
4. Marker for labeling egg trays with plot number and date
5. Cooler with ice for egg trays
6. Carpenter's bag for carrying egg trays and cutters into field

Laboratory:

1. Dissecting microscope
2. Glass slides
3. Fine brush
4. Small glass vials with screw-on lids
5. Gelatin capsules for holding eggs for wasp emergence
6. Paper punch
7. Hoyer's mounting medium, coverslips
8. Vials

Egg Trays:

Egg trays are constructed from the plastic grid panels (0.25 inches thick and 2 x 4 feet wide) that are used to diffuse light in light fixtures. They can be purchased at building supply stores. Sections about 5.3 x 3.5 inches (about 54 cells) are cut using a hacksaw. Ends of partitions are snapped off with pliers leaving smooth edges. Strips of 2 inch wide plastic tape are pressed against one side to form the bottom of the cells. Each cell is numbered on the inside upper corner using a ball point pen so each egg can be identified by number on the data sheet. A piece of glass slightly larger than the tray is placed over the top and held in place with large paper binders. Trays can be reused by replacing the tape bottoms.

Leaf Punch:

Bollworm eggs can be collected from leaves by using a leaf punch to cut a small disk of leaf containing the egg. A leaf punch can be constructed from a brass rod, spring, two nuts and a 4-inch length of copper tubing. Super weld epoxy can be used to weld the nuts in place. Construction of the leaf punch is described in detail in reference no. 25.

To collect an egg, the sharpened end of the punch is centered over the egg and pressed against the leaf. A neat leaf disk is cut by pressing and rotating the cutting edge through the leaf while the leaf is held against the forefinger of the other hand. Once cut, the leaf disk should be held inside the cutter and transferred to a cell in the egg tray. By depressing the plunger, the leaf disk is pushed from the punch and pressed against the sticky surface (tape) in the bottom of the cell. The tape holds the egg in place and prevents the drying leaf from curling around the egg and hiding it from view.

Plant Sampling Form

FIELD: **DATE:** **TREATMENT:** **TIME START:** **TIME END:**

		Bollworm	Bollworm Larvae			Squares		Bolls		Pirate Bug (Orius)		Big-eyed Bug		Ladybeetle		Lacewing Larva	Spiders	Other
	Plant[a]	Eggs	Small	Medium	Large	Total no.	Worm damage	Total no.	Worm damage	Imm.[b]	Adult	Imm.	Adult	Imm.	Adult			
PLOT No.	1																	
	2																	
	3																	
	1																	
	2																	
	3																	
	1																	
	2																	
	3																	
	1																	
	2																	
	3																	
PLOT No.	1																	
	2																	
	3																	
	1																	
	2																	
	3																	
	1																	
	2																	
	3																	
	1																	
	2																	
	3																	

[a]Do not sample the same plant for both bollworms and predators. See Protocols.

[b]Imm. = immature

Fate of Bollworm/Budworm Eggs

Field: _____ Collection date: _____

Cell	Egg stage*				Fate of egg		
	W	R	BH	P	Hatch	Paras	?
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							
23							
24							
25							
26							
27							

Cell	Egg color*				Fate of egg		
	W	R	BH	P	Hatch	Paras	?
28							
29							
30							
31							
32							
33							
34							
35							
36							
37							
38							
39							
40							
41							
42							
43							
44							
45							
46							
47							
48							
49							
50							
51							
52							
53							
54							

*On day of collection: W = white, R = ring, BH = black-head, P = parasitized (black)

Record of Releasing Trichogramma

Location _____ No. plants/acre _____

No. of acres _____ Planting date _____

Date	Time	No./acre	Temp.	Wind	Comments*

*Crop growth stage, etc.

Quality Control Data Form

Date shipment received_____

Adult Emergence

Disk or sample	No. wasps
1	
2	
3	
4	
5	
Avg. no./sample	

Sex Ratio (50 wasps)		Total	%
M			
F			

Wing Condition (50 wasps)		Total	%
Normal			
Abnormal*			

*Abnormal = wings absent or very short

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