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# Biological insecticide *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) strikes for caterpillar control

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**Abstract**
Mini-wasp *Trichogramma* is a biological insecticide that strikes only to target pests with no risk to other natural enemies, human health and environment. Once liberatedin fields, orchards and forests, tiny parasites would seek out and destroy eggs of the most feared caterpillars, such as sugarcane borers, codling moths, cotton bollworms, corn borers, spruce budworms and many others which cause damage to plants. This publication aims to serve as resource to pest management advisors, consultants, extension service agents and specialists, and others seeking information on biological control using *Trichogramma*. Correct timing of *Trichogramma* release requires good coordination between rearing facility, field staff and according to number of generations of pest. In one generation area, three times 50,000 adults per hectare are released, whereas in two generations area 150,000 adults per hectare are released only once per generation. The lifespan of these parasitoids is roughly 7 days in their immature stages, and then up to 10 days as adults for parasitism. For use of *Trichogramma* effectively, put them in field when first flight of moths is seen including species used, quality and parasite product, numbers and timing of release, release method and interactions between parasite, target pest, crop and environmental conditions.

**Keywords:** *Trichogramma,* Egg parasitoid, Parasitization, Biological control

## 1. Introduction
Trichogrammatids are the common group of egg parasitoids used for biological controland parasitoid modelling across the world. The genus *Trichogramma* is the most studied and successful taxa used in biological control programs. The members of genus *Trichogramma* are included in one of 80 genera in the family *Trichogrammatidae* ranging in size from 0.2 to 1.5 mm to act as parasites of insect eggs. The *Trichogramma* species most commonly collected from crops and orchards are *T. atopovirilia, T. brevicapillum, T. chilonis, T. deion, T. exiguum, T. fuentesi, T. minutum, T. nubilale, T. platneri, T. pretiosum*and *T. thalense*. The *Trichogramma* are minute wasps, whose adult female lays its eggs into moth eggs. When the wasp eggs hatch, the larvae devour the developing caterpillar inside the moth egg. The *Trichogramma* larvae pupate and grow into fully formed wasps inside the moth eggs. These turn characteristically black as the wasps develop inside the egg. Wasps emerge by chewing a hole in the moth's egg and are then ready to parasitize other moth eggs. This process takes from seven to ten days, depending on temperature and a female wasp can parasitize over fifty moth eggs during its lifespan of up to 2 weeks. Adult wasps feed on nectar and honey dew. The *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).The adult female wasp uses chemical and visual clues to locate a boll-worm egg. The chemical clues, called kairomones, are on the moth scales left near the egg by the female moth during oviposition. Some of these same chemicals are also bollworm sex pheromones, and egg shape and color also may be visual clues to the wasp [1, 2].

## 2. Lifestyle of *Trichogramma*
Today, *Trichogramma* species are the most widely used insect natural enemies in the world, partly because they are easy to mass rear and these attack many important crop insect pests. 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. The *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. The *Trichogramma pretiosum* 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. It attacks important pest species such as bollworms and budworms in cotton and tomatoes, corn earworms in corn and army-worms (Spodoptera) and loppers (Trichoplusia) in vegetables and other crops [3, 4].

Once a female finds a bollworm egg, it drills a hole through the chorion (egg shell) and inserts two to three eggs into the bollworm egg. The internal pressure of the bollworm eggs force 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. Eggs hatch in about 24 hours and the parasite larvae develop very quickly. During the 3rd instar (3 to 4 days after the host egg are parasitized) dark melanin granules are deposited on the inner surface of the egg chorion, causing the bollworm egg to turn black. The adult wasps can lay up to 300 eggs each, the wasp's larvae hatch from the eggs; attack the moth's eggs or embryos from within (endoparasitism). Larvae then transform to the inactive pupal stage. After about 4.5 days, the adult wasps emerge from the pupae and escape from the bollworm eggs by chewing a circular hole in the egg shell. 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 ºF) to as many as 17 days at 60 degrees F. With such a short life-cycle, these parasitoids can sometimes build up to 30 generations per year (many of which over winter). The conditions for optimum caterpillar control and moth control may be between 70-85 °F with a relative humidity of around 60%. But these are optimum conditions, and not necessarily essential for success, however, significantly cooler or warmer temperatures and humidity fluctuations may hamper reproduction and development of the *Trichogramma* to a certain degree. Large females parasitize more eggs than smaller females. Females provided with honey to feed and young bollworm eggs live on an average of 11 days, while females receiving only honey live for 3 days. Another study found the average adults life span as 24 days [5, 6].

The *Trichogramma* spp., as parasitoids, work by laying eggs in the eggs of many Lepidopteran pests and instead of pests hatching out, more mini-wasps emerge. The *T. brassicae, T. platner*or*iminutum*and *T. pretiosum*, are moth egg parasitoids capable of moth control as well as caterpillar control. These 0.9 mm. mini-wasps are very popular and very effective Lepidopteran egg parasitoids. The eggs of over 150 species of this order can be parasitized by the *Trichogramma* spp., in an orchard or tall crop setting field, greenhouse or short crop setting. In this case, short crop means one under the 8-10 foot range. Some popular hosts of these mini-wasps include the eggs of the gypsy moth; codling moth (*Cydiapomonella*); diamondback moth (*Plutellaxylostella*); Oriental fruit moth (*Graphiolithamolesta*); tomato pinworms (*Keiferialycopersicella*); cabbage loppers (*Trichoplusiani*); imported cabbage worms (*Pierisrapae*); tent caterpillars (*Malacosoma* spp.); and many, many more, even the grossly damaging tobacco/tomato hornworms (*Manduca*spp.) [7].

## 3. Use of *Trichogramma* in Biological Control

Benefits of these wasps are cheap and easy in rearing, and effective at controlling both caterpillars and moths. Because these parasitoids are supplied on cards that are perforated into squares, their even distribution is a sure thing. The one downfall for these parasitoids is ants; as they love to *Trichogramma* eggs and can rob them from the squares. Therefore, creativity is sometimes necessary such as stapling the squares to leaves, attaching them to a tree trunk and surrounding the small area with a sticky barrier product to help in ward off the ants. Another option is to pre-emerge the eggs by allowing the cards to remain in the bags they are shipped in (expand the bags before resealing them), wait for emergence, and checking the bags at least once daily. When the first sign of emerged parasitoids is evident, hang the cards and shake out the wasps in the bags. This will reduce the amount of time the eggs on the cards are exposed and threatened by marauding ants. Another drawback is their difficulty to scout and as mentioned before, the *Trichogramma* spp., have a wide host range. This means they can parasitize a great number of eggs belonging to a large number of species. These wasps are indiscriminate killers and as much as this is good and convenient to the grower, can be hazardous to nontarget organisms. Users should be cautious and aware of when employing these awesome parasitic mini-wasps. The pests' eggs have to be available, and one sure way to guarantee this is to know when they are being deposited. The *Trichogramma* spp., are only a preventive weapon for caterpillar and moth control, but they cannot parasitize the pests' eggs once they hatch into larvae [8, 9].

The *Trichogramma* spp., egg parasitoids generally remain absent in eggs of *Helicoverpaarmigera* (Hubner) collected from chickpea *Cicer arietinum*. In this study, the plant characters responsible for the absence of egg parasitoids and the feasibility of increasing parasitism levels on chickpea by mass-releasing *Trichogramma chilonis* Ishii have been investigated. The residence time of female *T. chilonis* on chickpea leaves has been affected by trichomes and the acidic trichome exudates secreted on all green parts of the plant. The parasitoids spent a longer time on chickpea leaves where the acidic trichome exudates have been washed off than on unwashed leaves, and longer on leaves of a glabrous chickpea mutant than on washed leaves. When placed on unwashed chickpea leaves, 6.8% of the parasitoids have been trapped and killed by the exudates. In a filter paper bioassay, females *T. chilonis*have been deterred by high concentrations of malic and oxalic acids, the major components of the trichome exudate. Acetone and hexane extracts from the surface of chickpea leaves did not elicit a response from the parasitoids in the bioassay. Similarly, the parasitoids did not respond to volatiles emitted by chickpea plants in a four-armed airflow olfactometer. No parasitized eggs have been collected from a chickpea field in which *T. chilonis* released five times at a weekly interval at a rate of > 137,000 females $ha^{-1}$. Sticky trap catches showed that no parasitoid population has been sustained in the release field [10, 11].

## 4. Scouting of *Trichogramma*

The *Trichogramma* are just too small and quick, so seeing these little wonders at work is normally out of the question. If growers can find the moth eggs, then they may be able to determine if these have exit holes made by the wasps. The only other indicator is less number of caterpillars upon their hatch, and less damage noticed shortly thereafter. Some caterpillar pests are more difficult to control than others. This can be a drawback, especially if the tough-to-kill pest is a serious annoyance, and the gypsy moth is a good example of this. With no significant numbers of natural enemies, *Trichogramma* spp., may have a difficulty in making an impact if used alone. With this reorganization, the implementation of multiple Integrated Pest Management (IPM) resources is necessary, but unfortunately, this costs time and money. Battling with ants, which are a real threat to biocontrol when it comes to these wasps, is sometimes very difficult too, which add complexity for the implementation of these parasitoids used for caterpillar and moth controls [12].

If growers are aware of an endangered species of moth or butterfly at release site, the release should be minimized. These wasps are not long-range flyers, and thus their release doesnot have too far movement from the wildflower meadow. Use common-sense that speaking of wildflower meadows, this points us toward an interesting side topic to consider trap-crops. Normally trap-crops can harbor the good bugs and pests, but in the case of these pests, a trap-crop probably would not lure them.

Moth and butterfly larvae seem to be such specific feeders, however, by using a close-range trap-crop, lure other natural enemies. Growers can see caterpillars being dragged off by wasps, birds and bugs all the time, so donot spray pesticides and lure the good folks. These two steps can do an awful lot and may lessen the necessity of purchased parasitoid use. Mainly, however, these parasitoids are used for caterpillar control in field and row crops, orchards, and gardens [13].

## 5. Release Strategies and Rates

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. The more *Trichogramma* are released the quicker the establishment could be. Quick establishment is desirable in all crops, but economic and time factors need to be considered. In high value vegetable crops, higher release rates (2 releases of 60,000+ per hectare) are recommended, and establishment is quick in about 2 weeks. In tomatoes, zucchinis, melons etc., start with two releases a week apart at the first sign of moth eggs. Apply 60 to 120 capsules per hectare per release depending on pest pressure. In crops where pressure is steady, this should be sufficient to ensure establishment for the life of the crop. In crops where egg pressure is highly variable, regular releases may be the best option. In sweet corn, make two releases into each planting as soon as eggs are present in reasonable numbers that is 1 egg per 5 plants is enough or else two weeks before silking. In field crops, inoculative releases as low as 30 capsules (or 1 gram) per hectare can enable establishment of *Trichogramma* if there is a good egg lay at the time of release. Two releases a week apart can increase the chances of establishment (more chance of catching an egg lay, more even generational distribution of wasps, less chance of adverse weather). Initially, expect low rates of parasitism (10 to 20%) but over the next 2 generations (3-4 weeks) the wasps can disperse and even out through the crop and parasitism typically increases to high levels. If necessary, use the soft insecticide options especially during the establishment period [14-16].

In cotton, releases can be made into earlier planted adjacent crops (e.g., maize, sorghum) or else directly into the cotton (or both). Releases can be made directly into cotton either it is GM or conventional. Releases into GM cotton can reduce resistance pressures, provide late season control and provide a *Trichogramma* refuge for nearby conventional crops. Releases into conventional crops should enable significant reductions in chemical use over the season. Releases into more advanced low insecticide needed crops near the cotton can create an in-field insectary from where the wasps can migrate into the young cotton crop. Lucerne, sorghum, maize and sunflowers are suitable crops, but chickpeas are not suitable for *Trichogramma*. If there is a steady egg laying, parasitism can increase with each generation of wasps (only 8 days in summer). It is not uncommon for parasitism to be close to 100% even under heavy pressure. The *Trichogramma* are best used in conjunction with other compatible and complimentary control measures e.g., biological insecticides and selective or safe chemical insecticides. Other local natural enemies, for instance, local parasitoids, lacewings, spiders, predatory beetles and bugs, are also likely to make a useful contribution [17, 18].

The study data revealed that both inundative and inoculative release methods of *T. chilonis* have appered more effective as compared with the control throughout the entire growing season. inundative release method of *T. chilonis*has been found the most effective technique against *Chiloinfuscatellus* (Snellen) infestation in both sugarcane plant and ratoon crops with minimum mean percent infestation of 3.50 and 6.50, respectively. This followed by inoculative release method, where 6.75 and 10.00 mean percent infestation has been recorded in sugarcane plant and ratoon crops, respectively. Maximum infestation has been recorded in control plots with mean percent infestation of 7.87 and 12.75 in sugarcane plant and ratoon crops, respectively. The data further revealed that inundative release method of *T. chilonis* in both sugarcane plant and ratoon crops also effectively controlled *Acigonasteniellus* (Hamp) with minimum borer’s infestation by recording 3.25 and 3.37 mean percent infestations in both sugarcane plant and ratoon crops, respectively. It has been followed by the plots where *T. chilonis* released as inoculative release method, where 4.87 and 6.25 mean percent infestation recorded in sugarcane plant and ratoon crop, respectively. Control plots showed maximum *A. steniellus* infestation in plant crop (7.27%) and ratoon crop (7.65%). The data further showed that no *Scirpophaganivella* Fabric infestation has been recorded in both sugarcane plant and ratoon crops. Maximum yield of sugarcane has been recorded in both plant and ratoon crops of sugarcane in plots treated with inundative release method (101.5t/ha in plant crop and 69.25 t/ha in ratoon crop) followed by inoculative release method (95.84t/ha in plant crop and 63.14t/ha in ratoon crop). The lowest yield of sugarcane has been recorded in control (91.14 t/ha and 58.33 t/ha) [19].

The egg parasite *T. maidis* is being mass reared and released against *Ostrinianubilalis* Hbn, in corn. According to the number of generations of the pest, different release strategies have been developed. In the one generation area, three times 50,000 adults per hectare are released, whereas in the two generations area 150,000 adults per hectare are released only once per generation. In order to get a long activity period by adult parasites, different developmental stages of the parasites are mixed. The average parasitization rate of the eggs of *O. nubilalis* varied between the years from 75% to 93% and the reduction of plant attack emained in the same order of magnitude. Biological control of *O. nubilalis* with *T. maidis* is nowadays well established in farmers practice and has become an important tool in integrated control in corn [20].

## 6. Trichogramma Release Techniques

Correct timingof *Trichogramma* release requires good coordination between the rearing facility and field staff. Preliminary, there can be two methods of field applications like release on cards and release as adults.

### 6.1. Release on Cards

The *Trichogramma* are shipped (as pupae) inside of grain moth eggs ready to emerge as adults. They can be loose or glued to perforated cards. Loose eggs can be divided into paper cups or other containers; there are approximately 20,000 eggs in a cubic centimeter (ml). The alternative of moth eggs glued on cards with 100,000 *Trichogramma* per card (up to 120,000) has benefits and limitations. Each card is broken into 30 hangable units with approximately 3,300 parasitized eggs to hang on twigs allowing ease of distribution especially in trees and vines. There can be approximately 100,000 *Trichogramma* per card and each card can be broken into 30 squares with 4,000 parasites per square inch that permits even distribution in fields and orchards.

Loose eggs can be divided into paper cups; there are approximately 20,000 eggs in a cubic centimeter. The card is perforated to cut into 30 tabs (3/4 X 2 inches), each with a door hanger style hook on one end, and each tab has a nominal 3,300 egg. The *Trichogramma* wasps emerge from cards in two to five days, depending on temperature, which should ideally be 80º to 90ºF. Emergence can be delayed by holding parasitized moth eggs at cooler temperatures (not less than 40 ºF) and emerging wasps are usually seen in the morning. To maximize pest fighting time, donot delay release after adult wasps emerge. Keep *Trichogramma* cards in the shade, out of the hot sun and application rate protocols for *Trichogramma*spp, can be changed, according to the height of the crop to determine the proper species to choose [21-23].

### 6.2. Release as Adults

For those who want to consider using loose *Trichogramma* wasps, the advantages are superior percentage emergence and survival in the field. The wasps emerge in two to five days, depending on temperature, which should ideally be 80º to 90 ºF. Emergence can be delayed by holding parasitized moth eggs at cooler temperatures (not less than 40 ºF). There could be some loss of emergence or searching ability after cold storage. Emerging wasps are usually seen in the morning, first males sitting quietly, then females and mating activity and at that point the males die. Don not delay releases after adult wasps emerge and keep *Trichogramma* in the shade, out of the hot sun. Loose parasitized eggs can be incubated in a variety of containers (preferably glass or paper over plastic). Jars or vials, bags or cups can be used and loose eggs are divided into roughly equal amounts among containers. Large containers can be opened at randomly spaced rows or trees releasing more where moth activity has been greater [24, 25].

#### 6.2.1. Glass Jar Incubation Containers

Any size jar can work from pint to gallon according to convenience and size of release. Use a tight-fitting lid. If using shredded paper or confetti as a vehicle, use a wide mouth jar. Use more jars when growers are doing this method the first time so they can get a feel for how the wasps behave. Putting shredded paper, yarn, string or thin strips of cloth in the container makes it easier to lift the live *Trichogramma* adults to the tree. If growers donot use shredded paper and they do not fly out of the jar easily, wrap the jar in a jar cloth or bag so they can be attracted to the mouth of the jar. The benefit of using a jar is a little more ability to see the wasps and the proportion that remains to be released [26].

#### 6.2.2. Paper Bag Incubation Containers

Any size paper bag can work from a lunch bag to a grocery bag, but there should not have holes in it. Again, put some material like shredded paper, short pieces of string or other material in the bag. If the bag and other material are white or light colored it is easier to see the moving specks that are the wasps. The benefit of using a paper bag is ease of handling and *Trichogramma* that is not walking on the paper shreds can be more likely to leave the bag than a clear jar [27].

#### 6.2.3. Honey Streak or Paper Gives Wasps an Energy Boost

A source of honey that is dry enough so the wasps cannot get stuck in it helps them to do a better job. The simplest method is to use a tool with a sharp point like a dissecting needle to make a very thin streak of honey inside each incubation container. Don't put too much or more will be likely to get stuck in it. A more complicated method is 'honey paper' put in with *Trichogramma* in jars or cups for dispersal. The wasps can access the honey without getting stuck in it. Mix a couple a drops of honey with a couple drops of water on a sheet of waxed paper. Fold and cut the waxed paper roughly 1/4 inch wide. Separate strips by pressing between thumb and fore finger. Place a pinch of loose *Trichogramma* or a cardstock tab or piece of a tab of *Trichogramma* card. This gives emerging wasps energy to go out foraging for moth eggs, they may live up to four times longer and lay more eggs [28].

## 7. Some More Tips for Adults Releasing

Releasing adults is ideal when growers can be ready to release while they are emerging. One limitation is that it is hard to predict how long it can take for all the wasps to emerge. A feature of the paper cup method is that growers can leave the paper cup with puncture holes in the field (or stapled to a leaf). They are not vulnerable to predators in the pupa stage while growers are waiting for all of the adults to emerge. If growers release from a jar or bag, the majority of wasps will be emerged, swarming and mating. If shredded paper is used, they will be evenly distributed on shreds that can be placed on trees. Otherwise, a wand of twisted paper can be used to pick them up and wave them into the foliage. Contents of jars or bags can be combined and kept for another 24 hours and released in an area of higher pest pressure. Place *Trichogramma* card pieces or loose eggs into the container, add strip of honey paper (optional), and close. Hold in a warm place at 70- 80 degree ºF and 60% RH, for 1 - 4 days until they are hatching. The males emerge first and wait for the females to emerge later in the day and wait for the day after first seeing the first wasps. Take the vials out to the field in a cooler with some ice packs to keep them from overheating. Avoid placing vials directly on the ice and use some crumpled newspaper to separate from the ice. Wasps can get stuck to condensation on the inside of the vials [29, 30].

In the field, open a vial next to a tree or area of plants and allow some of the wasps to fly away. Move onto another tree or area, release more wasp, use a 1-2 foot length of rubber hose to blow the remaining wasps from the vial, or leave the vial in the field for the rest of the wasps to fly out. Since *Trichogramma* prefer to attack freshly deposited moth eggs (up to 4 days old), the time to release *Trichogramma* is when moths are flying and laying eggs. Begin releases as early in the season as field and row crops provide shade for the parasites e.g., when tomatoes are 12-28 inches high. It is better to start releases early, as *Trichogramma* populations have the potential to grow geometrically each 7-10 days, and a long head start on pests is more likely to tip the ecological balance in favor of biological control. A few minor pest situations must be tolerated to obtain a natural enemy complex that controls major pest problems. Release rates depend on the species and strain and other factors, but some examples are, corn 1/10 card/acre 3 tabs/acre 150 mg loose sito/acre, avocado ½ card/acre 15 tabs/acre 750 mg loose sito/acre, and orchard crops 1 card/acre 30 tabs/acre 1.5 g loose sito/acre [31, 32].

## 8. Conclusion

The *Trichogramma* and *Trichogrammatoidea* are the most studied egg parasitoids in biologicalcontrol programs. The study concluded that it is feasible to manage bollworms and budworms in cotton by augmenting *Trichogramma* populations using the technology available. Constraints to the use of *Trichogramma* include 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. For development of *Trichogramma,* females wasp parasitize bollworm eggs (day 1) any time prior to black head stage, (days 1-3) after parasitism, parasitized egg is tan but has no ring, *Trichogramma* larva feeds internally on egg, (days 4-8) after parasitism, bollworm egg is black, *Trichogramma* is in pupal stage, and (days 8-9) after parasitism, adult wasps emerge. To support the release program, scientists have refined mass rearing methods to improve efficiency and reduce production costs. Equipment for releasing loose host eggs from aircraft has been devised. Methods have been also developed to temporarily halt development by refrigeration, so that *Trichogramma* could bestored for short periods of time. This ensured that adult wasps would emerge from host eggs within hours after release in the field. Rapid adult emergenceis necessary to reduce mortality caused by high soil temperatures, since host eggs applied by airplane are often deposited on the soil. These findings are valuable information for biological control programs. Further field investigation and laboratory tests are needed to verify the effectiveness of identified species as biological control agents.

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