# INUNDATIVE RELEASE OF THE EGG PARASITOID, *TRICHOGRAMMA MINUTUM* (HYMENOPTERA: TRICHOGRAMMATIDAE), AGAINST FOREST INSECT PESTS SUCH AS THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (LEPIDOPTERA: TORTRICIDAE): THE ONTARIO PROJECT 1982–1986

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# 1.0 BACKGROUND AND OVERVIEW OF PROJECT

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## PROTECTING CANADA'S FOREST RESOURCE

In the past decade, several economic, social, and biological factors have led to a dramatic increase in the management of Canada's productive forest lands. In 1980, the Canadian Council of Resource and Environment Ministers adopted a program to expand forest production to $210 \times 10^6$ m$^3$ by the year 2000 — a 35% increase in 20 years. Since then, federal–provincial Forest Resource Development Agreements in the 10 provinces have stimulated government agencies and the forest industry to expand silvicultural programs from 1200 ha in 1982–1983 to 551 000 ha in 1987–1988. This increased industrial pressure on the forest has been accompanied by a trend to withdraw forest land for non-industrial uses such as recreation, wildlife habitat, and wilderness reserves. The result has been a growing recognition of the need for protection of the forest resource from insects and other pests, so that the wood supply, wildlife populations, and recreational opportunities can be sustained over time.

To illustrate the above scenario, protection of softwood forests from devastation by insect pests such as the eastern spruce budworm, *Choristoneura fumiferana* (Clemens), and the jack pine budworm, *C. pinus pinus* Freeman, has become a routine forest management practice in eastern Canada. As the accessible supply of mature softwood dwindles, spraying programs have become more necessary to preserve the integrity of the standing inventory and ensure the future availability of wood. And, as Canada continues to invest more heavily in regenerating and tending new forests, the requirement to protect these investments, particularly on productive sites close to manufacturing facilities, becomes more critical.

As the need for protection has grown, so has the public challenge to the use of chemical pesticides in the forest environment. This challenge has resulted in periodic cancellation of all, or parts of, major forest spraying programs in most provinces in Canada, which, in turn, has created a highly uncertain situation in forest management planning and operations. This challenge has persisted and intensified over a quarter of a century, resulting in substantial changes to the technical design and cost of most provincial programs.

The large scale spraying programs of the early 1950's relied exclusively on chemical insecticides such as DDT applied at the relatively high rate of 1.14 kg/ha (Prebble 1975). As the adverse effects of the applications on non-target fauna became apparent, application rates were reduced and eventually DDT was replaced by organophosphorus insecticides such as phosphamidon and, later, fenitrothion. More recently, fenitrothion has been replaced largely by aminocarb which is generally considered to have less effect on non-target organisms. Imposition of restrictions on aerial spraying by regulatory agencies has resulted in the exclusion of much productive forest land because of its proximity to habitation, tourist operations, fish habitat, and drinking water sources. The result of these growing restrictions on chemical use in the forest environment has been a steady replacement of chemicals with microbial insecticides. In 1979, 99% of the forest area treated for insects was sprayed with chemical insecticide and only 1% with the "biological" insecticide, *Bacillus thuringiensis kurstaki* (*B.t.k.*) (Morris *et al.* 1986). By 1988, that situation had changed dramatically; of the nearly 750 000 ha sprayed, 64% was treated with *B.t.k.* and 36% with chemical insecticide. Five provinces — Ontario, British Columbia, Manitoba, Quebec, and Nova Scotia — have adopted a non-chemical approach to forest insect control. Only one province, New Brunswick, still uses significant amounts of chemical

insecticide. Newfoundland has recently used only *B.t.k.*, although chemicals might be used if spruce budworm again increases to very high levels.

Unfortunately, our current technical capability in non-chemical control is very limited. Apart from several *B.t.k.* products, only two other "biological" insecticides, both insect viruses, are registered in Canada: Virtuss® (or TM Biocontrol), for control of Douglas-fir tussock moth, *Orgyia pseudotsugata* McDonnough, and Lecontvirus®, for control of red-headed pine sawfly, *Neodiprion lecontei* (Fitch). Both are relatively specific and available only in limited quantities.

## The Challenge of a New Protection Technology

Although the insect control challenge in the agricultural sectors differs markedly from that in forestry, several problems have arisen in agriculture that will constrain our future dependence on conventional chemical insecticides and become increasingly important in the forest environment. Soil persistence, contamination of ground water, pesticide residues, and toxic waste disposal all pose serious technical and social problems which continue to elude acceptable resolution. Moreover, insect resistance to insecticides appears to be an unavoidable consequence of many insect control programs. Georghiou (1986) notes that 447 species of insects and mites, including most major pests, are now considered to be resistant to one or more class of chemical pesticide. Clearly, new insect control technologies which are socially and environmentally acceptable are badly needed so that agricultural and forestry investments can be protected, and future supplies of food, fibre, and other benefits can be assured.

The following papers describe a "new" technology which has been developed in Canada: the mass-production and inundative release of parasitoids. Of course, this is not a new concept nor a new technology. The tactic of inundative release of parasitoids, especially *Trichogramma* spp., has been used successfully for the control of agricultural and horticultural pests in the United States, the People's Republic of China, the USSR, and several countries in Southeast Asia, South America, and western Europe. In China and the USSR, *Trichogramma* is the most widely used beneficial insect on agricultural crops. About $15 \times 10^6$ ha are treated annually with *Trichogramma*, mainly on pests of cabbage, sugar beets, winter wheat, corn, and apples in the Ukraine, and sugar cane, rice, corn, and cotton in China (Li 1984; Cock 1985; Ridgway and Morrison 1985).

In the forest environment, releases of *Trichogramma* also have been considered successful in the USSR, China, and other Asian countries. In the USSR, *Trichogramma* spp. have been used with varying success against *Choristoneura muriana* (Hbn.) and such cone and seed insects as *Dioryctria ebeli* Mutuura and Munro (Belmont and Habeck 1983) and *Rhyacionia buoliana* Schiffermiller (Tsankov *et al.* 1980). In China, species of *Trichogramma* released against *Dendrolimus* spp. and other defoliating pests in pine plantations have reduced larval populations by up to 100% (Peng *et al.* 1984; Franz and Zimmerman 1984; Hsiao 1981). Effective use of *Trichogramma* has also been made against the teak skeletonizer, *Pyrausta machaeralis* Walker, in India (Patil and Thontadarya 1984).

In North America, very little research had been conducted on the feasibility of using inundative releases of *Trichogramma* in forestry prior to 1978, primarily because the need for new technology was not established. Until about 10 years ago, Canada enjoyed the luxury of a surplus of mature commercial stands to supply the industry; supply clearly exceeded demand, and much pest-caused loss was accepted. When threatening pest outbreaks did occur, damage could be controlled effectively with relatively inexpensive chemical insecticides. However, because of localized problems of wood supply, the growing concern regarding use of chemicals in the forest environment, and the recognition that pest damage thresholds are generally higher in forestry than in agriculture, interest in the development of alternatives to chemicals in forest pest management has increased greatly. This is reflected in one of the recommendations of the National Forest Sector Strategy for

Canada: "It is recommended that all elements of the forest sector... encourage development and use of effective alternative methods of pest control, including integrated pest management" (Canadian Council of Forest Ministers 1987).

The advantages of using *Trichogramma* in forest pest control programs are as follows:

1. it occurs naturally in spruce–fir forests and is self-regulating and, therefore, represents no apparent threat to the environment (Houseweart *et al.* 1984*a*);
2. it can be mass-produced with fairly simple, inexpensive technology and limited labour, thereby constraining the unit production cost in comparison with chemical insecticides (Morrison 1986);
3. it does not presently require federal registration nor provincial regulation;
4. it can be effectively applied using various techniques (Jones *et al.* 1977, 1979);
5. the host egg has no reported resistance to the parasitoid;
6. it is compatible with other control tactics, and therefore a potentially effective component of integrated pest management programs;
7. in sensitive areas, the technique will be much more acceptable, socially and politically, than conventional pesticides.

## The Spruce Budworm Problem and Use of *Trichogramma*

In Canada's boreal forest, the spruce budworm is the most devastating insect pest. From 1977 to 1981, defoliation by spruce budworm resulted in an average annual loss of $44 \times 10^6$ m$^3$, equivalent to two-thirds of the volume harvested in the same period (Gross 1985). In Ontario, spruce budworm losses from 1966 to 1985 averaged $13.3 \times 10^6$ m$^3$ of spruce and fir annually, considerably more than the volume of these species harvested ($9.1 \times 10^6$ m$^3$) each year. Currently, spruce budworm damage is controlled by the aerial application of microbial and chemical insecticides, but reliance on chemical insecticides as a management tactic is increasingly unacceptable, socially and politically. Although *B.t.k.* represents an attractive alternative to chemicals, new insect control technology is needed — a broader based technology which can be used as an alternative to *B.t.k.* in environmentally sensitive and inhabited areas, and in high value, intensively managed new forests. The scientific advances of the past decade make the technology of mass-production and inundative release of *Trichogramma* against spruce budworm an attractive potential alternative to insecticides.

Until recently, *Trichogramma minutum* Riley was the only known species to attack eggs of the spruce budworm (Morris 1963); however, in 1984, another species (*T.* near *nubilalae* Ertle and Davis) was collected from defoliating Lepidoptera in Maine (Houseweart *et al.* 1984*b*). Spruce budworm eggs are parasitized and destroyed by *Trichogramma* within the first 4 days of oviposition. Unfortunately, *Trichogramma* are not generally important in the natural control of spruce budworm because they are polyphagous and overwinter on alternate hosts, few of which are present in the forest environment (Houseweart *et al.* 1984*b*; Jennings and Houseweart 1983). However, it may be possible to manipulate *Trichogramma* because levels of parasitism (normally less than 15% in forests) as high as 77% have been reported (Anderson 1976).

## The Ontario Project (1982–1986)

Initial work on inundative releases of *T. minutum* against the spruce budworm in North America was conducted by W. Quednau at the Laurentian Forestry Centre (Forestry Canada), Ste.-Foy, Que., in the early 1970's (Varty 1984). Due to allergies associated with mass-rearing the parasitoid and the weak functional response by *T. minutum*, this work was discontinued in 1975 without clearly establishing its feasibility for control of spruce budworm (Varty 1984). Renewed interest developed following the formation of CANUSA (Canada – United States Spruce Budworm Program), with work being carried out in Maine from 1978 to 1981 (Houseweart *et al.* 1984*a*).

In 1982, a research project was initiated by the Ontario Ministry of Natural Resources (OMNR), Maple, Ont., with the Biological Control Laboratory (BCL) at the University of Guelph, Guelph, Ont., the Faculty of Forestry, University of Toronto, Toronto, Ont., and the Great Lakes Forestry Centre, Forestry Canada (FORCAN), Sault Ste. Marie, Ont., as cooperators. With major support from the OMNR, the Ontario Ministry of the Environment, and FORCAN, the cooperators undertook a project with three primary objectives:

1. to develop the technology for mass-production of *Trichogramma* in Canada;
2. to develop the technology for handling and releasing *Trichogramma* in the forest environment;
3. to determine whether inundative releases of *Trichogramma* could significantly increase the level of egg parasitism and decrease subsequent larval populations of the spruce budworm.

This monograph is a compilation of papers describing the research and the achievements related to these three objectives during the 5 years of the project. Section 2.0 describes the production system which was developed to mass-rear *T. minutum* in Canada. Section 3.0 consists of five papers outlining the actual field operations; specifically, the site used for all the studies, the systems developed for ground and aerial release of *Trichogramma*, the assessment of deposit using the technology for broadcasting the parasitized host eggs from aircraft, and the strategy developed to suppress populations of spruce budworm using *Trichogramma*. Section 4.0 summarizes the highlights of the project and outlines directives for the future.

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# 2.0 MASS-PRODUCTION OF *TRICHOGRAMMA MINUTUM* RILEY ON FACTITIOUS HOST EGGS

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## Abstract

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A system was developed to produce large numbers ($30 \times 10^6$ per week) of *Trichogramma minutum* Riley on eggs of the Angoumois grain moth, *Sitotroga cerealella* (Olivier). The parasitized host eggs were packaged and shipped in bulk for release against the spruce budworm, *Choristoneura fumiferana* (Clemens), and several other lepidopterous pests. Initial attempts to use the Mediterranean flour moth, *Ephestia kuehniella* (Zeller), as a host failed due to such factors as infestation by mites and parasitoids, low fecundity, long generation time, and the necessity for large labour inputs during moth collection. Eggs of *S. cerealella*, used to rear the host, remained viable when stored for up to 1 month at 9°C, but eggs stored at 2°C remained acceptable for parasitism for only 15 days. Rearing the host at high humidity (>70% RH) gave better percentage emergence and infestation of grain than rearing at low humidity (<30% RH). Developmental rates of *T. minutum* were determined over a range of temperatures and the developmental threshold and heat unit accumulations necessary for complete development were calculated. Using these values, *Trichogramma* were programmed, during the latter stages of production, to emerge within 24 h of exposure to light at room temperature. The programming facilitated shipment and handling of the parasitoids prior to their release.

## Résumé

Un système a été développé pour produire des oeufs de *Trichogramma minutum* en grands nombres ($30 \times 10^6$ par semaine) sur des oeufs de l'alucite des grains, *Sitotroga cerealella* (Olivier). Les oeufs-hôte parasités ont été emballés et envoyés en volume pour libération contre la tordeuse des bourgeons de l'épinette, *Choristoneura fumiferana* (Clemens), et contre plusieurs autres insectes nuisibles du Lépidoptère. Les premiers essais d'utiliser la pyrale méditerranéenne de la farine, *Ephestia kuehniella* (Zeller), comme hôte ont échoué, à cause de facteurs tels que l'infestation par des acariens et des parasitoïdes, la fécondité basse, la période prolongée des générations, et la nécessité d'investir énormément de travail pendant la collection des adultes. Les oeufs de *S. cerealella*, utilisés pour élever l'hôte, ont resté viables quand ils ont été entreposés pendant jusqu'à 1 mois à 9°C, mais les oeufs entreposés à 2°C n'ont resté acceptables pour le parasitisme que pendant 15 jours. L'élevage de l'hôte à l'humidité haute (>70% humidité relative) a donné une meilleure émergence et infestation de grain que l'élevage à l'humidité basse (<30% humidité relative). Les taux d'accroissement de *T. minutum* ont été déterminés par une grande étendue de température et le seuil de développement et les unités de chaleur accumulées nécessaires pour le développement complet ont été calculées. En utilisant ces valeurs, *Trichogramma* a été mis en marche, pendant les dernières étapes de production, d'éclore en dedans de 24 h à la température de la chambre, après exposition à la lumière. La mise en marche a facilité l'envoi et la manutention des parasitoïdes avant leur relâchement.

## INTRODUCTION

*Trichogramma* species have been mass-reared for inundative releases in many countries including Belgium, China, Columbia, France, Germany, Holland, India, Russia, South Africa, Switzerland, and the United States. Target pests for *Trichogramma* have varied among regions but have included insect pests of both agriculture and forestry.

A large number of *Trichogramma* are needed for inundative releases and, in most cases, the target pest is too difficult and costly to rear in sufficient numbers to satisfy the

requirements of the program. This is particularly true of the spruce budworm, *Choristoneura fumiferana* (Clemens), due to its long generation time and 18- to 35-week obligatory diapause (Grisdale 1970).

The selection of a production host is determined principally by its availability and relative ease and cost of rearing. The Mediterranean flour moth, *Anagasta kuehniella* (Zeller), and the Angoumois grain moth, *Sitotroga cerealella* (Olivier), are the most common factitious hosts for rearing *Trichogramma*. Throughout Asia, the rice moth, *Corcyra cephalonica* (Stainton), is often used and in China, the eri silkworm, *Philosamia cynthia* Donovan, and the oak silkworm, *Antheraea pernyi* Gurin-Meneville, produce large eggs on which *Trichogramma* are reared. Hosts with small eggs are desirable in this project because of their ease of handling during aerial release.

During the initial phase of this project, the only small-egg host available in Canada to mass-produce *Trichogramma minutum* Riley was *A. kuehniella*. Several problems arose in its culture, leading to poor levels of parasitoid production. This prompted a change to *S. cerealella*, also a small-egg host and one used to rear *Trichogramma* for many decades (Flanders 1929). An effective method for producing *S. cerealella* on wheat or barley which utilized miticides to suppress mite infestations was described by Morrison and Hoffman (1976). We employed techniques used by these authors to reduce the impact of the mite *Blattasocius tarsalis* (Berlese).

Morrison *et al.* (1978) devised an apparatus for mass-production of *Trichogramma pretiosum* Riley on *S. cerealella* that produced parasitoids unattached to a substrate and, thus, suitable for aerial release. Our attempts to use this apparatus failed because *T. minutum* was strongly attracted to areas receiving high light intensity; this left large numbers of host eggs in other areas not parasitized. We report on a production technique whereby host eggs are positioned between a light source and the *Trichogramma*, providing uniform parasitism of the host. The design of the apparatus also requires flight of the parasitoids to the host, thereby preventing runted parasitoids (those with poorly formed wings) from reaching the host eggs and reproducing. The transfer of runting to subsequent generations is thus reduced (Li 1983).

The objective of the present study was to develop a technique for mass-rearing large numbers of *Trichogramma* for aerial release on plantation forests. In the following section (Part I), we describe the rearing process developed at the Biological Control Laboratory at the University of Guelph, Guelph, Ont. In Part II, laboratory experiments designed to address specific research questions arising during production are discussed.

## PART I — MASS-REARING *TRICHOGRAMMA*

### Materials and Methods

#### Host Rearing

The method of rearing *S. cerealella* was a modification of that of Morrison and Hoffman (1976). Soft winter wheat was chosen as the rearing medium. Cribs to contain the wheat were made from wooden frames (120 by 30 by 2 cm), enclosed by hardware cloth (10 mesh per 2.54 cm) (Fig. 1*a*). S-shaped hooks, spaced evenly over the surface of the crib, prevented the two layers of hardware cloth from spreading more than 2 cm apart when filled with grain.

Ten kilograms of wheat was placed in each crib. Granary pests or their predators and parasitoids were killed by heat-sterilization of the cribs at 65°C for at least 24 h. Following sterilization, the cribs were sprayed with water to reduce their temperature and restore the grain to a favourable moisture content (ca. 15%). Prior to infestation, the wheat was treated with a solution (1.12 g/L) of dicofol (1.25 L per 10 kg wheat) to prevent infestation by the predaceous mite *B. tarsalis*.

Eggs used to infest wheat were treated with a 10% solution of Formalin for 4 min to destroy any mites present and then rinsed for 10 min with running water. On day 1, 15 g

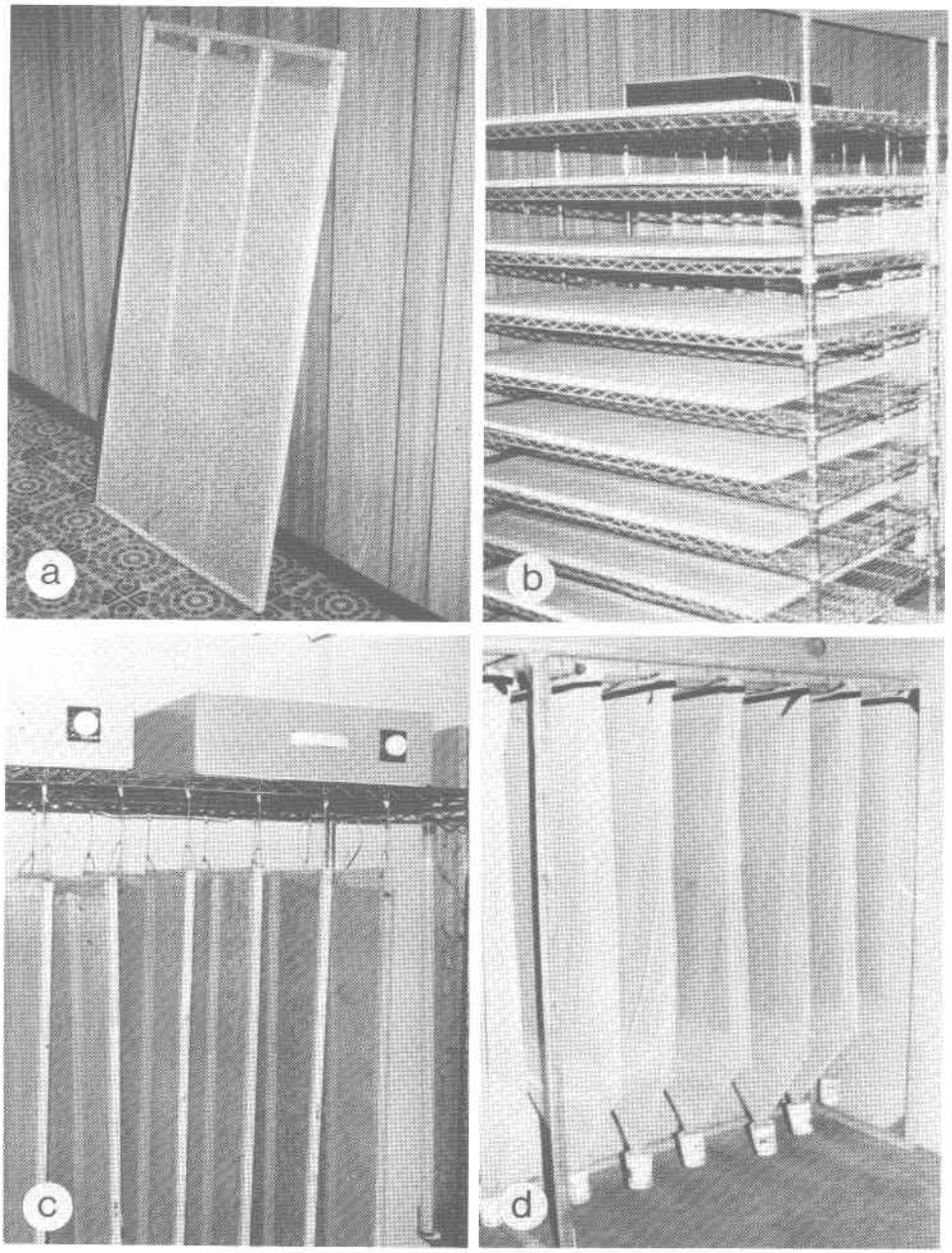


FIG. 1. (*a*) Single crib containing wheat; (*b*) horizontal storage rack; (*c*) vertical storage rack with cooling fans above; (*d*) adult collection cribs.

of fresh eggs of *S. cerealella* (ca. 750 000) was mixed with 250 mL of water and sprinkled on the cribs. The cribs, at 25 ± 2.5°C and 75 ± 10% RH, were placed 15 cm apart on horizontal storage racks (Fig. 1*b*) to allow complete egg hatch and early larval development. If fresh eggs were not available, eggs that were cold-stored at 9°C, 85% RH for less than 4 weeks were used. (Storage temperatures below 9°C caused severe mortality; see

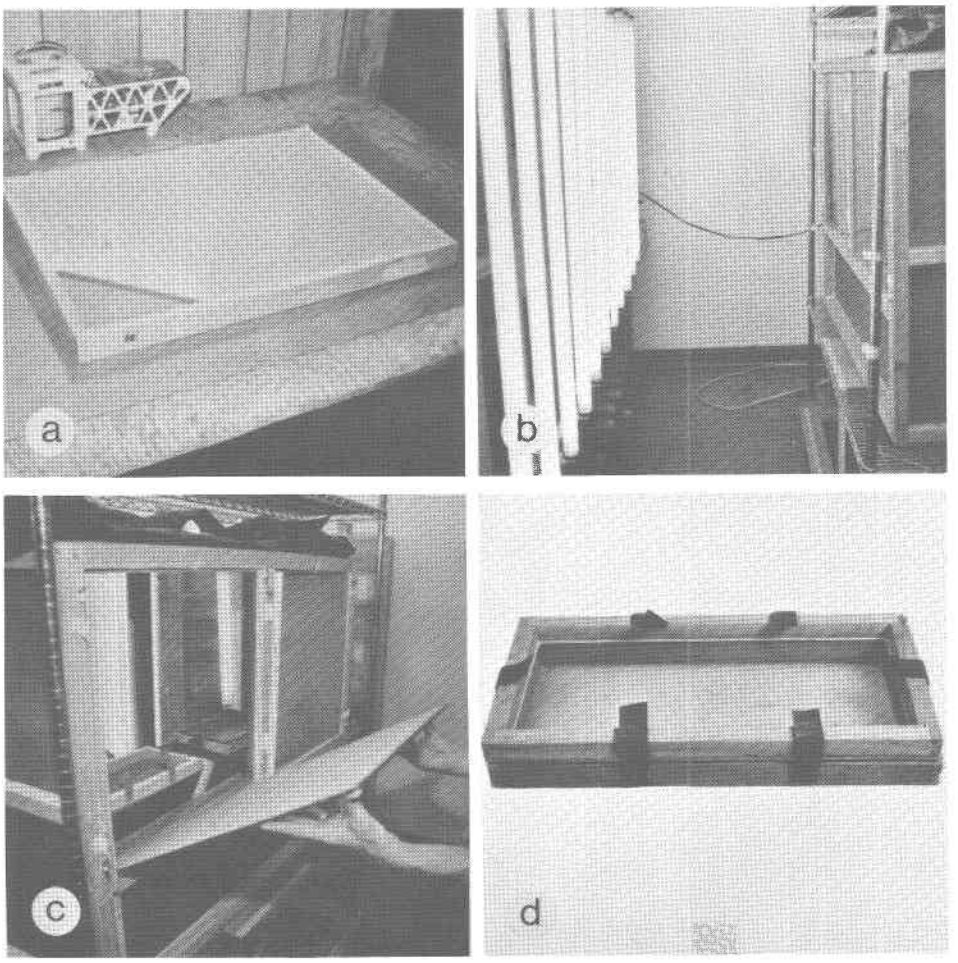


FIG. 2. (*a*) Oviposition tray; (*b*) lighting arrangement for parasitization unit; (*c*) loading glass plate into large parasitization unit; (*d*) holding tray for parasitized eggs.

Part II, Storage of Host Eggs.) The legs of the storage racks were placed in oil-filled trays to prevent infestation by predatory mites.

During mass-rearing, Morrison and Hoffman (1976) observed that excessive metabolic heat could cause larval aestivation, mortality, and adult sterility. Buildup of metabolic heat was avoided by dipping the cribs, once on day 15 (after set up), for 5 s in a water bath. The cribs then were hung vertically and cooled with overhead fans (Fig. 1*c*). On ca. day 22, these cribs were moved to a collection room (23 ± 4°C and 75 ± 10% RH), enclosed in sheer nylon bags, and hung on a rack (Fig. 1*d*) . Each bag was glued at the bottom to a collection cone which fit snugly into the top of a plastic container (1 L). Moths, emerging from the wheat, rested on the crib or collection bag and were dislodged easily with an air jet from a vacuum cleaner. Dislodged moths fell down into the containers where they could be removed and weighed.

For oviposition, the moths were placed in cages made of wooden frames (45 by 60 by 3.8 cm) covered on both sides by plastic screen (8 strand per centimetre) (Fig. 2*a*). The screen was glued and stapled to the frame with one corner left open. About

50 000 moths, 0 to 24 h old, with an average male:female ratio of 1:1, were placed in each cage and the open corner sealed with tape. The cage was placed horizontally on a piece of masonite in a storage rack for 72 h (at 23 ± 4°C and 75 ± 10% RH). Females readily oviposited in the cages with most of their eggs falling through the screen onto the masonite. Every 24 h during oviposition, eggs were brushed from the masonite. Those remaining on the screen were collected by vigorous brushing of the cage over a funnel inside a negative-pressure fume hood. The fume hood reduced the amount of moth scales entering the room and contacting the workers. The eggs were sieved through a screen (12 mesh per centimetre) and scales removed as they passed through the air current of a vacuum system, hand-held above the mesh screen. Clean eggs that were not immediately utilized were stored in a Koolatron® at 9°C. Koolatrons® are mobile environmental chambers with thermoelectric cooling/heating devices powered by AC or DC to maintain constant temperatures. By day 50, moth emergence from each crib was complete and the bagged cribs were sterilized for 24 h at 75°C to kill any remaining insects.

A major problem of rearing the Angoumois grain moth was the copious production of wing scales which created a hazardous work environment when moths were present in large numbers. Protective clothing and dust masks were mandatory when working in the moth collection area to prevent the inhalation of scales and reduce the risk of respiratory problems and allergic reactions.

### *Trichogramma* Rearing

The egg parasitoid, *T. minutum*, was obtained from parasitized egg masses of spruce budworm collected from northern Ontario (see Section 3.5) during 1980 and 1981. Throughout the project, small cultures (ca. 2000 individuals) of *Trichogramma* ecotypes were maintained in 30-mL glass vials stoppered with screen-vented corks. A cardboard strip (2 by 7 cm), holding up to 0.15 g (7500) of host eggs and attached with pesticide-free glue, was inserted into each vial. Usually, a parasitoid:host ratio of 1:5 was used in each vial. Colonies were allowed to multiply until there were sufficient *Trichogramma* to supply a large parasitization unit.

The large parasitization unit consisted of a plywood box (45 by 90 by 130 cm) on a rotatable platform (Figs. 2*c* and 3*a*). Each of the two larger sides contained two plate-glass windows (60 by 60 by 0.2 cm) (Fig. 3*b*), sealed tightly with turn-buckles against foam weatherstripping (Fig. 3*c*). Inside, all joints were sealed with silicone and all surfaces, except the glass, were painted flat black to minimize reflected light. A centrally located platform in the box, supported on pedestals and separated on all sides from the walls of the unit (Fig. 3*d*), supported the trays containing host eggs parasitized by *T. minutum* (Fig. 2*d*). This separation prevented flightless parasitoids from reaching the host eggs attached to the windows.

One side of the large unit faced a row of eight evenly spaced, 120-cm, fluorescent lights placed 52 cm from the surface of the glass (Fig. 2*b*). In addition, there were two 60-cm fluorescent lights mounted 10 cm from the corners of the parasitization unit. This arrangement encouraged the *Trichogramma* to distribute themselves evenly over the host eggs on the surface of the windows facing the light source.

Host eggs, sterilized by gamma radiation, were adhered with water to the inside of the glass plates. This required that a fine mist of water be applied to the glass with an artist's airbrush and eggs poured over the wet suface. Upon wetting, the natural glue-like properties of the eggs caused them to adhere to the glass. Depending on the amount of water used, or if the application of water was repeated once so that the eggs could be applied in two layers, 30–50 g of eggs ($1.5–2.5 \times 10^6$ eggs) were deposited on one side of each glass plate. Fresh eggs were used whenever possible but host eggs stored before and after gamma sterilization at 2°C, 85% RH for up to 30 days were also used.

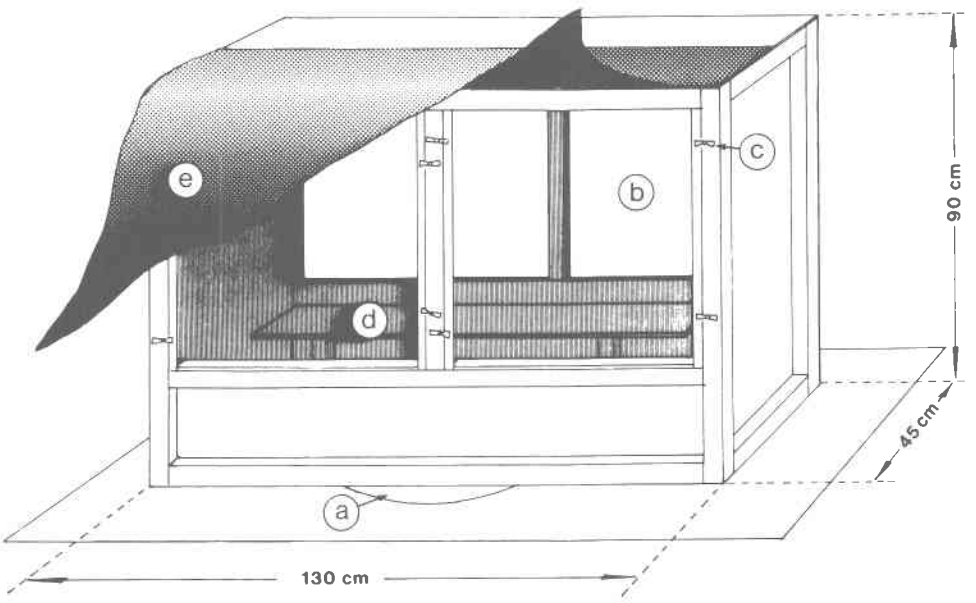


FIG. 3. Large parasitization unit: (*a*) rotating platform; (*b*) plate glass; (*c*) turn-buckles; (*d*) pedestal for parasites; (*e*) opaque drape.

Two glass plates with fresh host eggs were placed on one side of the parasitization unit (Fig. 2*c*). When the unit was opened to replace parasitized host eggs with fresh host eggs, additional parasitoids could be added. When the glass plates and parasitoids were in place, the unit was sealed and rotated so that the fresh host eggs were positioned toward the light source. A piece of opaque black plastic (Fig. 3*e*) was then draped over the opposing glass plate to create a dark and light side to the unit. Upon emergence, the parasitoids appeared to fly immediately toward the light side of the box; that side with the fresh host eggs adhered to the glass. After 30 min, the majority of the parasitoids had moved to the lighted side. A ratio of about 1:5 parasitoids:host eggs was maintained with the host eggs being exposed to the parasitoids for 24 h. If this period was reduced to 12 h (which was possible), a higher parasitoid:host egg ratio (2:5) was required to maintain high levels of parasitism.

Following this 24-h period of parasitization, fresh host eggs were placed on the two glass plates on the dark side of the unit and more parasitoids added to the centrally located platform. The unit was then rotated 180° and the opaque black plastic draped over the other side. This left those eggs, recently parasitized, on the dark side with the fresh eggs on the light side. The glass containing the parasitized eggs was then removed and replaced with a new glass plate, either with or without fresh eggs. The parasitized eggs were dislodged from the glass plate with a soft bristle brush and placed in a tray (2 by 40 by 40 cm) with a silkscreen bottom (60 mesh per centimetre). Each tray was held at 25°C for varying lengths of time of development (see Part II) and then moved to 17°C (dark) for programmed emergence of the parasitoids (Stinner *et al.* 1974). Parasitized eggs destined to be used as stock for continued parasitization were held in similar wooden trays (7 by 20 by 40 cm) with silkscreen bottoms (60 mesh per cm) and plexiglass lids (Fig. 2*d*). A foam strip, fastened to the wood frame between the lid and the base, provided a tight seal. The unit was held snugly together by Velcro® strips. Samples of eggs from each parasitization unit were glued to cards and placed in vials at 25°C and 75% RH to determine emergence, parasitism, runting, and sex ratio for each generation in the unit.

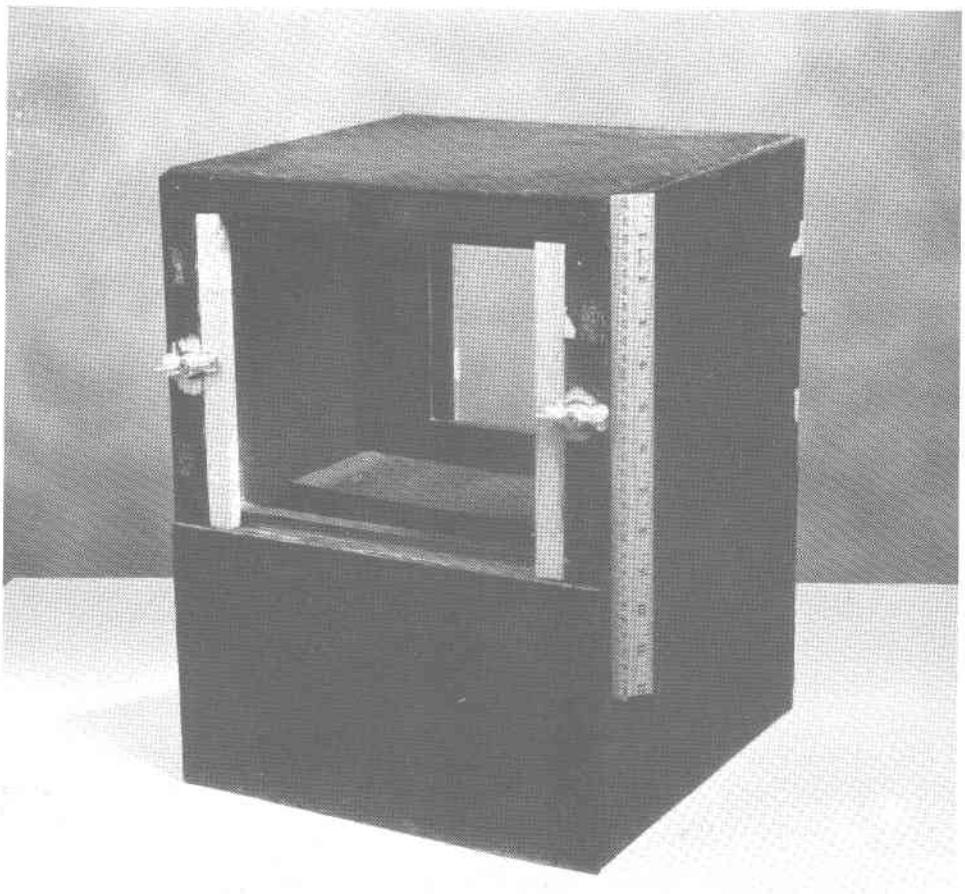

FIG. 4. A medium-sized parasitization unit for building up numbers of parasitoids.

A medium-sized rearing unit was used to expand the number of parasitoids from vial cultures to the large parasitization unit described above. Although similar to the large unit, this medium unit had only a single plate of glass (20 by 25 cm) on each side and a capacity of 4.5–11.5 g of eggs (225 000 – 575 000 individuals) (Fig. 4). The unit was operated in the same manner as the large parasitization unit.

## Programming and Shipping

All parasitoids intended for release were reared to the pupal stage in the laboratory and programmed according to Stinner *et al.* (1974). At the end of each programming schedule, the parasitoids were held at 17°C in the dark to prevent photo-stimulated emergence. At the time of shipping, the parasitized eggs were placed in shipment containers (29 by 23 by 2.5 cm) constructed of wooden frames with fine-mesh netting (60 mesh per cm) on the top and bottom to allow air and heat exchange. The netting on the bottom was securely stapled while the top netting was secured with Velcro® to facilitate removal of the parasitized eggs. This method of shipment prevented the eggs from clumping together because of condensation from metabolic activity.

The shipping containers were placed, along with gel ice packs and newspaper, in a Koolatron®. Ice packs were used to ensure that the contents were kept cool when a power source was unavailable. Parasitized eggs were shipped by commercial aircraft, either loose

in the above-mentioned containers or glued to cards (15 by 30) and placed in Ziploc® bags. At the field site, parasitized eggs were ready for emergence but could be held for an additional 24–48 h by maintaining the holding temperature at about 10°C.

## RESULTS

### Host Rearing

When *A. kuehniella* was used as the host, several factors made large scale production of host eggs difficult and costly. Severe infestations by the predaceous mite, *B. tarsalis*, and the parasitoid, *Venturia canescens* (Gravenhorst), and accidental sterilization of the culture by temperatures near 30°C prevented the rearing of large numbers. As well, *A. kuehniella* tended to have a long generation time and moth collection was very labour intensive. These difficulties prompted a change to a different rearing host, the Angoumois grain moth. This host was reared continuously from August 1983 to August 1986. A production schedule of 10 cribs, set up every 2 weeks, was initiated as the maintenance culture. This was based on original levels which showed that, on average, one crib (based on a mean of 17 cribs) would yield 667 g of moths (170 000 moths) and 9.7 g of eggs ($4.5 \times 10^6$ eggs). Moths would begin to emerge 27 days after infestation; emergence was essentially completed by day 47. Infestation of the Angoumois grain moth by *B. tarsalis* was low throughout production (<5%) because of the preventative measures described previously.

### *Trichogramma* Rearing

The production of *T. minutum* on the Mediterranean flour moth during 1982 and 1983 was small because of the previously mentioned problems (Table 1). When the host was changed to the Angoumois grain moth in 1983, however, *Trichogramma* production during 1984 and 1985 increased considerably. At full capacity, $30 \times 10^6$ *T. minutum* per week could be produced using the techniques outlined here. Production levels supplied sufficient host eggs for release of *Trichogramma* against the spruce budworm and other pest species: spruce budmoth, *Zeiraphera canadensis* Mut. and Free.; Douglas-fir conemoth, *Barbara colfaxiana* (Kearfott); codling moth, *Carpocapsa pomonella* (L.); jack pine budworm, *Choristoneura pinus pinus* Free.; and European corn borer, *Ostrinia nubilalis* (Hubner) (Table 1). The oviposition periods of most of these pests did not coincide with that of the spruce budworm and, therefore, production of *T. minutum* for release against spruce budworm was not diminished by production for the other species. In fact, the production cost of this parasitoid was reduced because rearing was continued throughout the whole growing season (May–August) for releases against these various pests.

From late 1983 to 1985, while *Trichogramma* was produced continuously on the Angoumois grain moth, rates of parasitism, runting, emergence, and sex ratio were determined to assess changes in parasitoid quality (Table 2). Between the two complete years of production, there was little change in the sex ratio or rate of emergence. The rate of parasitism of the Angoumois grain moth, however, declined over this period by about 10%, and the amount of runting in *Trichogramma* adults was reduced by 5%.

## PART II — PRODUCTION RESEARCH

## MATERIALS AND METHODS

### Storage of Host Eggs

The ability to stockpile host eggs for later use in periods of high demand is highly desirable for the mass-production of *Trichogramma*. During the first 2 years of the rearing program (1982 and 1983), host eggs were stored at 2°C for short periods (1–15 days) prior to infestation of the cribs. From 1984 to 1986, the storage temperature was maintained at 9°C and the effects of cold storage on hatching and infestation levels in the cribs were measured regularly during the rearing schedule. A sample of the Angoumois grain moth

Table 1. Production, user groups, target insects, and locality of releases of *Trichogramma minutum* from the University of Guelph from 1983 to 1985

| Year | User group* | Date | No. *T. minutum* ($\times 10^6$ ♀♀) | Target† and locality |
|---|---|---|---|---|
| 1983 | OMNR, | 4 July | 6.00 | SBW |
| | Pest Mgt. Section, Maple, Ont. | 11 July | 6.00 | Hearst, Ont. |
| | Univ. Toronto, | 22 July | 0.2 | SBW |
| | Fac. Forestry, Toronto, Ont. | | | Hearst, Ont. |
| | | Total | 12.25 | |
| 1984 | Agric. Canada, | 25 June | 0.50 | CM |
| | Vineland Stn., Ont. | 3 July | 0.30 | Vineland, Ont. |
| | | 9 July | 0.30 | |
| | | 16 July | 0.30 | |
| | | 23 July | 0.30 | |
| | | 30 July | 0.25 | |
| | | 1 August | 0.25 | |
| | | 7 August | 0.25 | |
| | OMNR, | 9 July | 47.00 | SBW |
| | Pest Mgt. Section, Maple, Ont. | 16 July | 47.00 | Hearst, Ont. |
| | Univ. of Toronto, | 20 July | 0.25 | SBW |
| | Fac. Forestry, Toronto, Ont. | 27 July | 0.25 | Hearst, Ont. |
| | CANFOR/MFC, | 31 July | 11.50 | SBM |
| | Fredericton, N.B. | | | New Brunswick |
| | | Total | 108.45 | |
| 1985 | FORCAN/PFC, | 26 April | 10.00 | DFCM |
| | Victoria, B.C. | | | Victoria, B.C. |
| | Agr. Canada, | 22 May | 0.25 | CM |
| | Vineland Stn., Ont. | 24 May | 0.25 | Vineland, Ont. |
| | | 3 June | 0.25 | |
| | | 10 June | 0.25 | |
| | | 17 June | 0.25 | |
| | | 24 June | 0.25 | |
| | | 1 July | 0.25 | |
| | | 8 July | 0.25 | |
| | | 16 July | 0.25 | |
| | | 29 July | 0.25 | |
| | | 12 August | 0.25 | |
| | | 19 August | 0.25 | |
| | OMNR, | 8 July | 30.00 | SBW |
| | Pest Mgt. Section, Maple, Ont. | 15 July | 30.00 | Hearst, Ont. |
| | FORCAN/MFC, | 29 July | 9.00 | SBM |
| | Fredericton, N.B. | 8 August | 9.00 | Fredericton, N.B. |
| | FORCAN/GLFC, | 8 September | 0.25 | JPBW |
| | Sault Ste. Marie, Ont. | | | SSM, Ont. |
| | Univ. of Guelph, | 16 July | 0.15 | ECB |
| | Environ. Biology, Guelph, Ont. | 2 August | 0.18 | Guelph, Ont. |
| | | 26 August | 0.25 | |
| | | Total | 91.83 | |
| | **Grand Total (1983–1985):** | | **212.53** | |

*OMNR, Ontario Ministry of Natural Resources, Pest Management Section, Sault Ste. Marie, Ont.; FORCAN/MFC, Forestry Canada, Maritimes Forestry Centre; FORCAN/PFC, Forestry Canada, Pacific Forestry Centre; FORCAN/GLFC, Forestry Canada, Great Lakes Forestry Centre.

†CM, codling moth, *Carpocapsa pomonella* (L.); DFCM, Douglas-fir conemoth, *Barbara colfaxiana* (Kearfott); ECB, European corn borer, *Ostrinia nubilalis* (Hubner); JPBW, jack pine budworm, *Choristoneura pinus pinus* Free.; SBM, spruce budmoth, *Zeiraphera canadensis* Mut. and Free.; SBW, eastern spruce budworm, *Choristoneura fumiferana* (Clemens).

Table 2. Parasitism, emergence, runting, and the sex ratio for *Trichogramma minutum* reared at Guelph, Ont., during 1984 and 1985 (annual mean values)

| | 1984 | 1985 |
|---|---|---|
| Parasitism of AGM* eggs (%) | 51.8 (2.0–75.0)† | 41.7 (13.7–72.0) |
| Emergence from AGM* (%) | 89.5 (49.0–100) | 91.6 (75.0–99.0) |
| Runting of adult *T.m.*‡ (%) | 7.5 (0.7–51.0) | 2.0 (0.0–6.9) |
| Sex ratio (♀:♂) | 1.86 (0.7–3.4) | 1.98 (0.7–4.4) |

*AGM = Angoumois grain moth.
†Numbers in parentheses indicate range of values.
‡*T.m.* = *Trichogramma minutum*.

eggs from each production period (one period = one set of 10 cribs every 2 weeks) was incubated at 25°C for 20 days. After this incubation, the percentage of eggs hatched and grain utilized in each production period was assessed.

The effect of storing host eggs at cold temperatures was also assessed by examining the performance of the emergent parasitoid. Newly laid (white) eggs of the host were collected daily and aged at 25°C for 4 days (during which time they turned red). Red and white eggs were each stored at 0, 2, and 5°C for periods of 0, 2, 4, 7, 14, and 21 days. From each of these 36 treatments, 200 eggs were attached with pesticide-free glue to cards (1 by 2 cm) and placed in 1.9-mL shell vials. A single mated female *T. minutum* was added to each vial and the top sealed with a cork stopper. Each treatment was replicated 10 times. After 48 h, the parasitoids were removed and the eggs incubated at 25°C. After 4 days, the black parasitized eggs were counted to determine the rate of parasitism. Host eggs turned black when the parasitoid reached the pre-pupal stage of development. The parasitoids emerged from the host eggs after 10 days and were examined to provide an estimate of percentage emergence and sex ratio. This allowed us to compare parasitism and emergence of *Trichogramma* according to the age of the host eggs as well as the duration and temperature of the egg stage.

### Effect of Relative Humidity on Survival of Angoumois Grain Moth

Morrison (1976) recommended that high humidity be maintained in the grain used for production of the Angoumois grain moth throughout rearing. This is normally achieved by wetting the grain before use. To assess the necessity of increasing the humidity during production, the eclosion and survival of the moths at different levels of humidity were examined.

The effect of relative humidity on the eclosion of host eggs was tested by using eggs adhered to paper towelling and placed at both 30 and 70% RH. Egg hatch at each humidity was determined after 20 days when it was presumed that all viable eggs had hatched. To assess host survival under different humidity treatments, eighteen 50-g samples were collected from winter wheat heat-sterilized at 65°C for 24 h (to kill insects and mites). Six of these samples were soaked in water for 4 h, six were dipped in water for 5 s, and six were left dry at air humidity, 75 ± 10% RH. Each sample was placed in an unwaxed paper cup (200 mL) and infested with 75.0 ± 0.1 mg of host eggs. The cups were then closed with drapery sheer. Three of the cups from each moisture treatment were stored at 25°C and 30% RH. The three remaining cups were stored at 25°C and 70% RH. A random sample of 50 kernels of wheat was taken from each cup, 21 days after infestation, and dissected to determine the percentage larval infestation. The cups were examined daily and emerged moths were counted and removed.

### Programming Emergence of *T. minutum*

Field releases of *Trichogramma* often require the ability to produce a predictable period of discrete adult emergence. To determine the development rate for *T. minutum* and, thereby, predict emergence, fresh eggs of the Angoumois grain moth were exposed

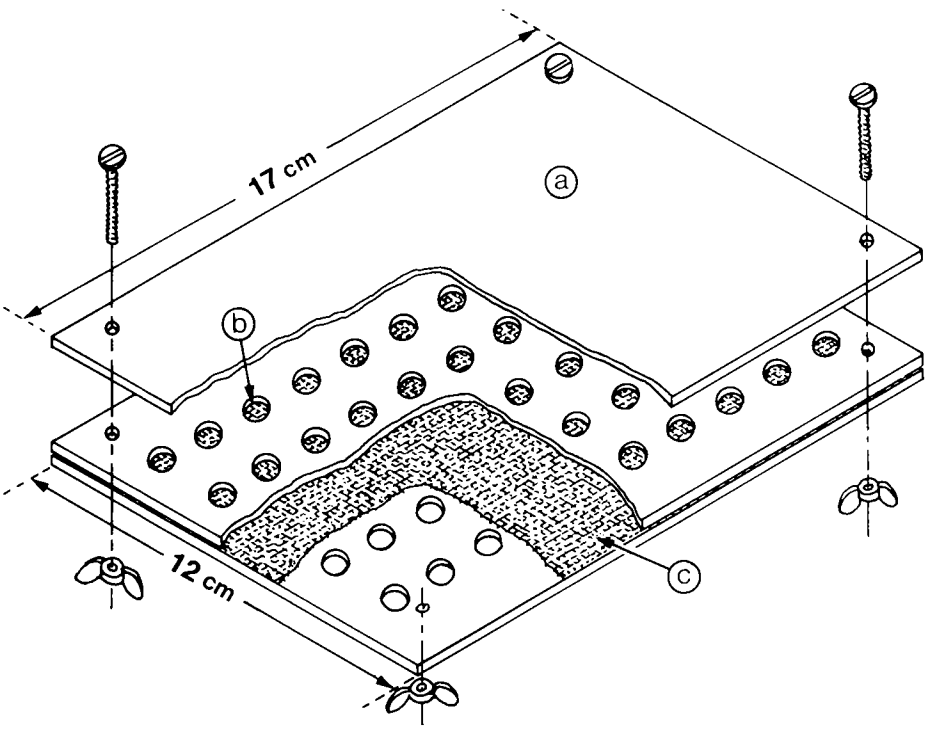


FIG. 5. Emergence tray used to confine individual parasitized eggs for observation: (*a*) plexiglass top; (*b*) well for parasitized eggs (diameter = 5 mm); (*c*) tricot nylon material.

to *Trichogramma* for 4 h at 25°C and then maintained at 15, 17, 20, or 25°C, 75% RH, and 16L:8D photoperiod. When the host eggs turned black, about 25 eggs from each temperature treatment were transferred into wells of an emergence tray, one egg per well (Fig. 5), and maintained at the same treatment temperature. When the first emergence was noted, the eggs in the emergence trays were examined four times a day until more than 50% had emerged. After 50% emergence, the eggs were examined daily until all parasitoids had emerged. Developmental rates were calculated for the combined egg–larval–pupal stages (from parasitization to adult emergence) and used to produce tables for programming the emergence of *T. minutum* or field releases.

## RESULTS

### Storage of Host Eggs

Storage at 2°C caused severe mortality to Angoumois grain moth eggs and this prevented them from being used to infest grain for production of hosts. The hatch rate of fresh eggs was 86%; at 2°C, however, egg hatch dropped to 69% after 24 h and 3.6% after 15 days. This susceptibility to low temperatures meant that although eggs stored at cold temperatures could not be used for infesting cribs, they could be killed and still used to rear *Trichogramma*. Unfortunately, this method of killing the host embryos for parasitization would require a minimum of 2 weeks of cold storage to obtain up to 95% egg mortality. When the cribs were infested with eggs stored at 2°C, less than 20% of the grain was utilized but 75% was utilized when infested with fresh eggs. Storage of host eggs at 2°C was stopped when it was identified as a mortality factor in April 1984. The storage

Table 3. Number of 1-day-old and 4-day-old eggs parasitized by individual female *Trichogramma minutum* after storage at 0, 2, or 5°C for 0, 2, 4, 7, 14, or 21 days

| Age of egg (days) | Temperature (°C) | Duration of storage (days) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0* | 2 | 4 | 7 | 14 | 21 | Mean |
| 1 (white) | 0 | 37.8 | 29.8 | 28.2 | 24.2 | 25.2 | 31.8 | 29.5 |
| | 2 | 33.4 | 30.3 | 28.0 | 36.8 | 29.9 | 32.6 | 31.8 |
| | 5 | 27.0 | 24.0 | 29.7 | 31.0 | 30.9 | 34.6 | 29.5 |
| | Mean | 32.7 | 28.0 | 28.6 | 30.7 | 28.7 | 33.0 | 30.3† |
| 4 (red) | 0 | 27.7 | 27.4 | 38.0 | 19.5 | 27.8 | 20.0 | 26.7 |
| | 2 | 24.5 | 23.7 | 24.3 | 25.1 | 23.4 | 22.9 | 24.0 |
| | 5 | 25.3 | 27.7 | 25.4 | 29.1 | 23.2 | 24.1 | 25.8 |
| | Mean | 25.8 | 26.3 | 29.2 | 24.6 | 24.8 | 22.3 | 25.5† |

*$n$ = 10 ♀♀ *T. minutum* per treatment.
†Overall fecundity of parasitoids attacking hosts stored as 4-day-old-eggs was significantly lower than that for parasitoids attacking hosts stored as 1-day-old eggs (Bonferroni $t$ test; $p = 0.05$).

temperature was changed to 9°C which allowed very slow development of the eggs. At 9°C, host eggs could be stored up to 1 month and still remain viable.

Neither storage temperature nor storage time had any significant effect on the acceptability of host eggs by *Trichogramma* (Table 3). There were no significant interactions between age of host eggs and storage temperature, between age of host eggs and storage time, or between storage time and storage temperature ($t$ test, $p = 0.05$). The age of host eggs, however, did significantly affect parasitism ($t$ test, $p = 0.05$). Fresh, white eggs were more acceptable than the partially developed, red eggs, regardless of temperature or length of storage.

These results suggest that freshly oviposited host eggs may be stored up to 3 weeks at 0–5°C and still remain suitable for parasitization and development of *Trichogramma*, although the eggs were not suitable for continuous rearing of the host due to high mortality. At more advanced stages of embryonic development, host eggs became less acceptable for successful parasitization.

## Effect of Relative Humidity on Survival of Angoumois Grain Moth

Relative humidity had no significant effect ($p<0.05$) on the eclosion of host eggs. Mean egg hatch at 30 and 70% RH was 88.3 ± 0.6% and 86.2 ± 0.7%, respectively. Rearing at high humidity, however, gave a significantly higher infestation and number of moths emerging (Table 4). At 70% RH, there was no significant difference between percentage infestation or total moths emerged from wheat that had been soaked for 4 h, dipped in water, or kept dry. At 30% RH, however, soaking the grain for 4 h significantly increased

Table 4. The effect of humidity (RH) and water treatment of the grain on the percentage infestation by the Angoumois grain moth and the mean number of moths emerging (treatment means)

| Relative humidity | Water treatment of grain | Infestation % | No. moths emerging |
|---|---|---|---|
| 70% | 4-h soak | 84.7 a* | 1210 a |
| | 5-s dip | 88.0 a | 1176 a |
| | Air-dry | 73.3 a | 1023 a |
| | Mean | 82.0 a | 1136 a |
| 30% | 4-h soak | 26.7 b | 16 b |
| | 5-s dip | 16.0 c | 6 c |
| | Air-dry | 1.3 d | 0 c |
| | Mean | 14.7 d | 7 b |

*Means in the same column followed by the same letter are not significantly different (Duncan's new multiple range test, $p = 0.05$).

Table 5. Effect of temperature on time to 50% emergence and development rate of *Trichogramma minutum* in eggs of the Angoumois grain moth

| Temperature (°C) | Days to 50% emergence | Development per day* (%) |
|---|---|---|
| 15 | 27.0 | 3.7 |
| 17 | 20.0 | 5.0 |
| 20 | 11.0 | 9.1 |
| 25 | 8.5 | 11.8 |

*Development = time taken from parasitization to adult emergence. Calculated from the regression line $y = -8.712 + 0.836x$, where $y$ is the rate of development (the reciprocal of developmental time) and $x$ is the temperature with a threshold temperature of 10.4°C.

both infestation and moth emergence. These data suggest that high humidity should be used to rear the Angoumois grain moth and that dipping or soaking the grain prior to infestation is beneficial for the production of this host.

**Programming Emergence of *T. minutum***

The rate of development of *T. minutum* was directly related to temperature (Table 5). By means of linear regression analysis, these data were converted to fit the equation: $y = ax + b$, where $y$ = reciprocal of developmental time, $x$ = °C, and $a$ and $b$ are constants. The estimate of the threshold temperature for development (10.4°C) was determined by $x$ when $y = 0$. This calculated rate of development ($y = -8.712 + 0.836x$) was useful as a predictive tool for estimating the emergence of adult *T. minutum* at various temperatures.

Emergence of *Trichogramma* has been shown to be synchronized to light cycles (Rounbehler and Ellington 1973) and emergence may be delayed for a short time (1–3 days) by holding the pupal parasitoids in the dark (Wolcott 1918). Stinner *et al.* (1974) stored *T. pretiosum* in complete darkness at 16.7°C for 4–10 days after rearing them for various periods at 25°C. After such storage, 93% of the adults emerged within 4 h of exposure to light at 26.7°C.

Using this information, a development table (Table 6) was constructed for rearing parasitoids at 25°C and a 16L:8D photoperiod, followed by a holding time at 17°C and OL:24D. Repeated tests of these time–temperature relationships have shown them to be a reliable way of programming parasitoid development so that they emerge shortly after release, provided the material is kept cool (10–17°C) and dark (OL:24D photoperiod) prior to release. The ability to hold parasitoids from 8.2 to 16.6 days allows the daily production for an entire week to be programmed to emerge at the same time. With further study, it is hoped that methods of storage of parasitized eggs for periods in excess of 1 month may be found.

Table 6. Programming schedule for *Trichogramma minutum* illustrating their development* and number of days to emergence when reared for various times at 25°C, 16L:8D photoperiod, followed by holding periods at 17°C, OL:24D photoperiod

| Days at 25°C (16L:8D) | Development (%) | Days at 17°C (OL:24D) | Development (%) | Total days to emerge |
|---|---|---|---|---|
| 8.2 | 100.0 | 0.0 | 0.0 | 8.2 |
| 7.0 | 85.4 | 2.6 | 14.6 | 9.6 |
| 6.0 | 73.3 | 4.7 | 26.5 | 10.7 |
| 5.0 | 61.0 | 6.9 | 38.9 | 11.9 |
| 4.0 | 48.8 | 9.1 | 51.3 | 13.1 |
| 3.0 | 36.6 | 11.3 | 63.7 | 14.4 |
| 2.0 | 24.4 | 13.4 | 75.6 | 15.4 |
| 1.0 | 12.2 | 15.6 | 87.9 | 16.6 |

*Development is defined as the time taken from parasitization to adult emergence.

## DISCUSSION

We have developed a system to produce sufficient *Trichogramma* for several experimental releases against various insect pests. From 1983 to 1985, over 212 $\times$ $10^6$ parasitoids were produced. The majority of these parasitoids (87%) were released against the spruce budworm; however, additional *Trichogramma* were produced for field tests against five other pests in forestry and agriculture.

Many successes were realized in the development of this mass-rearing system: the importation and rearing of large numbers of the Angoumois grain moth; the development of a new parasitization apparatus for *Trichogramma*; the development of a production system with a weekly capacity of 30 $\times$ $10^6$ parasitoids; and the successful production and shipment of over 200 $\times$ $10^6$ parasitoids. However, several changes will be necessary before this technology can be expanded into a production facility or transferred to the commercial sector. The main problems that prevented continuous large scale production were the high labour intensity required and the hazardous working conditions resulting from the proliferation of host scales. These two problems will have to be resolved through engineering design changes to the rearing system.

The change of alternate hosts from the Mediterranean flour moth to the Angoumois grain moth was essential to produce the number of *Trichogramma* required. Rearing the Angoumois grain moth was more efficient and less labour intensive than rearing the Mediterranean flour moth but when the former production system was expanded, it too became labour intensive and hazardous. Further research on the automation of mass-rearing the host is required. Some of the future objectives should include the following: development of automated equipment for rearing and collection of the host; greater efficiency in collecting moths and eggs; investigation into the effects of nutrition (different grain sources and treatments) on host emergence and fecundity; determination of the optimal rearing conditions (light, humidity, and temperature) for each stage of the host and parasitoid; and, ultimately, production of a wholly artificial diet for rearing *Trichogramma*.

Efficiency in a host rearing system could be greatly improved if hosts, produced during the times when they were not required, could be stockpiled until the demand for them was greater. Such technology would help greatly to lower the unit cost and improve the productivity of the system. Research should be directed toward improving storage technology as well as the effect of stored material on parasitoid effectiveness.

Research is also needed on the potential for storing parasitoids at low temperature in the pre-pupal stage. It has been suggested that pre-pupal parasitoids can be stored for periods of up to 300 days with a high percentage emergence of fit parasitoids (Quednau 1957). This successful, long-term storage, however, will be dependent on both the species of *Trichogramma* and host used (Marston and Ertle 1973).

Most *Trichogramma* species are non-discriminating and attack several species of insect eggs. By initiating research to find additional species of *Trichogramma* and other *Trichogramma*-susceptible species, the range of hosts suited to this means of pest control could be widened. This has important implications in increasing the efficiency of parasitoid production. *Trichogramma* produced throughout the growing season could be used against a variety of host species whose eggs are susceptible to *Trichogramma* at different times. The development of a system capable of handling these various pest problems would improve production efficiency.

Research into the production of parasitoids of consistently high quality (e.g. optimum longevity, fecundity, host-finding ability) is also fundamental to the increased efficiency of mass-rearing and inundative release. The quality of parasitoids is related to their physiological status. Nutrients (e.g. properties of the host egg) can have a significant effect on

the longevity and fecundity of *Trichogramma* and, as such, may be important for enhancing production capabilities. It may also be possible to provide nutrients to emerging parasitoids in the field, thus improving their fecundity, longevity, and vitality and, ultimately, parasitism of pest populations.

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# 3.0 FIELD RELEASE AND SUPPRESSION OF SPRUCE BUDWORM

## 3.1 THE FIELD TEST SITE

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### SELECTION CRITERIA FOR FIELD-RELEASE SITE

Four criteria were used to select the field site used for an inundative release of *Trichogramma* against the spruce budworm, *Choristoneura fumiferana* (Clemens).

(1) The presence of a spruce budworm population of suitable density and stability. Moderate-to-high densities of spruce budworm were required to test the efficiency of inundative releases. Although a spruce budworm outbreak period may persist for 20–30 years across Ontario (Brown 1970; Blais 1983), an outbreak in one specific location is not expected to last more than 10 years. Thus, it was necessary to establish that the spruce budworm population was reasonably stable and would not collapse over the 5-year period of the proposed study.

(2) The presence of large, uniform stands of high value host trees. If *Trichogramma* proves to be a feasible control technology, it will likely be used first in managed, high value plantations. Release blocks of at least 8 ha of 20- to 40-year-old white spruce trees were used to facilitate sampling and monitoring, as well as aerial application. The initial design required that there be at least 1.6 km between release and control blocks, but as the study progressed, it became apparent that a distance of as little as 500 m was more than adequate to eliminate the possibilities of *Trichogramma* drifting between release and non-release sites during application or of adult dispersal between blocks.

(3) A low level of parasitism by naturally occurring *Trichogramma*. Parasitism of spruce budworm eggs by *T. minutum* is highly variable among years and locations (Miller 1953; Morris 1963; Thomas 1966), and parasitism rates tend to be higher in areas where the diversity of non-host tree species is greater (Kemp and Simmons 1978; Houseweart *et al.* 1984). For these trials, a low level of attack by naturally occurring *Trichogramma* was desirable to minimize masking and confusion of treatment effects.

(4) Logistical suitability. Ease of ground access and transport of biological research material to the site were included among the factors taken into consideration; airport facilities were needed to receive parasitoids from Guelph, Ont., and Sacramento, CA, and railway access was required for the transportation of sentinel egg clusters from Sault Ste. Marie, Ont.

### THE TEST SITE

An area in Rogers Township in northeastern Ontario was chosen as the field test site, which was located at about 50°N latitude, 84°W longitude, ca. 45 km northwest of Hearst.

#### Physiography

Rogers Township (Fig. 1) straddles a ridge between the Nagagami and Kabinakagami rivers within the James Bay watershed. It consists of a gently to moderately rolling clay–till plain (Boissoneau 1966; Anon. 1965). The height of land ranges from about 290 m above sea level near the southern boundary of the township to 152 m above sea level in the northwestern quadrant of the area.

#### Climate

The study site was located within the Northern Clay Belt climatic region, which is characterized by a short frost-free season and very low winter temperatures (Chapman

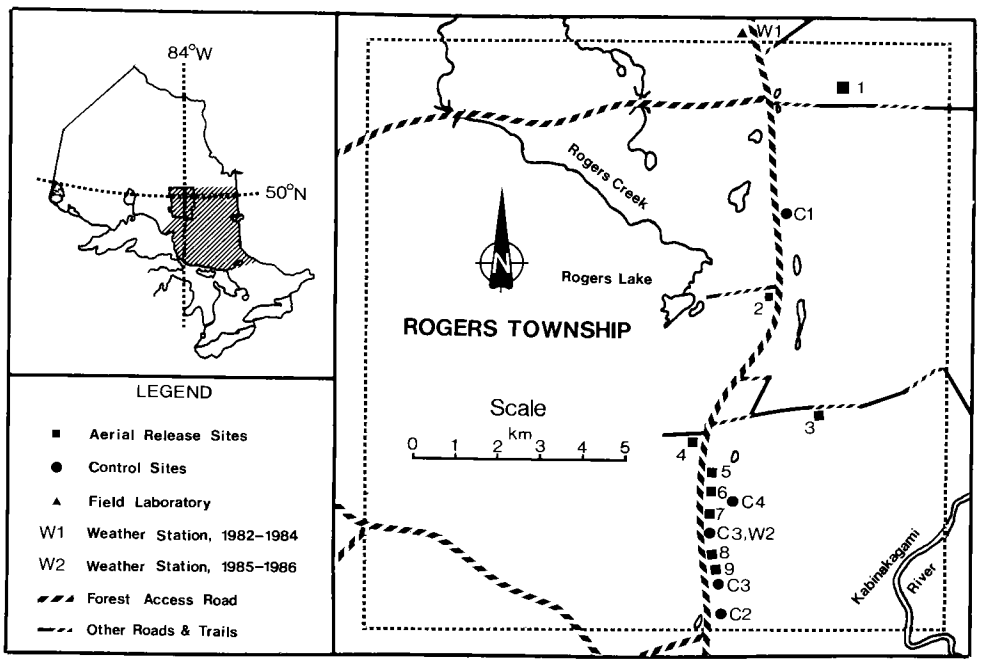


FIG. 1. Map of Rogers Township in the Hearst District of Ontario (hatched area represents northeastern Ontario) and the location of plots used for experimental releases of *Trichogramma minutum* from 1982 to 1986. Numbers on the map indicate plot numbers for studies carried out in the following years: No. 1, 1982 and 1983; Nos. 2–4, 1984; Nos. 5–9, 1985; Nos. 5 and 7, 1986. Controls for these plots are prefaced with a "C".

1953). The site is dominated by the Arctic airstream for more than half the year (Bryson and Hare 1974), particularly from February to June. During late summer, there is a strong influence of warm, moist tropical air that originates over the Gulf of Mexico, and in the fall, the Pacific airstream sweeps across the region. Figure 2 illustrates these climatic features for Kapuskasing, which is representative of the Northern Clay Belt; data are from the nearest Atmospheric Environment Services station (Hare and Thomas 1974).

## Forest Type

The study area was within the Central Plateau Section of the Boreal Forest Region (Rowe 1959), just west of the Northern Clay Belt and at the southern edge of the Hudson Bay Lowlands, a low-lying basin of swamp, bog, and muskeg. The old forest was a mixedwood type, with jack pine on the elevated portions and stands of white spruce, trembling aspen, balsam poplar, balsam fir, and white birch on the lower areas.

## Forest History

Rogers Township was logged extensively in past years. the majority of the stands were about 140 years old and were in the black spruce working group. The older cutover areas received silvicultural treatment from the time of planting. By 1982, about 22% of the township had been planted with white spruce, black spruce, and pine. Over time, many of the plantations have developed into mixed-species stands, with heavy natural regeneration of balsam fir and deciduous species such as poplar (Table 1).

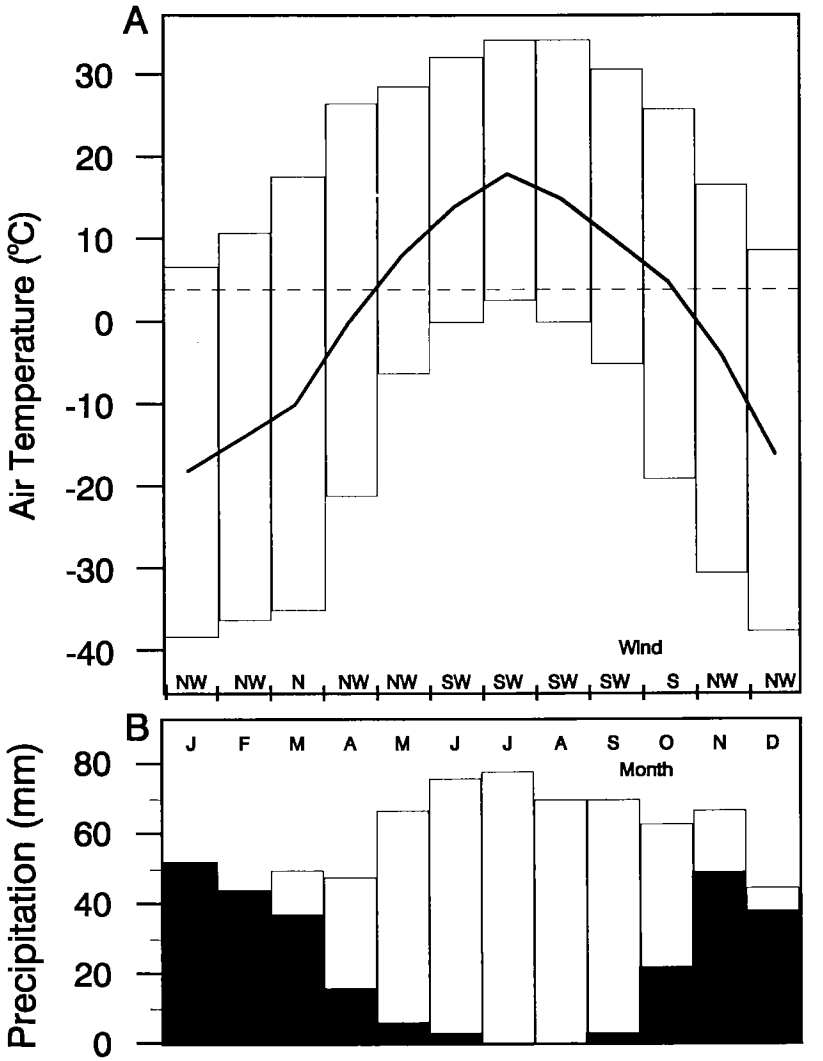


FIG. 2. Climatic data for Kapuskasing, Ont. (data were obtained from Hare and Thomas [1974, p. 227]): (A) mean daily temperature (°C), white bars indicate mean daily minimum and maximum (1941–1970); (B) total precipitation (mm), white bars indicate rainfall (mm) (1941–1970), shaded bars indicate snowfall (1941–1970). The prevailing sector of the wind is shown along the bottom axis of the graph.

## Description of Plots and Sample Trees

Most of the spruce in the sample plots (Fig. 1) were 18–23 years old in 1982. Table 1 shows data on stand composition as well as measurements of sample trees used during the release experiments. The sample trees represented approximately the median of the population (Fig. 3). In most plots, the balsam fir showed severe defoliation and some branch and tip mortality as a result of attack by spruce budworm.

## Eastern Spruce Budworm History

The current outbreak of spruce budworm began in 1967. A large outbreak area developed in the northeastern part of Ontario (Fig. 1), and by 1970, susceptible host trees within

Table 1. Stand density and species composition of plots in Rogers Township used for inundative releases of *Trichogramma minutum*, 1982–1986. Measurements of trees used for release-related sampling are included (mean values)

| Year | Stand density* (trees/ha) | Stand composition (%)† | | | | Sample trees‡ | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Bf | Sw | Po | Other | DBH (cm) | Height (m) | CW (m) | Defoliation index§ |
| 1982 | 3273 | 79 | 21‖ | — | — | 7.7 | 7.1 | 2.7 | — |
| 1983 | 3273 | 79 | 21 | — | — | — | — | — | — |
| 1984 | 2584 | 55 | 29 | 9 | 7 | 8.1 | 5.8 | 2.1 | 2.3 |
| 1985 | 2099 | 39 | 43 | 9 | 9 | 0.4 | 7.3 | 2.2 | 1.6 |
| 1986 | 1598 | 42 | 42 | 13 | 3 | 7.9 | 7.2 | 2.4 | 1.3 |

*Spruce budworm host trees ≥2 cm in diameter at breast height. Non-hosts were not tallied.
†Bf, balsam fir, *Abies balsamea* (L.) Mill.; Sw, white spruce, *Picea glauca* (Moench) Voss; Po, poplar, *Populus* spp.
‡DBH = diameter at breast height; CW = crown width.
§Current-year defoliation by spruce budworm: 1, 0–20%; 2, 21–50%; 3, 51–80%; and 4, 81–100%. Damage to spruce was generally moderate in 1982; however, many of the balsam fir had very little foliage as a result of repeated attacks.
‖Black spruce, *Picea mariana* (Mill.) B.S.P., is included in 1982 only.

a $2.1 \times 10^6$ ha area were moderately to severely defoliated. The net area of moderate-to-severe damage in northeastern Ontario increased to about $17 \times 10^6$ ha between 1979 and 1981 (Table 2), followed by a drop to ca. $7 \times 10^6$ ha in 1982, when the populations declined in the central area of the outbreak. Marginal areas of the outbreak persisted and increases in area were recorded to the north and northwest. By 1985, only pockets of the outbreak

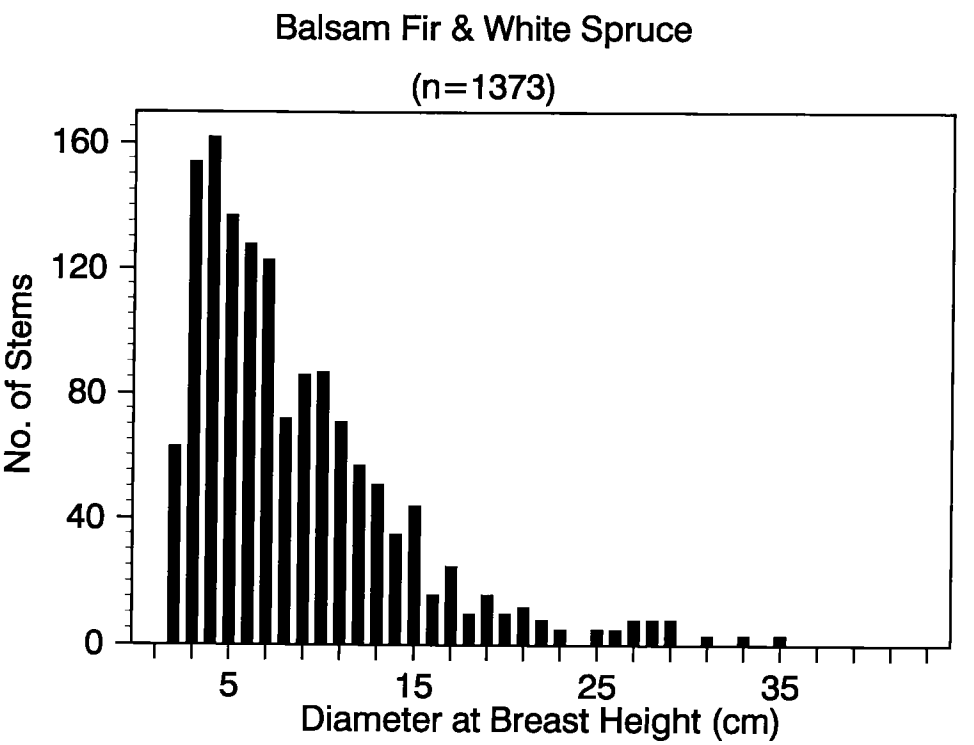


FIG. 3. Frequency distribution (number of stems in each diameter class) for all spruce budworm host trees in a 20-ha block encompassing the 1982–1983 treatment plots (No. 1 in Fig. 1). Pooled data from thirty-one 13-$m^2$ quadrants, comprising a 6.5% sample of the block, are represented.

Table 2. History of spruce budworm defoliation, population densities, and parasitism for various locations in Ontario from 1968 to 1986*

| | Area of moderate-to-severe defoliation (million ha) | | | Hearst District | | Rogers Township | | Algonquin |
|---|---|---|---|---|---|---|---|---|
| Year | All Ontario | NE Ontario | Hearst | No. egg masses per 9.29 $m^2$ foliage | % egg mass parasitism† | No. egg masses per 9.29 $m^2$ foliage | % egg mass parasitism | % egg mass parasitism |
| 1968 | 0.3 | 0.2 | 5.4 | 0 | — | 0 | — | — |
| 1969 | 1.0 | 0.7 | 11.0 | 0 | — | 0 | — | — |
| 1970 | 2.8 | 2.1 | 19.9 | 3 | 18.2 | 0 | — | — |
| 1971 | 5.3 | 0.5 | 45.8 | 6 | 0 | 0 | — | — |
| 1972 | 7.7 | 5.4 | 113.5 | 5 | 0 | 0 | — | — |
| 1973 | 7.4 | 5.1 | 125.4 | 19 | 0 | 0 | — | — |
| 1974 | 9.6 | 7.5 | 137.2 | 100 | 0 | 0 | — | 5.2 |
| 1975 | 13.3 | 11.0 | 374.2 | 162 | 0.7 | 0 | — | 10.7 |
| 1976 | 14.4 | 14.0 | 574.9 | 113 | 0 | 0 | — | 36.1 |
| 1977 | 14.1 | 13.5 | 1204.0 | 61 | 1.9 | 0 | — | 34.9 |
| 1978 | 15.2 | 14.8 | 1298.7 | 261 | 0.7 | 27 | 0 | 18.4 |
| 1979 | 18.4 | 16.9 | 1827.9 | 605 | 0.8 | 1040 | 0 | 0 |
| 1980 | 18.9 | 17.1 | 1854.9 | 349 | 0.2 | 150 | 0 | 20.8 |
| 1981 | 18.2 | 17.0 | 1845.4 | 353 | 0.5 | 195 | 0.4 | 43.8 |
| 1982 | 8.0 | 6.7 | 349.1 | 346 | 0.9 | 819 | 1.0 | 11.8 |
| 1983 | 9.0 | 6.5 | 780.0 | 518 | 0.8 | 730 | 1.8 | — |
| 1984 | 8.7 | 4.0 | 784.2 | 572 | 0.5 | 340 | 2.2 | — |
| 1985 | 12.3 | 2.7 | 1173.7 | 304 | 0.7 | 199 | 2.0 | — |
| 1986 | 8.9 | <0.1 | 32.4 | 51 | 5.7 | 18 | 37.5 | — |

*Data supplied by G. Howse and J. Meating, Forest Insect and Disease Survey Unit, Forestry Canada, Sault Ste. Marie, Ont.
†At least 50% of eggs in an egg mass had to be parasitized for the mass to be classified as parasitized (Sanders 1980).

remained in the eastern portion of the area, but a major area of moderate-to-severe damage expanded to the northwest and coalesced with the expanding northwestern Ontario outbreak, which had been building since about 1976.

In the Rogers Township area, moderate-to-severe defoliation was first present in 1977, and the outbreak continued every year through 1986 (Table 2). Until 1986, when the populations in Rogers Township collapsed, the level of infestation was adequate for all aspects of the release experiments.

## Level of Parasitism by Naturally Occurring *Trichogramma*

Until 1986, parasitism by *Trichogramma* in the Hearst District and Rogers Township was very low (Table 2). Parasitism of egg masses varied from 0 to 1.9% in the Hearst District during the decade prior to 1986 and from 0 to 2.2% in Rogers Township from 1978 to 1985. In 1986, parasitism of egg masses increased to 5.7 and 37.5% in the Hearst District and in Rogers Township, respectively.

## Management History

Since 1981, annual spraying operations against the spruce budworm, using both chemical and microbial insecticides, were conducted in Rogers Township. These control programs were conducted in late May and early June, and therefore posed no hazard to the releases of *Trichogramma* that took place 6–8 weeks later.

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# 3.2 GROUND SYSTEMS FOR RELEASING *TRICHOGRAMMA MINUTUM* RILEY IN PLANTATION FORESTS

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## Abstract

*Mem. ent. Soc. Can.* 153: 31–37 (1990)

Two systems for releasing the egg parasitoid, *Trichogramma minutum* Riley, from ground level in forest stands are described: (1) a gridded point-source release using parasitized host eggs attached to cards, and (2) a hand-held leafblower distributing parasitized eggs in bulk. Neither technique affected the emergence of the parasitoids released. Parasitoids emerging from eggs released in bulk had a similar sex ratio, longevity, and fecundity to those not released. Both methods of ground release achieved uniform parasitoid distribution and resulted in levels of parasitism similar to those achieved with aerial releases. Parasitism was greater in the mid- to upper canopy of trees than in the lower canopy. The difficulties associated with each technique and its comparative usefulness in experimental and operational programs are discussed.

## Résumé

Deux systèmes pour le relâchement du parasitoïde de l'oeuf, *Trichogramma minutum* Riley, à partir du niveau de la terre dans les terrains forestiers sont décrits: (1) un relâchement d'un point-source dans une superficie quadrillée en utilisant les oeufs parasités de l'hôte attachés aux cartes et (2) un relâchement en utilisant une souffleuse de feuilles tenue dans la main pour disperser en volume les oeufs parasités. Les parasitoïdes éclos des oeufs relâchés en volume avaient le rapport sexuel, la longévité et la fécondité semblables à ceux non relâchés. Les deux méthodes de relâchement terrestre ont accompli une distribution uniforme de parasitoïdes et ont abouti aux niveaux de parasitisme semblables à ceux accomplis par les relâchements aériens. Le parasitisme était plus important aux niveaux moyens et hauts des voûtes de feuillage qu'au niveau bas. Les difficultés associées à chaque méthode et l'utilité comparative de chacune aux programmes expérimentaux et opérants sont discutées.

## INTRODUCTION

The application of pupal *Trichogramma* from the ground is a standard method of release in countries such as China where production and labour costs are low (Huffaker 1977). In North America, early studies relied exclusively on this approach (Smith 1929; Van Steenburgh and Boyce 1938; Jaynes and Bynum 1941) but, now, the widespread use of ground releases is precluded by the large areas requiring treatment and the high cost of labour. In agriculture, this has led to the development of aerial release systems for treatment of large areas (Jones *et al.* 1979; Bouse *et al.* 1980; Bouse and Morrison 1985; also Section 3.3).

Although aerial systems have made releases of *Trichogramma* more cost-effective, there remains a need for ground applications. As shown by Parker *et al.* (1971), Oatman and Platner (1971, 1978), and Smith *et al.* (1987), ground release may be more appropriate for research where controlled applications on a number of small plots are required. As with liquid insecticides, ground releases of parasitoids reduce the chance of drift and, thus, allow the researcher to use smaller buffer zones; this, in turn, reduces the cost of establishing field experiments. Where the supply of *T. minutum* Riley is limited, ground releases make more efficient use of available parasitoids. Ground releases are also particularly useful in specific conditions: small discrete areas, areas of human habitation or high public use, environmentally sensitive areas, and private plantations and woodlots.

Jones *et al.* (1977) developed a mechanized technique for ground release of *T. pretiosum* Riley in cotton; however, forest stands have greater biomass, require more *Trichogramma*, and are more difficult to work in than cotton fields. This paper describes two systems developed for ground release of *T. minutum* in forest stands. Specifically, our interest was to develop systems that would (1) provide a uniform distribution of parasitoids simulating aerial release, (2) have little or no effect on the quality of parasitoids, (3) apply low quantities of parasitoids on specific areas in small forest plots, and (4) be easy to use in the forest environment.

## Materials and Methods

In 1983 and 1986, two ground systems were examined for the release of *Trichogramma* against the spruce budworm, *Choristoneura fumiferana* (Clemens). The studies were conducted in those forest stands described in Section 3.1.

The parasitoids used in 1983 were reared on eggs of *Sitotroga cerealella* (Olivier) by a commercial insectary, Rincon Vitova, Oak View, CA; in 1986, they were produced by the biological Control Laboratory, University of Guelph, Guelph, Ont. (see Section 2.0). Parasitoid pupae within eggs of the rearing host were transported by aircraft to the field. Prior to release, a subsample (ca. 1000 host eggs) was taken from each production batch to determine emergence, sex ratio, longevity, and fecundity. Parasitism of spruce budworm following releases was assessed with sentinel egg masses. Each sentinel egg mass consisted of a single egg mass laid in the laboraory on a balsam fir twig (5–10 cm in length) by spruce budworm reared on artificial diet. Each twig was tied to a pulley system which moved between the ground to the top of a single sample tree (see Section 3.5 and Smith 1985). These fresh egg masses were placed on randomly selected trees in the plots at three different heights, 1.25, 2.25, and 3.25 m above ground, and changed every 3 days throughout the summer. Weather conditions were monitored 15 km from the study sites.

### Grid Point Releases

*Trichogramma minutum* were released on 7, 14, and 21 July 1983 at the rate of $12 \times 10^6$ ♀♀ per hectare per release from point sources in a grid pattern. Host eggs were attached to cardboard sheets using diluted Elmer's white glue. The cards were placed in cone-shaped paper cups (25–150 mL), with openings at the top and bottom to permit escape of the parasitoids, and taken, under cool conditions (<15°C), to the field. The cups were pinned 25 cm above the ground on release stakes in five plots (50 by 50 m; 0.25 ha). To achieve a uniform grid distribution, the stakes were located in a 7 by 7 m spacing (Smith 1985). The distance from each stake to the nearest sample tree with sentinel egg masses was measured (Fig. 1*a*). Three weeks after each release, 10 cards (ca. 10 000 host eggs per card) were selected randomly from each plot and successful emergence of the parasitoids was calculated to compare with emergence from the subsample collected prior to the release. Because the parasitoids had already emerged, no assessment of sex ratio and female longevity or fecundity could be made post-release.

### Broadcast Releases

An electric hand-held leafblower (Model No. PB 100C-50, Allegretti Manufacturing Co. [Canada] Inc., Tilbury, Ont.) powered by a portable generator was used to release *T. minutum* from the ground at a rate of $12 \times 10^6$ ♀♀ per hectare per release. Two releases, one each on 5 and 12 July 1986, were made on a single plot (25 by 25 m). The parasitoids were shipped in bulk from the production facility where the number of female *T. minutum* in a given volume of host eggs was estimated. A shoulder strap was used to maintain the blower at a 45° angle from the applicator. This provided freedom for one of the applicator's arms to control the direction of the nozzle and the flow rate of the parasitized material.

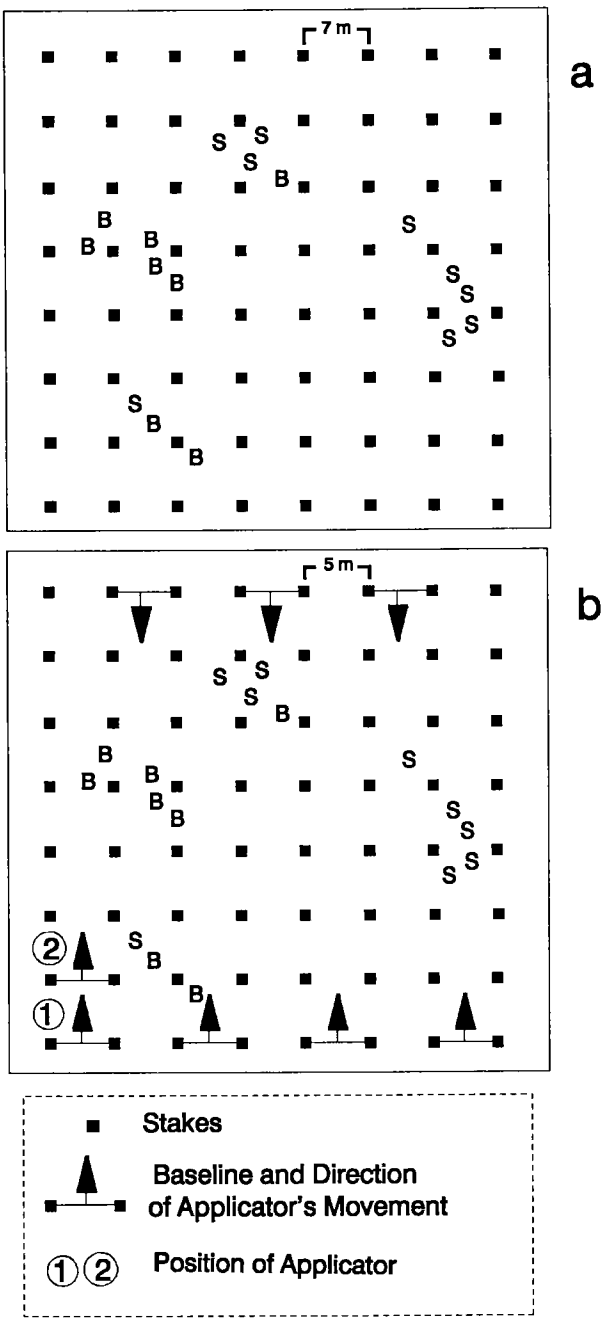


FIG. 1. Grid design for release stakes on experimental forest plots receiving ground releases of *Trichogramma minutum* in (*a*) 1983 and (*b*) 1986. Sample trees for monitoring parasitism with sentinel egg masses designated as follows: B = balsam fir; S = white spruce.

An initial test was made of the distribution pattern from the leafblower by placing deposit cards (625 $cm^2$) sprayed with Tanglefoot® in a 180° arc at intervals of 1 m from the applicator. Unparasitized eggs of the Angoumois grain moth were blown through the mechanism, directly in front of the applicator for ca. 10 s.

On each 0.0625-ha plot in the field (25 by 25 m), a grid pattern of stakes, 5 by 5 m, was established (Fig. 1*b*) and used to guide the application with the leafblower. Parasitized host material was divided equally, by volume, into 5-mL coffee creamer cups. With the applicator positioned on the baseline mid-way between two stakes (Fig. 1*b*, position 1), the contents of one container were dropped slowly in front of the air flow of the nozzle as the blower was rotated through a 180° arc. The applicator then walked to the next baseline (Fig. 1*b*, position 2) and repeated this procedure until all 25 grids had been treated. For each release date, small samples of the parasitized material that was distributed were collected randomly in funnels (see Section 3.5) on one plot to compare emergence, sex ratio, female longevity, and fecundity of the released parasitoids with those from the subsample taken before the release.

## Results and Discussion

Both methods of ground release were effective. With the point release system, parasitism of sentinel egg masses on sample trees close to the point of release ($X$ = 89.6% at 0–1.75 m) was not significantly higher than on trees furthest away ($X$ = 85.3% at 1.75–3.5 m; $\chi^2 = 1.73$, df = 1, $p > 0.05$). At the same application rate, both the point and broadcast releases resulted in similar levels of maximum parasitism (1983 and 1986 in Table 1). Although slightly higher, both types of ground release also produced rates of maximum parasitism similar to those on plots where *T. minutum* was released aerially during 1984 and 1985 (Table 1; also see Section 3.5). Irrespective of the release technique, *T. minutum* tended to parasitize significantly more egg masses and eggs in the mid- to upper canopy than in the lower canopy (Table 1). The data suggest that the parasitoids released on the ground were deposited uniformly and dispersed into the upper canopy upon release. Previous studies also have shown that, even when released from grid points on the ground, female *T. minutum* will disperse vertically into the upper canopy where the majority of host eggs are laid (Smith 1985, 1988). The natural predominance of parasitism by *T. minutum* on eggs of spruce budworm in the upper canopy has been noted by Kemp and Simmons (1978) and Houseweart *et al.* (1984).

Neither type of ground release affected parasitoid emergence (Table 2). In the broadcast release (1986), the sex ratio and female longevity and fecundity were not significantly different ($p$ = 0.05, Student's $t$ test) for both pre- and post-release samples. The point releases were a particularly good technique for releasing emerged parasitoids because there was no release mechanism to damage the adults. This technique would be useful where shipping schedules are uncertain. However, point release sources required more preparation time and were subject to predation by small mammals after 3 days in the field. Point releases are suited to well-managed plantations or individual trees in urban areas where the release can be controlled. The broadcast method is better suited to natural or unmanaged stands where dense brush may make the release of carded material difficult.

The leafblower distributed parasitized host eggs up to 7 m from the applicator; the majority were deposited at 3–5 m.

The gasoline-powered generator provided sufficient electricity to operate the blower continuously for 4 h. The exhaust fumes produced by the generator could be kept at least 50 m from the leafblower with extension cords, reducing their possible effect on the performance of the parasitoids. Because the blower could be directed upward, *T. minutum* were more likely to be placed directly in the canopy with this technique than with the point releases.

Table 1. Parasitism of sentinel spruce budworm egg masses following inundative release of $12 \times 10^6$ female *Trichogramma minutum* per hectare from the ground and by helicopter in forest stands near Hearst, Ont., from 1983 to 1986

| Year | Method of release | No. of plots | Maximum parasitism* (%) | No. obs. | Mean parasitism of egg masses (%) | | | Mean parasitism of eggs (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Upper canopy | Middle canopy | Lower canopy | Upper canopy | Middle canopy | Lower canopy |
| 1983 | Ground: grid point | 5 | 86.6 | 1683 | 74.3a† | 65.4b | 55.4c | 80.0ab | 83.1a | 75.7b |
| 1984 | Aerial | 2 | 84.1 | 1350 | 40.2a | 38.9b | 32.4c | 83.9a | 84.6a | 76.5b |
| 1985 | Aerial | 1 | 77.5 | 1800 | 40.5a | 35.3ab | 31.0b | 80.0a | 76.2a | 67.7b |
| 1986 | Ground: broadcast | 1 | 84.2 | 1444 | 22.5a | 22.5a | 19.0a | 80.3a | 78.8a | 73.6a |

*Parasitism of sentinel spruce budworm egg masses placed in the field for 3 days.
†Means for either egg mass parasitism or egg parasitism, followed by the same letter within each row, are not significantly different at the $p = 0.05$ level (Duncan's multiple range test 1955).

Table 2. The quality of *Trichogramma minutum* before and after point (1983) or broadcast (1986) release from the ground in forest stands near Hearst, Ont.

| | | Emergence (%) | | Sex ratio (% ♀♀) | | Longevity (days)* | | Fecundity (no. SBW† eggs parasitized per ♀) | |
|---|---|---|---|---|---|---|---|---|---|
| Year | Release | Pre-release | Post-release | Pre-release | Post-release | Pre-release | Post-release | Pre-release | Post-release |
| 1983 | 7 July | 85 | 81 | 58 | — | 2.5 | — | 19.8 | — |
| | 14 July | 83 | 86 | 55 | — | 1.5 | — | 4.2 | — |
| | 21 July | 87 | 86 | 57 | — | 2.0 | — | 4.5 | — |
| 1986 | 5 July | 81 | 75 | 45 | 52 | 1.8 | 1.8 | 5.7 | 5.4 |
| | 12 July | 86 | 87 | 53 | 52 | 1.3 | 1.6 | 4.6 | 5.3 |

*Longevity of adult female parasitoids from time of emergence from host egg to death.
†SBW = spruce budworm.

Our study shows that ground releases of *T. minutum* have practical use in forestry. In some cases, such as urban environments, these techniques may be the most appropriate. Both point releases in grid patterns and broadcast releases provided uniform coverage. Results from the ground release plots were comparable with those from plots treated by aerial releases but without the associated high costs of releasing large numbers of parasitoids or large scale field expenses. The ground applications were relatively easy to use in the forest environment, their greatest cost being that for labour. In small woodlots or in urban areas, control measures are often implemented by the landowner and, thus, labour costs can be considered negligible. The broadcast technique needs further refining before it can be considered on a commercial scale.

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# 3.3 AERIAL RELEASE SYSTEM FOR *TRICHOGRAMMA MINUTUM* RILEY IN PLANTATION FORESTS

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## Abstract

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An aerial dispersal system was developed for inundative release of insect eggs, *Anagasta kuehniella* (Zeller) and *Sitotroga cerealella* (Olivier), parasitized by *Trichogramma minutum* Riley. The equipment was used successfully in field tests over a 4-year period (1982–1985) in a coniferous plantation forest near Hearst, Ont. The release equipment consisted of simple electrical components, mechanical components from a small grain planter, and a centrifugal slinger used for aerial seeding of jack pine. The equipment was mounted on a Bell® 47 helicopter, flown at about 25 m above the ground. A swath width of 15 m was attained using this system. Application rates ranged from 12.3 to 263.0 g ♀ ♀ parasitoids per hectare ($0.6–25 \times 10^6$ ♀ ♀ parasitoids per hectare) during the 4 years of tests.

## Résumé

Un système de distribution a été développé pour le relâchement d'inondation des oeufs d'insectes, *Anagasta kuehniella* (Zeller) et *Sitotroga cerealella* (Olivier), parasités par *Trichogramma minutum* Riley. L'appareil a été utilisé avec succès pour les essais au champs pendant une période de 4 ans (1982–1985) dans un forêt de conifères de plantation, près de Hearst, Ont. L'appareil de relâchement s'est composé des composants électriques simples, des composants mécaniques d'une petite planteuse de grains, et d'une jeteuse centrifuge utilisée pour ensemencement aérien de pin gris. L'appareil a été monté sur un hélicoptère de marque Bell® 47, qui a volé à 25 m près au-dessus de la terre. Une largeur de bande de 15 m a été atteinte avec ce système. Les taux d'applicage ont varié de 12,3 à 263,0 g ♀ ♀ par hectare ($0,6–25 \times 10^6$ ♀ ♀ de parasitoïdes par hectare) pendant les 4 ans d'essais.

## INTRODUCTION

The extent and inaccessibility of many forest insect outbreaks in Canada make it necessary to apply control agents by aircraft. The control of spruce budworm, *Choristoneura fumiferana* (Clemens), populations, using *Trichogramma*, presents a particularly complex challenge. The physical nature of the spruce budworm habitat in spruce–fir forests (Morris 1963), the limited searching ability of *Trichogramma* (Smith 1988), and the need to obtain significant control of spruce budworm populations (Prebble 1975; Sanders *et al.* 1985) necessitate the release of large numbers of parasitoids ($>2 \times 10^6$ per hectare) on relatively inaccessible areas.

We describe the release system developed by the Ontario Ministry of Natural Resources to distribute aerially large numbers of the egg parasitoid, *T. minutum* Riley, against populations of spruce budworm. The deposit and distribution of parasitoids and the operational implications of this system in suppressing spruce budworm populations are discussed in Sections 3.4 and 3.5.

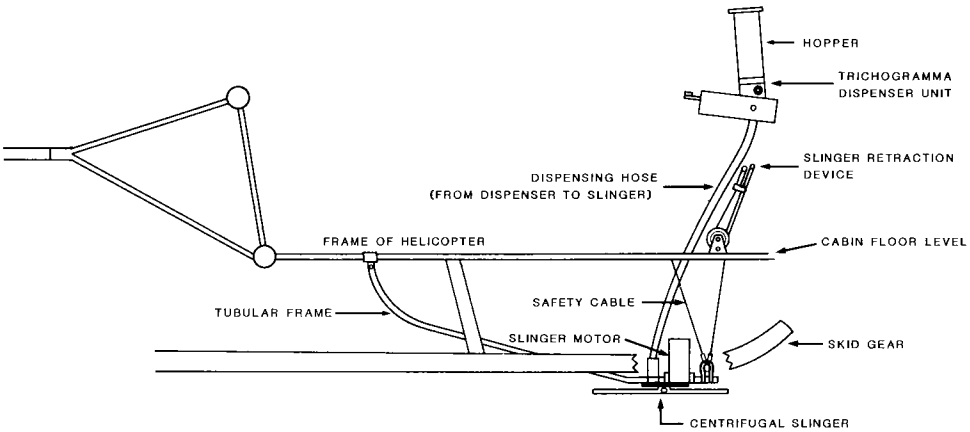


FIG. 1. Inside unit and outside slinger for dispensing *Trichogramma* from a Bell® 47 helicopter.

## MATERIALS AND METHODS

### Egg Storage Method

The parasitized eggs of the Mediterranean flour moth, *Anagasta kuehniella* (Zeller), or the Angoumois grain moth, *Sitotroga cerealella* (Olivier), were received in mesh-netting shipment containers that were placed, with gel ice packs and newspaper, in Koolatrons® (see Section 2.0). The shipping containers were kept refrigerated at about 10°C in the dark until release.

### Release Equipment

The unit used for the aerial release of *T. minutum* in host eggs consisted of two main components, a hopper (Fig. 1) and egg dispenser (Figs. 1, 2, 3 [*top*]) located inside a Bell® 47 helicopter; and a centrifugal slinger on a tubular frame bolted to the bottom of the helicopter (Figs. 1, 3 [*bottom*]). The slinger was located below the dispenser on the outside of the aircraft. A clear flexible hose (2 cm inside diameter) carried the eggs from the dispenser to the slinger where they were discharged (Fig. 1).

The egg dispenser (Figs. 2 and 3 [*top*]) consisted of a hopper bottom and seed plate which controlled the rate of flow. Seed plates 5459 and 5460 from a Planet Jr.® drill seeder were used for the releases. The hopper was constructed from a clear acrylic tube (length 30 cm, 8.6 cm inside diameter) with a machined base that fit securely into the supporting collar (Fig. 2). An acrylic lid (not shown) was constructed to fit on the upper, open end of the hopper.

An acrylic plate (25 by 25 by 4 mm) was located directly below the orifice of the seed plates and the opening at the bottom of the hopper was manually positioned with an external handle (activation handle, Fig. 2). When the plate was pushed in, the opening at the bottom of the hopper was cleared and a micro-switch activated to provide power from the helicopter's electrical system to a 28-V DC drive motor. The motor, coupled to a Boston gear (2:1 gear ratio), powered a shaft running through the lower part of the hopper and a wobble plate or rotary brush (interchangeable), located mid-way along the shaft, revolved at 22 rpm above the orifice in the hopper bottom maintaining an even flow of eggs (Fig. 2).

The centrifugal slinger (Figs. 1 and 3 [*bottom*]) was part of a Brohm seeder developed by the Ontario Ministry of Natural Resources for the aerial distribution of jack pine seed. A 24-V DC motor powered the slinger which turned at about 1500 rpm. As the eggs passed through the clear dispensing hose, they were directed toward the centre of the slinger cavity

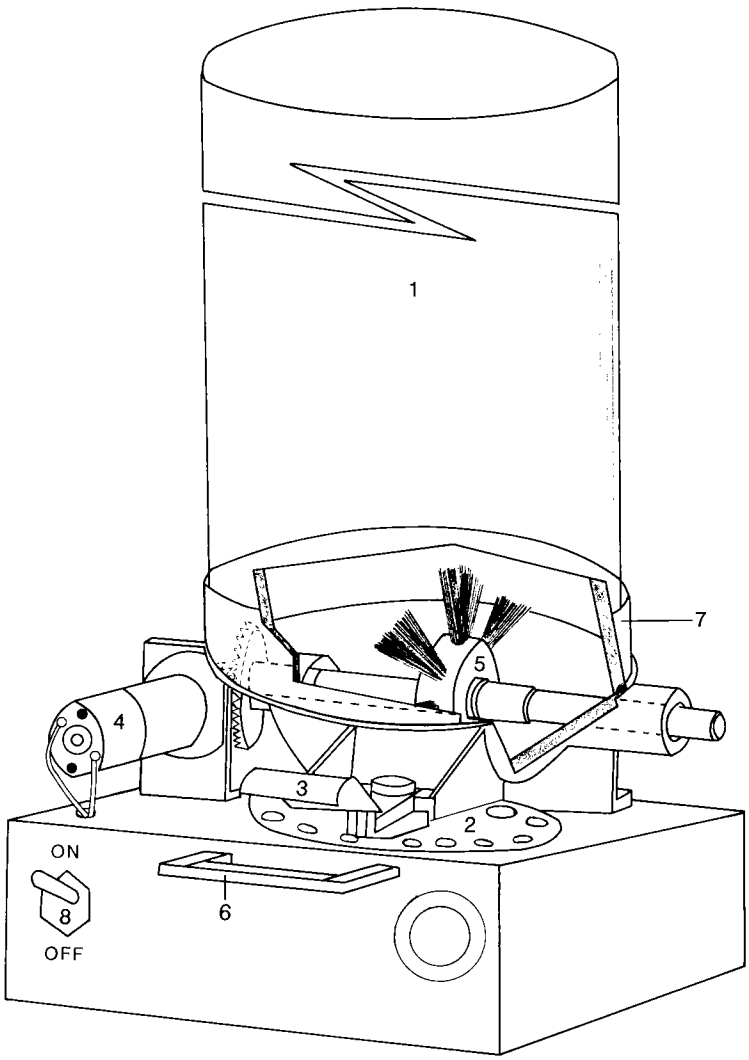


FIG. 2. Inside unit for dispensing *Trichogramma* (cut-away view of rotating brush and shaft). Components are labelled as follows: (1) hopper; (2) seed plate; (3) orifice selection lever; (4) 28-V motor; (5) rotary brush; (6) activation handle; (7) supporting collar; and (8) main power switch.

and discharged horizontally through four plastic tubes (30 cm long by 16 mm inside diameter). The slinger could be lowered or retracted from inside the helicopter with a mechanical device. During the aerial releases, the slinger was extended below the skid gear of the helicopter to minimize interference with the discharged eggs. When landing, the slinger mechanism was retracted to its protected position above the skid gear.

## Calibration

The pattern of egg deposition was assessed, using a series of aerial releases carried out in an open field under calm conditions. To determine swath width, a line of paper deposit cards (25 cm by 25 cm) sprayed with Tanglefoot® was laid out on the ground at 1.5-m intervals over a distance of 60 m. The mid-point of the cardline was marked with

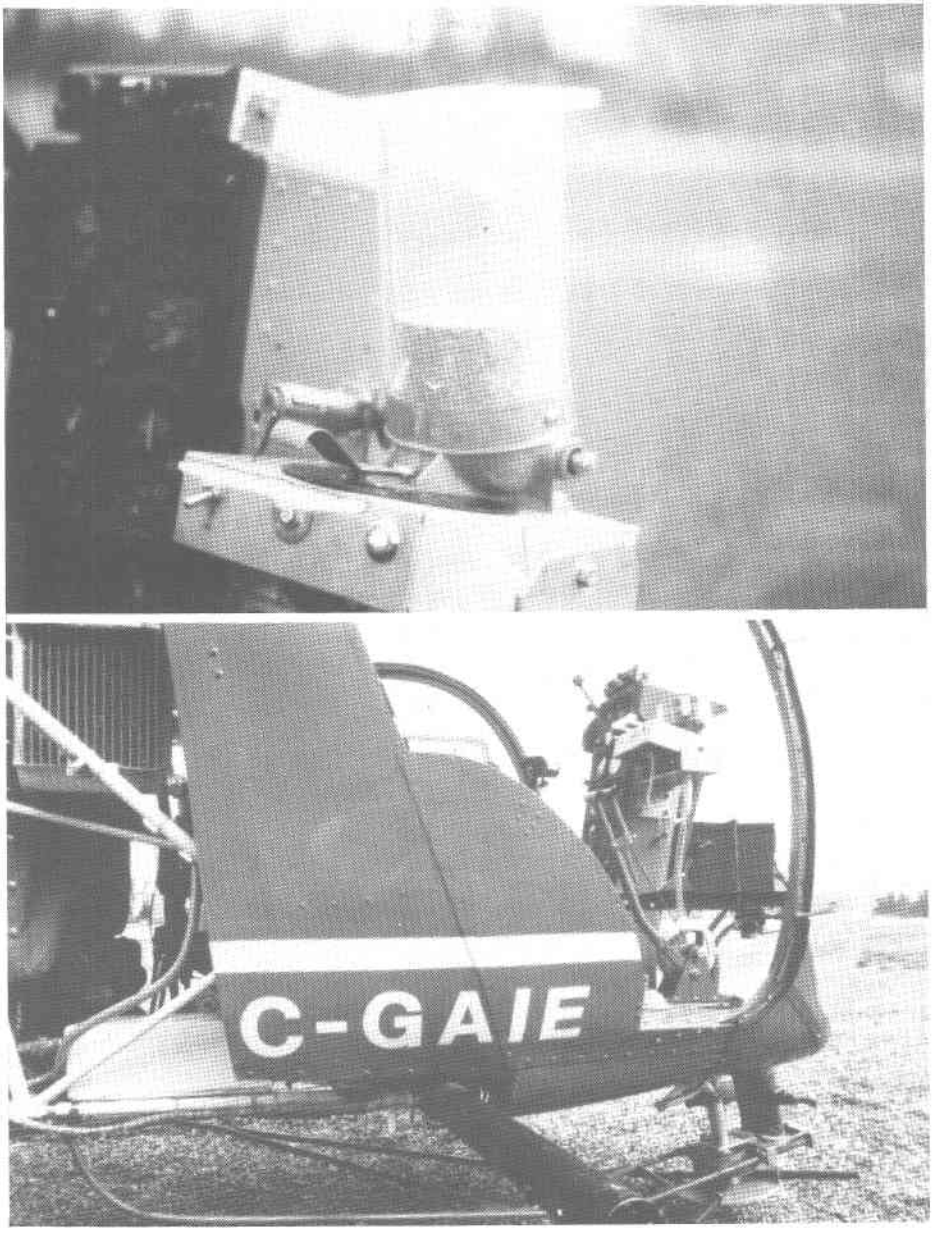


FIG. 3. (*Top*) Dispenser unit for *Trichogramma minutum* mounted in a Bell® 47 helicopter. (*Bottom*) Dispenser unit for *T. minutum*, including a slinger, mounted on a Bell® 47 helicopter.

a helium-filled meteorological balloon. The helicopter was flown over the balloon, perpendicular to the cardline, releasing a swath of non-parasitized host eggs. The aircraft flew about 25 m above ground at an air speed of about 60 km/h. This procedure was repeated over the same line until a swath of eggs on the cards was clearly visible. Using this technique, a swath width of 15 m was estimated.

The dispenser unit was calibrated to release the required number of parasitoids per hectare. The parasitoid flow rate was regulated by selecting different orifice sizes on the

Table 1. Flow rates for non-parasitized eggs of *Sitotroga cerealella* from the release system on a Bell® 47 helicopter

| Seed plate (No. 5459) | | | Seed plate (No. 5460) | | |
|---|---|---|---|---|---|
| Orifice No. | Diameter (mm) | Flow rate (g/min)* | Orifice No. | Diameter (mm) | Flow rate (g/min)† |
| 14 | 4.00 | 30 | 27 | 7.75 | 180 |
| 15 | 4.25 | 34 | 28 | 8.00 | 188 |
| 16 | 4.50 | 35 | 29 | 8.50 | 256 |
| 17 | 4.75 | 41 | 30 | 9.00 | 295 |
| 18 | 5.00 | 48 | 31 | 9.50 | 349 |
| 19 | 5.50 | 58 | 32 | 10.00 | 386 |
| 20 | 6.00 | 82 | 33 | 10.50 | 443 |
| 21 | 6.25 | 85 | 34 | 11.00 | 467 |
| 22 | 6.50 | 92 | 35 | 12.00 | 511 |
| 23 | 6.75 | 94 | 36 | 13.00 | 585 |
| 24 | 7.00 | 99 | 37 | 14.00 | 755 |
| 25 | 7.25 | 107 | 38 | 16.00 | 877 |
| 26 | 7.50 | 108 | 39 | 18.00 | 1000 |

*Based on five 15-s and five 30-s tests.
†Based on ten 10-s tests.

seed plate (Table 1). Approximate flow rates were determined for non-parasitized eggs of the Angoumois grain moth and the same values were used later for parasitized host eggs in the field releases. The number of female parasitoids per gram of parasitized eggs was estimated by the Biological Control Laboratory at the time of shipment to the field. Therefore, a calculated flow rate, expressed in grams per minute, could be used to determine the number of female parasitoids that should be dispersed. By changing the seed plate or the orifice size, or both, or by altering the aircraft speed, the desired application rate (number of female parasitoids per hectare) was obtained.

## Results of Field Application

All aerial releases were carried out on the spruce–fir plantation forest described in Section 3.1. The application specifications and numbers of *Trichogramma* released from 1982 to 1985 are summarized in Table 2. For all releases, the number of female parasitoids released per hectare was based on emergence rates and sex ratios determined by the Biological Control Laboratory, University of Guelph, Guelph, Ont., at time of shipment.

In 1982, only 82 g of host eggs (12.3 g ♀♀ parasitoids) were available for release (Table 2). This small quantity was added to a larger volume of timothy seed to provide bulk and ensure an adequate distribution of the expected $0.8 \times 10^6$ ♀♀ *Trichogramma* over the 1.3-ha plot. Using orifice No. 26, about 1 min of dispersing time (boom time) was required to release the parasitized material at a rate of $0.6 \times 10^6$ ♀♀ per hectare (12.3 g AI per hectare).

In 1983, 441 g of parasitized host material was available for release on the designated 2-ha plot but the No. 26 orifice on the No. 5459 seed plate was too small to distribute this large volume evenly in a single pass. Therefore, a smaller orifice (No. 21) was used which provided only one-third the required flow rate, necessitating three overlapping swaths to release all the parasitized material over the plot. A second seed plate with larger orifices (No. 5460) was used for the releases in 1984 and 1985, to avoid overlapping swaths in the plots.

In 1984, three 1-ha plots were treated with rates ranging from 13.5 to $25 \times 10^6$ ♀♀ per hectare; in 1985, five 1-ha plots were treated with rates from 3 to $12 \times 10^6$ ♀♀ per hectare. In both 1984 and 1985, two separate releases, about 1 week apart, were conducted on each plot.

Table 2. Specifications for the aerial release of *Trichogramma* from a Bell® 47 helicopter (1982–1985)

| Year | Plot* | Flying speed (km/h) | Orifice No. | Area treated (ha) | Weight of released material (g) | | Expected application rate (AI per hectare)‡ | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Host eggs† | Parasitized eggs | grams ♀♀ | × $10^6$ ♀♀ |
| 1982 | 1 | 50 | 26 | 1.3 | 82 (BCL and B)§ | 30 | 12.3 | 0.6 |
| 1983 | 1 | 50 | 21‖ | 2.0 | 441 (RV) | 394 | 80.4 | 16.7 |
| 1984 | | | | | | | | |
| 1st Release | | | | | | | | |
| | 2 | 56 | 33 | 1.0 | 385 | 347 | 148.9 | 13.5 |
| | 3 | 56 | 33 | 1.0 | 385 | 347 | 148.9 | 13.5 |
| | 4 | 56 | 35 | 1.0 | 400 (RV) | 380 | 125.1 | 15.0 |
| 2nd release | | | | | | | | |
| | 2 | 56 | 35 | 1.0 | 460 | 414 | 204.1 | 16.1 |
| | 3 | 56 | 33 | 1.0 | 457 | 421 | 207.6 | 16.2 |
| | 4 | 56 | 33 | 1.0 | 350 (RV) | 332 | 158.2 | 25.0 |
| 1985 | | | | | | | | |
| 1st release | | | | | | | | |
| | 5 | 64 | 27 | 1.0 | 130 | 117 | 66.0 | 3.0 |
| | 6 | 64 | 27 | 1.0 | 130 | 117 | 66.0 | 3.0 |
| | 7 | 64 | 31 | 1.0 | 260 | 334 | 188.4 | 6.0 |
| | 8 | 64 | 31 | 1.0 | 260 | 334 | 188.4 | 6.0 |
| | 9 | 64 | 36 | 1.0 | 518 | 466 | 263.0 | 12.0 |
| 2nd release | | | | | | | | |
| | 5 | 64 | 27 | 1.0 | 152 | 137 | 43.6 | 3.0 |
| | 6 | 64 | 27 | 1.0 | 152 | 137 | 43.6 | 3.0 |
| | 7 | 64 | 29 | 1.0 | 304 | 274 | 85.2 | 6.0 |
| | 8 | 64 | 29 | 1.0 | 304 | 274 | 85.2 | 6.0 |
| | 9 | 64 | 33 | 1.0 | 610 | 549 | 174.6 | 12.0 |

*See Section 3.1 for plot locations and descriptions.
†All parasitoids were provided by the Biological Control Laboratory (BCL) at the University of Guelph unless otherwise indicated as: B, Biogenesis Inc., Mathis, TX, USA; RV, Rincon Vitova, Oakview, CA, USA.
‡Application rate reflects expected values for emergence and proportion of female parasitoids used to determine the flow rate and flying speed: no. female parasitoids = (no. parasitized eggs) × (% expected female parasitoids) × (% expected emergence) at time of shipping; active ingredient (AI) = emerged female parasitoids.
§For 1982, 77 g of the parasitoids were reared from the Mediterranean flour moth in Guelph and the remaining 5 g of parasitoids were reared from the Angoumois grain moth at Biogenesis. Timothy seed (220 g) was mixed with the parasitoid material for a total of 302 g. Flow rates for timothy seed were used to determine the correct orifice size.
‖Three successive applications were made on this plot using orifice No. 21; this orifice allowed only one-third of the required output.

## Discussion and Conclusions

The release equipment performed well on the small scale applications carried out from 1982 to 1985. The tests showed that aerial release of insect eggs parasitized by *Trichogramma* can be successfully conducted over forested land without adversely affecting the parasitoid (see Section 3.4).

Despite good performance by the release equipment under these small scale conditions, its potential for use over larger areas is limited. The dispenser unit would not be suitable for larger areas because of the limited capacity of the hopper. In addition, a larger program would require a more sophisticated dispenser unit incorporating a cooling apparatus. This would prevent the buildup of metabolic heat in the hopper and reduce the chance of the *Trichogramma* emerging prior to release.

The Brohm-style centrifugal slinger designed to release jack pine seed with a minimum of physical/mechanical damage lends itself well to parasitoid application. Research into the most appropriate slinger/distribution mechanism (i.e. arch of distribution, methods and materials of construction, etc.) however, should be considered to ensure cost-effectiveness. Furthermore, investigation should be conducted into the most appropriate choice of aircraft and distribution mechanisms for larger scale applications of parasitoids.

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# 3.4 DEPOSIT AND DISTRIBUTION OF *TRICHOGRAMMA MINUTUM* RILEY FOLLOWING AERIAL RELEASE

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## Abstract

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The egg parasitoid, *Trichogramma minutum* Riley, was distributed by helicopter over forest stands near Hearst, Ont., to control the spruce budworm, *Choristoneura fumiferana* (Clemens). The quality of the parasitoids in terms of emergence, proportion of females, longevity, and fecundity was not affected by aerial release. Based on monitoring with deposit cards, at 10 m above ground, the helicopter had an effective swath width of ca. 10 m. Aerial release provided an uneven distribution of deposit on 1.0-ha plots, with significantly less parasitized material reaching the outer edges of each plot than in the centre; parasitism of sentinel egg masses within the plots corresponded to the distribution of deposit. Over 50% of the released material was deposited on the ground. Drift outside the plots was generally less than 25 m, never exceeding 100 m. The extent of drift was dependent on the application technique, and to a lesser extent, wind direction. Deposit cards provided an extensive rather than an intensive sampling method for monitoring the aerial distribution of *T. minutum*.

## Résumé

Le parasitoïde des oeufs, *Trichogramma minutum* Riley, a été distribué par hélicoptère au-dessus des terrains forestiers près de Hearst, Ont., pour maîtriser la tordeuse des bourgeons de l'épinette, *Choristoneura fumiferana* (Clemens). La qualité des parasitoïdes en fonction d'éclosion, de proportion de femelles, de longévité et de fécondité n'a pas été touchée par le relâchement aérien. Selon le contrôle sur les cartes de dépôt, à 10 m au-dessus de la terre, l'hélicoptère a eu une bande d'efficacité d'une largeur de ca. 10 m. Le relâchement aérien a donné une distribution de dépôt inégale aux lotissements de terre de 1,0 ha, fournissant significativement moins de matières parasitées autour des bords qu'au centre de chaque lot; le parasitisme des masses d'oeufs posées en sentinelles en dedans des lots ont été en conformité à la distribution des matières déposées. Au-dessus de 50% des matières relâchées ont été déposées à terre. La diffusion en dehors des lots a été généralement moins de 25 m et n'a jamais excédé 100 m. L'étendue de la diffusion dépendait de la technique d'applicage, et à un degré moindre à la direction du vent. Les cartes de dépôt n'ont fourni qu'une méthode extensive pour contrôler la distribution aérienne de *T. minutum*.

## INTRODUCTION

In China and the USSR, *Trichogramma* species are the most widely used beneficial insects for biological control of pests on agricultural and forest crops (Beglyarov and Smetnik 1977; Li 1983). In these countries, the success of this approach has been due mainly to the comparatively low costs of labour; parasitoids are produced in factories and distributed in the field either manually or by limited mechanical methods. However, automated techniques for the production and distribution of *Trichogramma* would be required to ensure the economic feasibility of inundative releases in North American markets (Ables

*et al.* 1979). Initial attempts in the United States employed hand application methods and were followed by more mechanized ground level releases (Jones *et al.* 1977; also see Section 3.2). In recent years, systems have been developed for attaching parasitized eggs of *Sitotroga cerealella* (Olivier) to wheat carriers and applying them by aircraft (Jones *et al.* 1979; Bouse *et al.* 1980).

During 1983, outbreak populations of the spruce budworm, *Choristoneura fumiferana* (Clemens), occurred in Ontario on more than $12.1 \times 10^6$ ha of boreal forest (Howse and Applejohn 1983). At this time, *Trichogramma minutum* Riley was the only known egg parasitoid of the spruce budworm and was considered for use in Canada. The magnitude of forest infestations requires aerial release of the parasite and in 1982 the Pest Management Section of the Ontario Ministry of Natural Resources developed a helicopter system for distributing *T. minutum* uniformly on forest stands (see Section 3.3). Application rates, distribution of deposit, and meteorological and biological factors influence the success of such a system (Joyce 1985). We examined the distribution and quality of *T. minutum*, aerially released on plantation forests near Hearst, Ont., under different weather and stand conditions from 1983 to 1985 (see Section 3.5). These factors also were measured for an orchard release near Guelph, Ont., in 1985.

## Materials and Methods

### Parasitoid Quality

*Trichogramma minutum* were reared at the Biological Control Laboratory, University of Guelph, Guelph, Ont., and transported to the field (see Section 2.0). Parasitoid quality was assessed following aerial release in 1985 (Table 1). Prior to the release of $10 \times 10^6$ *T. minutum*, a sample of ca. 2000 parasitized eggs was taken from (A) the glass of the parasitization unit, (B) the bulk supply of parasitized eggs, and (C) the hopper of the release mechanism (see Table 2). The remaining eggs were aerially released in September from a height of 20 m over an orchard at Guelph, Ont. Deposits from this release were determined by sampling eggs with (D) 45-cm-wide cardboard funnels, (E) cloth-covered trays (60 by 120 cm), both placed at ground level, and (F) a sheet (250 by 150 cm) held horizontally 1 m above the ground. All samples of parasitized eggs (A to F) were incubated at 25°C. Twenty females from each sample were placed in vials (1 ♀ per vial) and provided daily with 50 gamma-sterilized, fresh host eggs. Emergence from the samples and longevity and fecundity (e.g. total number of host eggs parasitized) for each emerged female parasitoid were determined.

Parasitoid quality was assessed under field conditions following releases near Hearst, Ont., in 1983 and 1984. Samples of (ca. 500) parasitized host eggs were collected prior to being placed in the dispenser unit (pre-release), and after the eggs had passed through the dispenser unit (post-release). Emergence and sex ratio of parasitoids from these two samples were compared. In 1984 and 1985, the post-release sample was collected with funnels (30 cm diameter) placed 1 m above ground level on the release plots. Seven funnels on one plot in 1984 and five funnels in each of five plots in 1985 were used. In a field laboratory, pre- and post-release longevity and daily fecundity were measured for 20 ♀ ♀ *T. minutum* by isolating each insect in a 1.9-mL vial and providing her with two fresh spruce budworm egg masses. Fecundity was estimated from the number of eggs parasitized and the number of progeny emerged.

### Release in an Orchard

In 1985, deposit cards (25 by 25 cm) were used to assess the width of a single swath in a dwarf apple orchard at Guelph, Ont. Cards sprayed with aerosol Tanglefoot® were placed on the ground in a line perpendicular to the flight path of the helicopter. The cards were spaced at intervals of 1.75 m for 15 m on either side of the intended flight path. The Bell® helicopter, described in Section 3.3, flew one swath over the orchard, releasing

parasitized host eggs at ca. 10 m above the ground. After the release, the number of parasitized and non-parasitized host eggs on each card was counted.

**Release in Plantation Forests**

From 1983 to 1985, aerial releases were made between 0600 and 0800 hours on the stands described in Section 3.1. All study areas were oriented in the same cardinal direction to standardize the flight path of the helicopter. Stand composition and condition were measured in each area and weather conditions were monitored 15 km from the release sites. As in the orchard, deposit cards were used to assess rates of deposit and distribution. Prior to release, each card was stapled to a plywood board, horizontally on top of a 3-m stake. This allowed the cards to be disassembled and collected rapidly. After the release, each card was covered with Saran Wrap®, removed from the board, and taken to the laboratory for enumeration of parasitized and non-parasitized host eggs.

Only small quantities of parasitized host material were available in 1983; consequently, only one 2.0-ha plot was used. On this plot, the *Trichogramma* was released in three adjacent diagonal swaths with 20 deposit cards placed randomly along the flight path. Parasitized eggs were released on 14 July at $20.1 \times 10^6$ per hectare. In addition to the deposit cards, six branches, three each of balsam fir and white spruce, were sprayed with Tanglefoot® and placed on the ground in the study area prior to release. The surface area (Sanders 1980) and number of parasitized eggs and unparasitized host eggs were recorded for each branch.

During 1984 and 1985, additional studies were conducted with larger numbers of parasitoids. In each year, 1.0-ha study plots (100 by 100 m) were established: three in 1984 and five in 1985. The distribution pattern within each plot was examined both years by placing deposit cards on stakes, at the centre and at 10-m intervals for 50 m in each of the four cardinal directions (21 cards per plot). This provided an extensive sample of deposit. Egg deposit between plots was compared using ANOVA and Duncan's new multiple range test (Duncan 1955). Drift outside each plot was also assessed both years. In 1984, deposit cards were placed at 25, 50, 100, 150, and 200 m in the four cardinal directions outside the plots, and in 1985, cards were located at 25-m intervals from the four edges of each plot up to 100 m.

Parasitism of naturally laid spruce budworm egg masses in each plot was compared with relative deposit within each plot. In each plot, nine groups of two balsam fir and two white spruce trees were selected. One group was located at the plot centre, one at 10–20 m from the centre, and one at 40–50 m from the centre in each cardinal direction. During early August, in both 1984 and 1985, one 45-cm branch tip was taken from the upper mid-crown of each of the balsam fir and white spruce trees in each group (total 36 branches per plot). This design allowed us to compare parasitism with deposit by both direction and distance from the centre of the plot. Student's $t$ test was used to examine deposit at the centre versus the edges of the plot while ANOVA and Duncan's new multiple range test tested significance of deposit by cardinal direction. Ostle and Mensing's (1975) $\chi^2$ test for proportions with binomial distribution was used to compare parasitism of the egg masses on the branches.

In 1984, two releases were conducted on each plot at $19.8 \times 10^6$ parasitized eggs per hectare on 10 July and $22.9 \times 10^6$ parasitized eggs per hectare on 16 July. One plot was used to determine the sampling intensity for parasitoid deposit. Deposit cards were placed on 3-m stakes at 2.5-m intervals from the centre of the plot up to 10 m in each cardinal direction. These 17 cards, in an area of 314 $m^2$, provided intensive samples to obtain more precise information on the effective swath width as well as an estimate of variability in deposit between cards. This allowed us to determine the minimum number of cards needed to measure parasitoid deposit on these stands accurately.

Table 1. Emergence, longevity, and fecundity of *Trichogramma minutum* before and after release by helicopter in an apple orchard at Guelph, Ont., during 1985

| | Pre-release | | | Post-release | | |
|---|---|---|---|---|---|---|
| | A* | B | C | D | E | F |
| Emergence (%) | 89.7† | 87.5 | 90.9 | 87.9 | 88.5 | 87.2 |
| Female longevity (days) | 6.3 | 7.1 | 4.7 | 7.3 | 7.5 | 6.1 |
| First-day fecundity (no. parasitized eggs per ♀) | 38.2 | 31.0 | 32.9 | 36.0 | 34.2 | 32.8 |
| Total fecundity (no. parasitized eggs per ♀) | 68.2 | 65.4 | 58.5 | 77.7 | 68.8 | 73.7 |

**Trichogramma* taken from (A) glass of window box parasitization unit, (B) bulk parasitized eggs; (C) hopper; (D) cardboard funnel; (E) cloth trays, and (F) sheet on ground.
†Means in each row are not significantly different at the $p = 0.05$ level (Duncan's new multiple range test).

To examine vertical deposition, additional deposit cards were placed on the ground, beneath each of the 17 cards described above. The number of parasitized eggs deposited at 3 m and at ground level could then be compared with the number released from the helicopter (10 m).

In 1985, the 21 extensive cards on the plots were also used to examine the effect of different release rates on the distribution pattern of parasitized eggs. Parasitized eggs were released on these plots at 4.8, 9.6, and $19.2 \times 10^6$ per hectare on 9 July and at 4.8, 9.5, and $19.0 \times 10^6$ per hectare on 19 July. The highest rate was applied on a single plot while the two lower rates were each replicated on two plots.

## RESULTS

### Parasitoid Quality

The orchard study at Guelph showed that parasitoids were unaffected by aerial release (Table 1). There were no significant differences in emergence, longevity, or first-day or total fecundity before and after release ($p = 0.05$, Duncan's new multiple range test, Duncan [1955]). Similarly, in forest stands near Hearst during 1983 and 1984, the quality of parasitoids passing through the release mechanism was relatively unchanged (Table 2). Where slight reductions did occur, they were not significantly different ($p = 0.05$, Student's

Table 2. Biological parameters of parasitoid quality measured before and after aerial release of *Trichogramma minutum* near Hearst, Ont., in 1983 and 1984

| | | First release | | | Second release | | |
|---|---|---|---|---|---|---|---|
| Year | Parameter | A* | B | C | A | B | C |
| 1983 | Emergence (%) | — | 85.8† | 79.5 | — | — | — |
| | Sex ratio (% ♀♀) | — | 56.0 | 50.9 | — | — | — |
| 1984 | Emergence (%) | 94.5 | 73.5 | 78.8 | 89.5 | 84.7 | 88.7 |
| | Sex ratio (% ♀♀) | 65.2 | 58.0 | 65.2 | 61.4 | 57.5 | 61.4 |
| | Female longevity (days) | — | 2.5 | 2.4 | — | 2.0 | 2.3 |
| | Total fecundity (no. parasitized eggs per ♀) | — | 14.7 | 16.6 | — | 17.6 | 11.5 |

*Values determined (A) prior to shipping from Biocontrol Laboratory, Guelph, Ont., (B) pre-release sample, and (C) post-release sample.
†Means between pre-release (B) and post-release (C) columns are not significantly different for either year or release at the $p = 0.05$ level (Student's $t$ test).

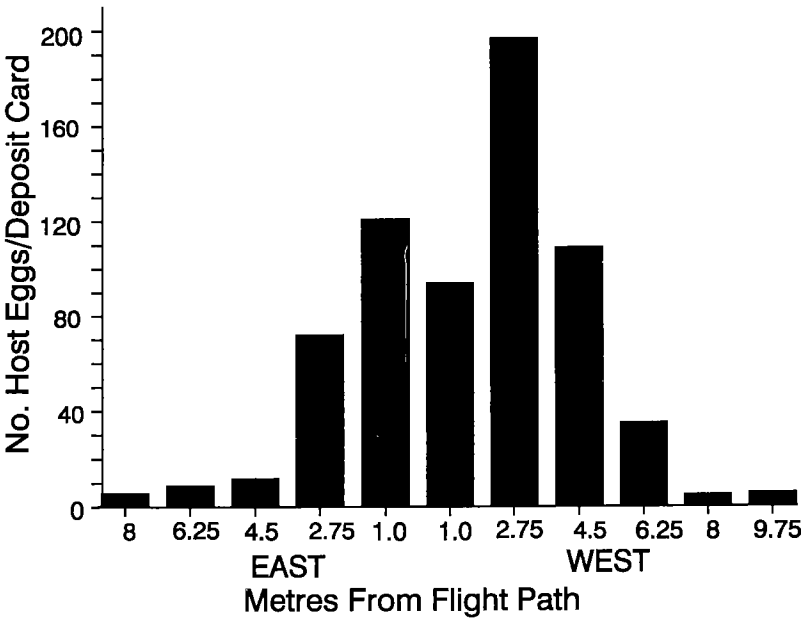


FIG. 1. The number of host eggs collected on deposit cards under the flight path of the helicopter releasing *Trichogramma minutum* over an apple orchard near Guelph, Ont.

*t* test). These results clearly show that the mechanism for aerially releasing *T. minutum* from 10 m in height over forest stands was not harmful to the viability of the parasitoids.

**Distribution of Deposit**

(*i*) Orchard: Host eggs released over an orchard from a single helicopter swath were deposited normally. Peak deposition occurred ca. 3 m to the west of the flight path and was probably the result of a 5- to 10-km/h east wind at the time of release (Fig. 1). Although host eggs were deposited over a width of about 18 m, the effective swath width (within which over 90% of the eggs were deposited) was about 11 m. For application purposes, an effective swath width of 10 m was used.

(*ii*) Forest: Parasitized material was deposited somewhat unevenly across individual plots in forest releases (Figs. 2 and 3). This effect was more apparent in 1984 (Fig. 2) when the aerial technique was still being perfected than in 1985 (Fig. 3). Infrequently, on all plots, no deposit was recorded in 10- to 20-m sections, particularly near the plot edges. More uniform distribution was achieved in 1985 at all three rates of release (Fig. 3). The different release rates, however, were not clearly evident on the deposit cards. For both releases, the greatest number of eggs were collected on plot 9 ($X = 19.1 \times 10^6$ per hectare released); however, a clear trend in deposit relative to release rate was observed only for the second release.

Parasitism of spruce budworm egg masses in the plots in 1984 and 1985 was usually lower on the edges (30–50 m) than in the centre (0–20 m) (Table 3). Although the level of this reduction varied (0–35%), it was similar to the reduction in deposit (Table 3).

Cardinal direction did not significantly affect deposit of host eggs. In each directional quadrant, the mean number of eggs deposited was relatively uniform within the plots (frequency for each quadrant, $X = 25\%$, range = 9–40%). In 1984, the deposit for both releases in north, south, east, and west quadrants was 28, 33, 18, and 21%, respectively ($F = 2.51$; df = 23; $p = 0.05$) and in 1985 it was 22, 16, 36, and 26%, respectively ($F = 2.14$;

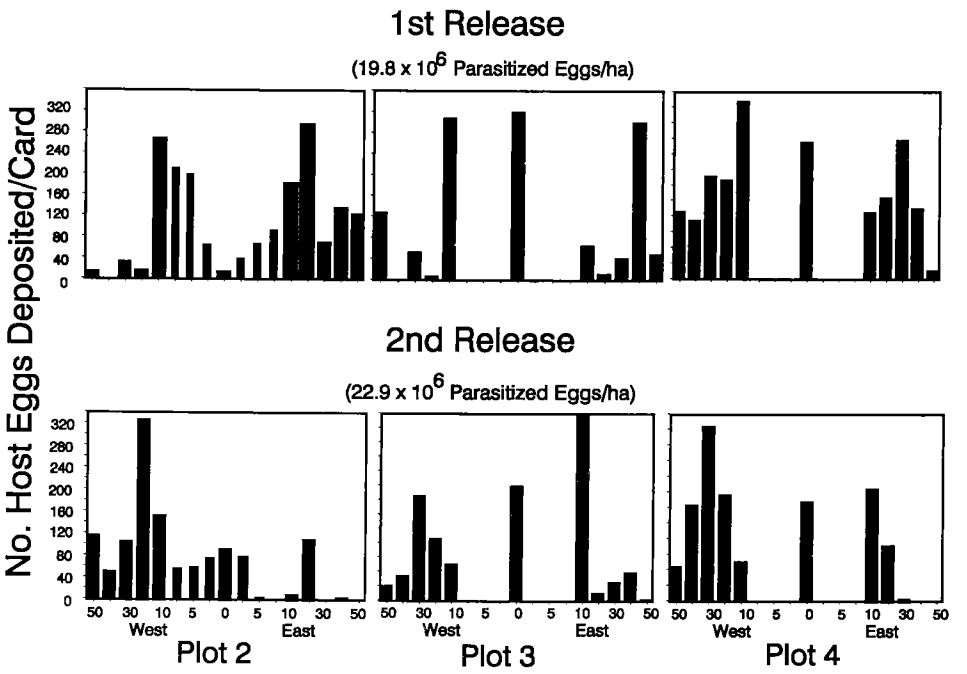


FIG. 2. Distribution of deposit on forest plots receiving aerial releases of *Trichogramma minutum* near Hearst, Ont., in 1984.

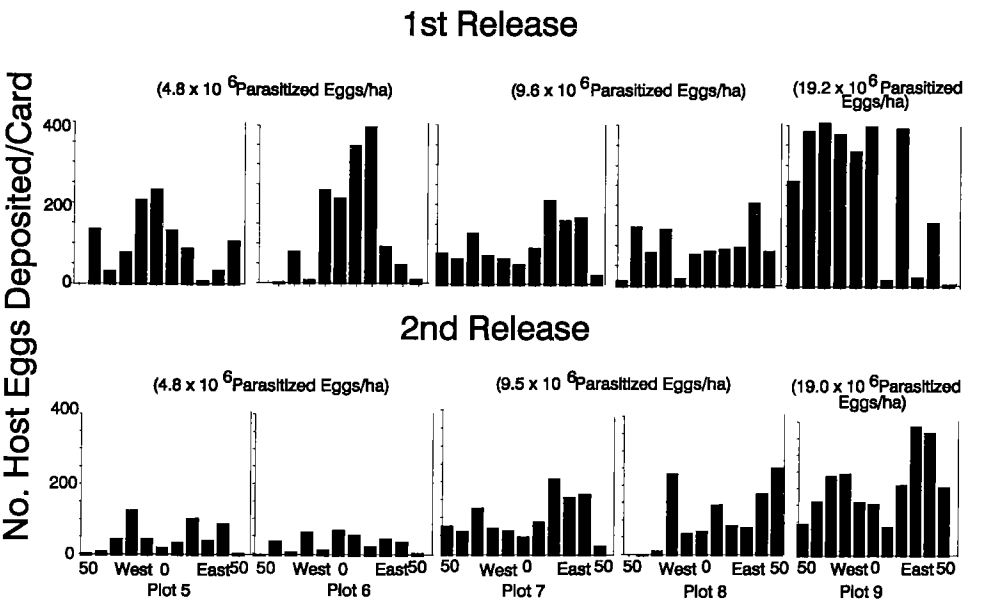


FIG. 3. Distribution of deposit on forest plots receiving three different rates of *Trichogramma minutum* near Hearst, Ont., in 1985. Release rates and number of parasitized eggs per hectare for each plot are indicated in parentheses.

Table 3. Parasitism of naturally laid spruce budworm egg masses and deposit by distance on forest plots receiving *Trichogramma minutum* reared at the Biocontrol Laboratory, Guelph, Ont., in 1984 and 1985

| Year | Plot | Variable | Distance from centre of plot (m) 0–20 | 30–50 | Reduction* (%) |
|---|---|---|---|---|---|
| 1984 | 2 | Parasitism† | 99 | 90 | 9 |
| | | Deposit‡ | 100 | 99 | 2 |
| | 3 | Parasitism | 95 | 79 | 17§ |
| | | Deposit | 113 | 86 | 23§ |
| | 4 | Parasitism | 42 | 29 | 32§ |
| | | Deposit | 145 | 99 | 32§ |
| 1985 | 5 | Parasitism | 49 | 41 | 16 |
| | | Deposit | 49 | 39 | 21§ |
| | 6 | Parasitism | 50 | 50 | 0 |
| | | Deposit | 42 | 50 | 0 |
| | 7 | Parasitism | 46 | 43 | 7 |
| | | Deposit | 44 | 39 | 12 |
| | 8 | Parasitism | 62 | 43 | 31§ |
| | | Deposit | 71 | 46 | 35§ |
| | 9 | Parasitism | 60 | 61 | 0 |
| | | Deposit | 67 | 50 | 27§ |

*Percentage reduction in variable between 0–20 m and 30–50 m.
†Percentage of viable, naturally occurring spruce budworm egg masses parasitized by *T. minutum*.
‡Mean number of parasitized eggs deposited on a 0.0625-$m^2$ card.
§Significant reduction at the $p<0.05$ level for values between 0–20 m and 30–50 m. (Parasitism, $\chi^2$ test for proportions with binomial distributions, Ostle and Mensing [1975]; Deposit, Student's $t$ test.)

df = 39; $p = 0.05$). Similarly, parasitism of budworm egg masses was not affected by cardinal direction. In 1984, parasitism in the north, south, east, and west quadrants was 65, 62, 65, and 64%, respectively ($F = 1.52$; df = 107; $p = 0.01$) while in 1985 parasitism was 45, 53, 53, and 48%, respectively ($F = 2.07$; df = 164; $p = 0.05$).

Wind speed was always below 3 km/h at the time of each release in 1984 and 1985 (Table 4). The deposit data collected by cardinal direction inside each plot indicated that wind had little effect on the distribution of deposit within each plot although it may have had a limited effect on deposit drifted outside the plots.

In 1985, drift at 25 m was higher downwind than upwind (Table 4). Over 90% of this drift material was within 25 m of a plot boundary, with no drift found beyond 100 m. In three instances, the number of eggs on a card 25 m outside the plot was as high as on any card inside that plot. In addition, the mean for each directional quadrant at 25 m was always associated with a high standard error. This suggests that the plot edges received irregular deposit, more likely a result of the application technique than the direction of the wind. The location of the drift with respect to the direction of the application supports this theory. Parasitoids were applied in swaths on the plots running from north to south in 1984 and east to west in 1985. In each year, significant drift at 25 m was observed only along that transect flown by the helicopter. The corners of the plots were marked with helium balloons; thus, it may have been difficult for the applicator to identify the plot edge when closing the release mechanism.

Vertical deposit of parasitized host eggs in the forest canopy was assessed in 1984 (Table 5). In both releases, 50–55% of the parasitized eggs released from the helicopter remained in the canopy. The majority of these eggs (36.9–40.6%) were deposited between the helicopter (10 m) and 3 m above ground (height of the deposit cards) but the remaining 9.8–19.8% were trapped in the lower crown and shrubs. This suggests that about 50% of those eggs released aerially remain in the canopy and, of that material, at least 35% will be found in the upper crown.

Table 4. Direction and distance of parasitized host eggs on deposit cards outside plots receiving aerial releases of *Trichogramma minutum* near Hearst, Ont., in 1984 and 1985

| | | Wind at release site | | | Mean no. parasitized host eggs per card outside plot | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Distance (m) | | | | | |
| Year | Release | Speed (km/h) | Direction | Direction | 25 | 50 | 75 | 100 | 150 | 200 |
| 1984 | First | 0 | — | North | 64.1* | 6.4 | — | 0.9 | 0 | 0 |
| | Second | 2.9 | Southwest | South | 0.7 | 0 | — | 0 | 0 | 0 |
| | | | | East | 0 | 0.3 | — | 0 | 0 | 0 |
| 1985 | First | 1.6 | Northeast | North | 0 | 0 | 0.4 | 0 | — | — |
| | | | | East | 2.0* | 0.2 | 0 | 0 | — | — |
| | | | | West | 17.2 | 1.4 | 0 | 0 | — | — |
| | Second | 2.1 | West | North | 0 | 0 | 0 | 0.4 | — | — |
| | | | | South | 0.2 | 0.2 | 0.4 | 0 | — | — |
| | | | | East | 43.4* | 1.4* | 0.6* | 0 | — | — |
| | | | | West | 2.2* | 1.0 | 0 | 0.2 | — | — |
| Frequency of occurrence (%) | | | | | 90.5 | 7.2 | 1.2 | 1.1 | 0 | 0 |

*Standard errors associated with each mean are equal to the mean unless designated by an asterisk where they are equal to 50% of the value of the mean.

Table 5. Deposit of parasitized host eggs by vertical position in the canopy following aerial release of *Trichogramma minutum* near Hearst, Ont., in 1984

| Height above ground (m) | Plot(s) | No. cards | First release: Mean no. eggs counted ($\times 10^6$ per hectare) | First release: Difference (%) | Second release: Mean no. eggs counted ($\times 10^6$ per hectare) | Second release: Difference (%) |
|---|---|---|---|---|---|---|
| 10* | — | — | 19.8 | | 22.9 | |
| | | | | 36.9 | | 40.6 |
| 3† | 1,2 | 54 | 12.5 | | 13.6 | |
| 3‡ | 1 | 17 | 9.6 | | 16.3 | |
| | | | | 19.8 | | 9.8 |
| 0‡ | 1 | 17 | 7.7 | | 14.7 | |

*Height of helicopter during both releases of parasitized host eggs.
†Height of sticky cards used to monitor deposit extensively.
‡Height of sticky cards used to monitor deposit intensively.

In 1984, deposit on the cards was compared by intensive and extensive sampling (Table 6). As expected, mean deposits of both parasitized and non-parasitized eggs were associated with lower standard errors for intensive samples (SE = 24% of mean) than for extensive samples (SE = 39% of mean). Both sampling systems were highly variable, with any single card providing an estimate with a confidence interval of 77% or less. To provide an estimate with 95% confidence in these stands, 250 deposit cards would be required within an area of 314 $m^2$.

The deposit of host eggs on branches of balsam fir and white spruce was compared in 1983. A mean of $4.0 \pm 4.0$ (SE) host eggs was found on each branch of balsam fir whereas $50.1 \pm 22.2$ (SE) eggs were collected on white spruce. Branches from the two species were similar in length and width; therefore, the 10-fold difference observed in deposit was probably due to the greater density and more cylindrical shape of needles on white spruce than balsam fir. This suggests that species composition may affect estimates of deposit as well as the amount of parasitized material deposited in the upper canopy.

## Discussion and Conclusions

Information on the deposit of parasitized host eggs derived from deposit cards was extremely variable; only extensive sampling to determine where the parasitized material has been deposited is recommended. The cards provided little information on the exact amount of material applied, although they did indicate relative amounts and those areas that had been missed in the application or where drift had occurred outside the plots.

Table 6. Comparison of intensive and extensive sample cards to monitor deposit of host eggs released aerially on forest plots near Hearst, Ont., in 1984

| Release | Height above ground (m) | No. host eggs per card: Parasitized, Intensive ($\bar{X} \pm$ SE)* | Parasitized, Extensive ($\bar{X} \pm$ SE) | Non-parasitized, Intensive ($\bar{X} \pm$ SE) | Non-parasitized, Extensive ($\bar{X} \pm$ SE) |
|---|---|---|---|---|---|
| First | 3 | $60 \pm 19$ | $85 \pm 46$ | $32 \pm 8$ | $39 \pm 17$ |
| | 0 | $48 \pm 13$ | $56 \pm 29$ | $23 \pm 8$ | $30 \pm 11$ |
| Second | 3 | $102 \pm 22$ | $122 \pm 49$ | $34 \pm 6$ | $39 \pm 15$ |
| | 0 | $92 \pm 19$ | $102 \pm 33$ | $31 \pm 5$ | $29 \pm 6$ |
| Mean | | $76 \pm 18$ | $91 \pm 39$ | $30 \pm 7$ | $34 \pm 12$ |

*$N$ = 17 cards in 314 $m^2$ for intensive samples and 21 cards in 7850 $m^2$ for extensive samples. Extensive samples for 0 m above ground included the single card at the centre of the plot and the five cards at 10-m intervals from the centre in each cardinal direction.

Further work, in different stands, using more intensive sampling with either cards or branch samples is needed to characterize the distribution of aerially released *T. minutum*.

Little drift was observed in this study. Where parasitized material was deposited outside the plots, it was associated with poorly defined plot boundaries or inadequate control of the release mechanism at the edges of the plot. The little wind that was present determined the location of drift only to a limited extent. This suggests that aerial releases of *T. minutum* can be conducted with a minimal buffer zone of 100 m between experimental areas or release sites provided that wind speeds at the time of release are less than 3 km/h.

The present study has shown that aerial releases do not affect parasitoid quality, as measured by emergence, sex ratio, longevity, and fecundity. In addition, when released aerially over stands, almost half of the parasitized material is deposited in the canopy. It appears, therefore, that uniform releases of *Trichogramma*, either aerially or from the ground, will provide similar information on parasitism if the predation of parasitized eggs is unaffected by their vertical location in the stand.

The helicopter release system developed by the Ontario Ministry of Natural Resources deposited parasitized eggs on forest stands in effective swaths of ca. 10 m but with a relatively uneven swath pattern. This uneven pattern is characteristic of small aircraft and can be corrected through altered flight patterns as described by Fleming *et al.* (1985). In addition, almost half of the material released fell to the ground. Despite this concentration of parasitoids near the ground, greater parasitism of spruce budworm egg masses was always observed in the upper canopy (also see Smith 1985; Smith *et al.* 1987; and Section 3.5). In contrast with insecticides, *T. minutum* is an active control agent which moves from its point of application to locate specific target pest(s) in forest stands. This makes uniform coverage of foliage, essential with insecticides, less important for aerial releases of *T. minutum*.

Houseweart *et al.* (1984) recommended the use of broadcast, aerial releases of *T. minutum* for control of the spruce budworm. The decision to release *T. minutum* either by broadcast (aerial or ground) or in a series of points on the ground will probably be based upon (1) size, type, and value of the areas to be covered, (2) access to the stand(s), (3) number of releases and density of parasitoids required, and (4) relative costs of manual labour versus a helicopter. In the present study, parasitism of egg masses was lower at the edges of the plots, where fewer parasitized eggs were deposited, than in the centre. Female parasitoids, therefore, dispersed horizontally only a short distance from the point of release. Smith (1988) has shown that *T. minutum* released in these stands will move less than 5 m. Thus, for aerial releases, *T. minutum* should be deposited directly on target areas and if point releases are to be used, they should be spaced at 10 m or less to provide relatively uniform parasitism.

The cost of aerial application was a major component of the total cost of releasing *T. minutum*, due to the small areas treated. With larger treatment areas, the unit area cost of application per unit area would be less. Costs may be reduced further and the pattern of distribution improved if the system were modified through increased mechanization. Gross *et al.* (1981) described an improved system for applying, concurrently, both *Trichogramma* and kairomones for the management of *Heliothis zea* (Boddie). In the USSR, parasitized eggs were effectively dispersed from aircraft in suspensions of water (Sokhta *et al.* 1984; Pas'ko *et al.* 1982). Because of the experimental success shown by *T. minutum* in suppressing pest populations (Section 3.5), continued research in this area of release technology is warranted to make this approach more cost-effective in North America.

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# 3.5 SUPPRESSION OF SPRUCE BUDWORM POPULATIONS BY *TRICHOGRAMMA MINUTUM* RILEY, 1982–1986

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## Abstract

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The ability of the egg parasitoid, *Trichogramma minutum* Riley, to suppress outbreak populations of the spruce budworm, *Choristoneura fumiferana* (Clemens), was studied annually near Hearst, Ont., from 1982 through 1986. Timing of broadcast parasitoid-releases was linked to spruce budworm moth emergence and oviposition. These phenological relationships were predicted from a regression based on larval development at least 2 weeks before expected emergence; this allowed sufficient time to regulate (program) parasitoid emergence during mass-rearing. Emergence of caged spruce budworm adults was used to monitor moth eclosion in the field. Pheromone traps provided daily information on the activity of male moths and helped to synchronize the parasitoid releases with spruce budworm oviposition. Information on parasitoid activity was obtained from sentinel (laboratory-reared) and naturally occurring spruce budworm egg masses. A curvilinear relationship between the rate of parasitoid release and parasitism of sentinel egg masses was developed. Two parasitoid releases, 1 week apart, early in the oviposition period of spruce budworm, significantly increased parasitism of host eggs by 14–83% and reduced larval populations correspondingly from 42 to 82%. Single releases were less effective and increased parasitism by 0.3–52% (single ground release, 1986). Two parasitoid releases, combined with a spring application of *Bacillus thuringiensis* Berliner to larval populations, was the most effective strategy and resulted in 83% egg parasitism and 93% larval reduction. Release rates greater than $12–16 \times 10^6$ ♀♀ *T. minutum* per hectare were not warranted based on impact and costs. The effects of release timing, weather, host density, and parasitoid quality on the future successful use of *T. minutum* are discussed.

## Résumé

La capacité du parasitoïde de l'oeuf, *Trichogramma minutum* Riley, à supprimer les populations éruptives de la tordeuse des bourgeons de l'épinette, *Choristoneura fumiferana* (Clemens), a été étudiée annuellement, près de Hearst, Ont., à partir de l'année 1982 jusqu'à l'année 1986 comprise. Le réglage de relâchements de parasitoïdes à la volée a été lié à l'éclosion et la ponte de la tordeuse. Ces rapports phénologiques ont été prédits d'une régression basée sur le développement larvaire au moins 2 semaines avant l'éclosion attendue; ceci a donné du temps suffisant à régler (à mettre en marche) l'éclosion des adultes de la tordeuse dans les cages a été utilisée pour contrôler l'éclosion du Lépidoptère dans les champs. Les pièges de phéromones ont fourni des renseignements journaliers concernant l'activité des mâles adultes et ont aidé à synchroniser les relâchements de parasitoïdes avec la ponte de la tordeuse. Les renseignements concernant l'activité des parasitoïdes ont été obtenus des masses d'oeufs de référence (élevages du laboratoire) et de celles qui se présentaient en nature. Un rapport curviligne entre la vitesse de relâchement du parasitoïde et le parasitisme des masses d'oeufs de référence a été développé. Deux relâchements de parasitoïdes, séparés de 1 semaine, au début de la ponte de la tordeuse, ont augmenté significativement le parasitisme des oeufs de l'hôte de 14–83% et ont réduit les populations larvaires également de 42–82%. Les relâchements simples ont été moins efficaces et ont augmenté le parasitisme de 0,3–52% (relâchements terrestres simples en 1986). Deux relâchements de parasitoïdes, joints à un traitement printanier des populations larvaires avec *Bacillus thuringiensis* Berliner, se sont montrés le stratège le plus efficace et ont eu comme résultat un parasitisme d'oeufs de 83% et une réduction de larves de 93%. Les taux de relâchement plus extensifs que de $12–16 \times 10^6$ ♀♀ *T. minutum* par hectare n'ont pas été

justifiés d'une base d'effet et de frais. Ont été discutés les effets du relâchement, du réglage, du temps, de la densité de l'hôte et de la qualité du parasitoïde sur l'utilisation réussie à l'avenir de *T. minutum*.

## INTRODUCTION

The effect of egg parasitoids on populations of forest defoliating Lepidoptera is not completely understood (Anderson 1976), although many reports of their interactions have been published. In North American forests, the major families of egg parasitoids are Trichogrammatidae, Scelionidae, and Encyrtidae, and within these families *Trichogramma* Westwood is the most important genus associated with forest insects in northeastern North America (Houseweart *et al.* 1984*b*). Its seasonal distribution in North American forests has been described by Thorpe (1984).

Augmentative releases of native biological control agents against native pests has a long history in forest protection, although most experiments have been too fragmentary and short to be conclusive (Pschorn-Walcher 1977). Parasitoids such as *Trichogramma* generally are considered poor candidates for inundative releases because of their lack of selectivity or synchrony with specific host pests. In North America over the past 10 years, however, a concerted effort has been made to use a native species of egg parasitoid, *T. minutum* Riley, in inundative releases against a specific native pest, the spruce budworm, *Choristoneura fumiferana* (Clemens) (Houseweart *et al.* 1984*a*; Smith *et al.* 1987).

Initial studies showed little evidence that *T. minutum* could cause significant reductions in larval populations of spruce budworm. Houseweart *et al.* (1984*a*) concluded the following: (1) a native strain of *T. minutum* from a local area (Maine) performed better than a strain from California; (2) broadcast and multiple releases from the ground would be better than point releases; (3) closely timed aerial releases might significantly increase rates of parasitism; and (4) very high numbers of parasitoids ($>2\times10^6$ ♀♀ *T. minutum* per hectare) would be necessary to suppress natural populations of spruce budworm.

Recent research on ground releases of *T. minutum* has shown that two applications of ca. $12\times10^6$ ♀♀ per hectare, synchronized with the host's oviposition, could have a significant impact on overwintering larval populations (Smith *et al.* 1987). The extent to which such releases could reduce late larval feeding and protect foliage remains to be determined. As suggested by Van Hamburg and Hassell (1984), the relative success of reducing larval populations with an egg parasitoid will be dependent on the following: (1) the levels of egg parasitism achieved; (2) the subsequent level of early larval losses; and (3) the degree to which these two are density-dependent. Factors affecting the efficacy of *Trichogramma* following inundative release in agricultural crops have been dealt with by Lopez and Morrison (1985), Ridgway *et al.* (1981), Kot (1968, 1979), and Knipling (1977). Essentially no information is available on factors affecting inundative releases in the forest environment.

In 1982, the Ontario Ministry of Natural Resources developed an aerial application system for broadcast release of *T. minutum* on forested areas (Section 3.3). This aerial release system, combined with the large numbers of parasitoids made available through the rearing facility at the Biological Control Laboratory, University of Guelph, Guelph, Ont. (Section 2.0), allowed us to test the efficacy and feasibility of using aerial releases of *T. minutum* to suppress spruce budworm populations in northern Ontario. Specifically, the objectives were to determine the impact of inundative releases of *T. minutum* at varying rates on late larval populations of spruce budworm and to develop the operational use of such a strategy under forest conditions, in terms of aerial distribution, and host–parasitoid prediction and synchronization.

## MATERIALS AND METHODS

A description of the sample plots is provided in Section 3.1. As outlined in Section 3.3, plot sizes were 1.3 ha in 1982, 2.0 ha in 1983, 1.0 ha in 1984 and 1985, and 0.063 ha in 1986. Parasitoids were released aerially in all years except 1986 (see Section 3.3). Because studies in previous years showed that aerial release did not affect parasitoid quality (Section 3.4) and little parasitoid material was available in 1986, *T. minutum* was released from the ground. The design of this ground release simulated aerial release (Section 3.2).

### Source of Parasitoid Material

*Trichogramma minutum* collected from Plummer Township (49°N, 86°W) in 1981 were reared in the production facility of the Biological Control Laboratory at Guelph, Ont., and used for all releases. New parasitoid material from Rogers Township (50°N, 87°W) was added to the stock colony held at Guelph in 1983 and in 1984, from recollected release material in Rogers Township. The parasitoid stock was maintained on factitious hosts during 1982: on the Mediterranean flour moth, *Ephestia kuehniella* (Zeller), at Guelph, Ont. (see Section 2.0), and on the Angoumois grain moth, *Sitotroga cerealella* (Olivier), at Biogenesis, Mathis, TX, USA. Rincon Vitova (Oak View, CA, USA) provided all parasitoids released during 1983 and one-third of those released in 1984. Parasitoids reared outside Canada were the original native strain (Plummer) collected from northern Ontario during 1981 and sent to facilities in the United States for mass-rearing. Parasitoids reared at the Biological Control Laboratory in Guelph, Ont., using the Angoumois grain moth as the host egg, comprised the remaining two-thirds of those released in 1984, as well as all parasitoids released in 1985 and 1986.

In 1982 and 1983, a sample (ca. 1000 parasitized eggs) was retained from each shipment prior to release to assess parasitoid quality, i.e. emergence, percentage of females, longevity, and fecundity. In 1984, 1985, and 1986, parasitoids were obtained after release by placing 26-cm funnels, with collection vials attached, in relatively open areas of the plots prior to release. Immediately following release, parasitized eggs were collected from these funnels and daily emergence, longevity, and fecundity of emergent female parasitoids determined. Because only female parasitoids are active in destroying host eggs, release rates were expressed as the number of female parasitoids per hectare per release. Longevity and fecundity were determined for 20 females from each rearing source. Each female was isolated in a 1.9-mL vial and provided with two fresh spruce budworm egg masses. Fecundity was determined from the number of host eggs parasitized and the number of progeny produced.

The parasitoids were shipped in bulk at about 10°C in Angoumois grain moth eggs and had been programmed during rearing so that >50% would emerge in 48 h (unless refrigerated) with the remainder emerging over 4–5 days. Parasitized material was held in cardboard containers in the dark at 5–10°C until required.

### Timing of Parasitoid Releases

It was essential that the expected time of adult spruce budworm emergence and parasitoid release be accurately forecast because the Biological Control Laboratory could produce very large numbers of parasitoids for only a limited time. Thus, the releases had to coincide closely with the appearance of spruce budworm adults and oviposition; specifically, the first days and peak period of oviposition. For releases in early July, initial timing estimates were required by early March, to be followed by up-dating throughout the developmental period of the spruce budworm.

Due to the shortage of parasitized material, a single non-replicated release at peak budworm oviposition (maximum daily number of egg masses) was made in both 1982 and 1983. In the remaining years, based on studies by Smith *et al.* (1987), two releases were made early in the ovipositional curve (between the start and peak of oviposition) for each

year. The number of parasitoids available determined the number of replicated plots that could be included.

## Spruce Budworm Development

Development of spruce budworm was monitored by directly sampling egg masses, larvae, and pupae on branches (1982, 1985, and 1986) and catches of male moths in pheromone traps (1982–1986). Branch sampling (as described for each year of study) was conducted to monitor larval, pupal, and ovipositional stages and provide data that could be used to predict moth emergence. Sample trees, from which the branches were taken, were randomly selected within each plot. These trees were divided between balsam fir and white spruce according to the relative stocking density of each species. The surface area of each branch was measured (Sanders 1980) and counts made of all spruce budworm stages (larvae, pupae, and egg masses) as well as pupal emergence (%) and egg hatch (%) (McGugan 1954). Parasitized egg masses and pupae collected from these branches were held in a field laboratory to estimate parasitoid emergence and the onset and duration of adult budworm eclosion in the field. In 1982, 1985, and 1986, branch samples were collected every 3 days from mid-June until early August to assess pupal development and moth emergence.

In all years, larval development was used to predict moth emergence, 2–4 weeks before expected flight. One collection of branch samples (as described above) was specifically made in mid-June of each year, prior to pupation of spruce budworm. Ten years of data from Kapuskasing, Ont. (48.5°N, 82.5°W), suggested that the first adult moths would be collected in black-light traps at the earliest on 30 June and at the latest on 15 July (unpublished data). A process-oriented phenology and ovipositional model developed for spruce budworm by Régnière (1982, 1983) was used to refine this expected date of moth emergence taking into account site-specific information in each year. The model predicted first moth emergence from the percentage of fifth-instar or older larvae on a given sampling date by means of a regression equation. The equation followed the form $Y = a + bX$, where $Y$ is the predicted date of first adult occurrence and $X$ is the date on which the $i$th (0.05–0.9) proportion or greater of fifth-instar larvae were recorded. The regression coefficients, $a$ and $b$, changed systematically as the proportion of fifth-instar larvae changed: "$a$" inversely with an increasing proportion and "$b$" directly. The regression coefficients themselves were regressed against the proportion of fifth-instar and older larvae and the resulting functions substituted back into the original function. This produced the equation used for updating the predicted date of first adult emergence:

$$Y = 72.474 + 0.693X + 0.23608XZ - 47.554Z \quad [1]$$

where $Y$ = the predicted date of first adult emergence; $X$ = the larval sampling date (Julian date); and $Z$ = the proportion of fifth-instar or older larvae on the sampling date (within the range 0.05–0.9).

## Adult Moth Emergence

Pheromone traps baited with polyvinyl chloride cylinders (4 by 10 m) containing 0.03% of a 95% (*E*)-11-tetradecenal : 5% (*Z*)-11-tetradecenal mixture were used to monitor the emergence and activity of adult male spruce budworms (Sanders 1981). Both sticky traps (Pherocon 1CP®) and bucket-type Uni-traps (Sanders 1986) were used according to availability (see description for each year of study). The traps were spaced at least 40 m apart in each plot, 2–3 m above ground level. They remained in the field from mid-June until early August and were examined daily before 1000 hours EST for the number of male moths captured. When sticky traps were used, the bottom liners were changed daily to prevent saturation.

In 1985, the hourly activity of male moths in this area was monitored with an activity meter using the same type of pheromone lure at the centre of the trap. The meter contained a circular sticky card (75 cm diameter), divided into 24 equal parts, which was replaced daily. Each of the 24 sticky parts was exposed for only 1 h each day. The trap was run for 27 consecutive days (12 July to 5 August) during peak spruce budworm moth activity.

### Assessment of Release Effect

The impact of *Trichogramma* releases on populations of spruce budworm was assessed using four criteria: in the year of release, (1) parasitism of sentinel egg masses and (2) parasitism of natural egg masses; and in the year following release, (3) density of spruce budworm larvae and (4) parasitism of natural egg masses. Data were compared between control and release plots using ANOVA with Duncan's new multiple range test (where applicable) and Ostle and Mensing's (1975) test for proportions with binomial distributions.

**Sentinel egg masses**: Sentinel egg masses were used to determine levels of natural parasitism on the non-release control plots as well as the extent and duration of parasitoid activity (temporal parasitism) on release plots. Sentinel eggs consisted of fresh spruce budworm egg masses laid on twigs of balsam fir by females reared in the laboratory on both artificial diet (young larvae) and natural foliage (older larvae) (Smith 1985). The egg clusters were shipped twice weekly from the laboratory in Sault Ste. Marie, Ont., to Hearst, Ont., in ice-cooled styrofoam boxes.

Sentinel egg masses were placed at varying heights in the crown of each sample tree by means of a pulley system (Smith 1985). The sample trees were balsam fir or white spruce, located in random clumps of two to four trees, at least 5 m away from the plot edge. Tree height, crown width, and condition were assessed yearly for each tree (see Section 3.1; Table 1). Because spruce budworm eggs are acceptable for parasitism by *T. minutum* only at a relatively young physiological age, before the head capsule of the embryo appears (Houseweart *et al.* 1982), the egg masses were changed every 3 days from mid-June until early August each year. This ensured a continuous supply of fresh susceptible egg masses for parasitism in the field. Following exposure of these egg masses to *Trichogramma* in the field, rates of parasitism were determined by holding them at the field laboratory in individual containers, usually size 00 gelatin capsules. Freshly laid egg masses of spruce budworm are bright green, but they turn gray as the head capsule of the developing larvae within the chorion becomes pigmented. Eggs parasitized and killed by *Trichogramma* turn shiny black within a few days at room temperature, thus making diagnosis of parasitism by *Trichogramma* relatively simple. For those egg masses parasitized, the number of eggs per egg mass that were either parasitized, missing, partially eaten, or not hatched were recorded as well as the date and sex ratio of emergents.

The number of egg masses parasitized out of the total number of viable egg masses placed in each plot constituted a measure of percentage egg mass parasitism for the 3-day period on that plot. Percentage egg parasitism was calculated by multiplying the proportion of egg masses parasitized by the proportion of viable eggs parasitized within each egg mass.

**Natural egg masses**: Parasitism of natural egg masses laid in the current year was determined by sampling annually at the end of the ovipositional period. Whole branches of balsam fir and white spruce were cut from the upper mid-crown of sample trees in each plot, one branch per tree (Dorais and Kettela 1982). Branch length and width were measured yearly, to calculate surface area (Sanders 1980), and in 1985 and 1986 each branch was weighed. Needles containing egg masses were removed from each branch and parasitism was calculated as for sentinel egg masses.

**Carryover effect**: The survival of *T. minutum* following inundative release was evaluated in those years following release. Throughout the period of oviposition by spruce

Table 1. Daily mean temperatures, sunshine, and wind speed, and total rainfall for the week following releases of *Trichogramma minutum* in Rogers Township from 1982 to 1986 inclusive

| Year | Release date | Temperature (°C) | | Sunshine (h) | Wind speed (km/h) | Total rainfall (mm) |
|---|---|---|---|---|---|---|
| | | Maximum | Minimum | | | |
| 1982 | 14 July | 23.0 | 10.0 | 5.7 | 9.5 | 19.6 |
| 1983 | 14 July | 28.8 | 13.7 | 11.3 | 8.3 | 19.7 |
| 1984 | 10 July | 24.6 | 12.1 | 7.3 | 4.9 | 25.5 |
| | 16 July | 24.7 | 12.2 | 9.0 | 5.7 | 25.5 |
| 1985 | 9 July | 20.9 | 9.1 | 6.7 | 7.4 | 32.8 |
| | 19 July | 21.0 | 11.0 | 4.9 | 11.8 | 79.5 |
| 1986 | 5 July | 24.1 | 10.2 | 10.5 | 3.4 | 0 |
| | 12 July | 27.7 | 12.6 | 7.7 | 2.9 | 0 |

budworm, sentinel egg masses were placed on those plots that had received the highest release rate of *T. minutum* in the previous year. The egg masses were changed every 3 days, to ensure a constant supply of fresh, acceptable host eggs. Carryover effect was measured as the percentage of these egg masses that were parasitized.

**Larval populations**: To assess the impact of egg mass parasitism on larval populations of spruce budworm, 45-cm branch samples were collected in the spring of the year following each release. Samples were taken from balsam fir and white spruce in both the release plots and control areas. The branches were collected in mid-June when the majority of budworm were in the fourth to sixth larval instar. The number of larvae per branch was counted to compare larval populations on the release and control plots. The expected level of defoliation was then derived for each population according to the relationship outlined by Dorais and Kettela (1982). A measure of population reduction was calculated using a modified Abbott's formula to account for natural mortality in the control plots (Fleming and Retnakaran 1985).

## Annual Releases: Methodology and Results

### 1982 Releases

*Trichogramma* were released on only one 1.3-ha plot (plot 1) in 1982 because of the low number of parasitoids available. A control plot, similar to the release plot, was established ca. 3 km away (plot C1). Weather conditions during the sampling period in 1982 were relatively cool, overcast, windy, and wet (Table 1; see also Section 3.1).

To assess development of spruce budworm and estimate initial oviposition for timing the releases, whole branch samples of balsam fir and white spruce were taken from sample trees in 1982. One branch was taken from the lower, mid- and upper crown of each sample tree and the number and stage of each larva determined (McGugan 1954; Sanders 1980).

Larval development in 1982 was predicted by selecting three branches from each of 90 balsam fir and 30 white spruce on 9 June. This sampling indicated that the development of budworm larvae was further ahead on balsam fir (83% were fifth instar or older) than on white spruce (69% were fifth instar or older) (Table 2). Based on the regression equation, moth emergence was predicted for 25 June. Beginning on 7 July and on every 3rd day until 28 July, three branches from each of 180 balsam fir and 90 white spruce trees were taken to monitor spruce budworm phenology. Populations of pupae were highest on the first sample date, 7 July, and all adult moths had emerged by 28 July (Fig. 1). The daily activity of male moths was monitored with 30 sticky pheromone traps (Pherocon 1CP®), 15 in each of the release and control plots. The first male moth was captured on 4 July (Table 2), 9 days after the predicted date; relatively cool weather during June (daily mean = 12.7°C) delayed pupation and adult emergence. Moth flight peaked on 26 July, at about the same time as total adult emergence was reported (Fig. 2). Natural egg masses

Table 2. Phenological sampling of spruce budworm (SBW) on plots receiving inundative releases of *Trichogramma minutum* near Hearst, Ont., from 1982 to 1986 inclusive

| Year | Sample date | Host tree species | No. SBW collected | SBW larval development (%) | | | Predicted date of emergence* | First empty pupal case | First ♂ moth in pheromone trap |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 5th instar | 5th–6th instars | 6th instar | | | |
| 1982 | 9 June | Balsam | 921 | 73 | — | 10 | 25 June | 10 July | 4 July |
| | | Spruce | 912 | 42 | — | 27 | 25 June | 10 July | |
| 1983 | 16 June | Balsam | 50 | 80 | 20 | 0 | 6 July | — | 2 July |
| | | Spruce | 50 | 95 | 5 | 0 | 6 July | — | |
| 1984 | 27 June | Balsam | 396 | 7 | 82 | 11 | 12 July | — | 9 July |
| | | Spruce | 408 | 7 | 76 | 17 | 12 July | — | |
| 1985 | 29 June | Balsam | 169 | 4 | 94 | 2 | 11 July | 8 July | 7 July |
| | | Spruce | 445 | 1 | 91 | 8 | 11 July | 8 July | |
| 1986 | 20 June | Balsam | 154 | 4 | 95 | 1 | 3 July | 29 June | 30 June |
| | | Spruce | 136 | 5 | 93 | 2 | 3 July | 2 July | |

*Predicted from the equation $Y = 72.474 + 0.693X + 0.23608XZ - 47.554Z$ where $Y$ = the predicted date of first moth emergence, $X$ = the larval sampling date (Julian), and $Z$ = the proportion of fifth-instar or older larvae on the sampling date (within the range 0.05–0.9).

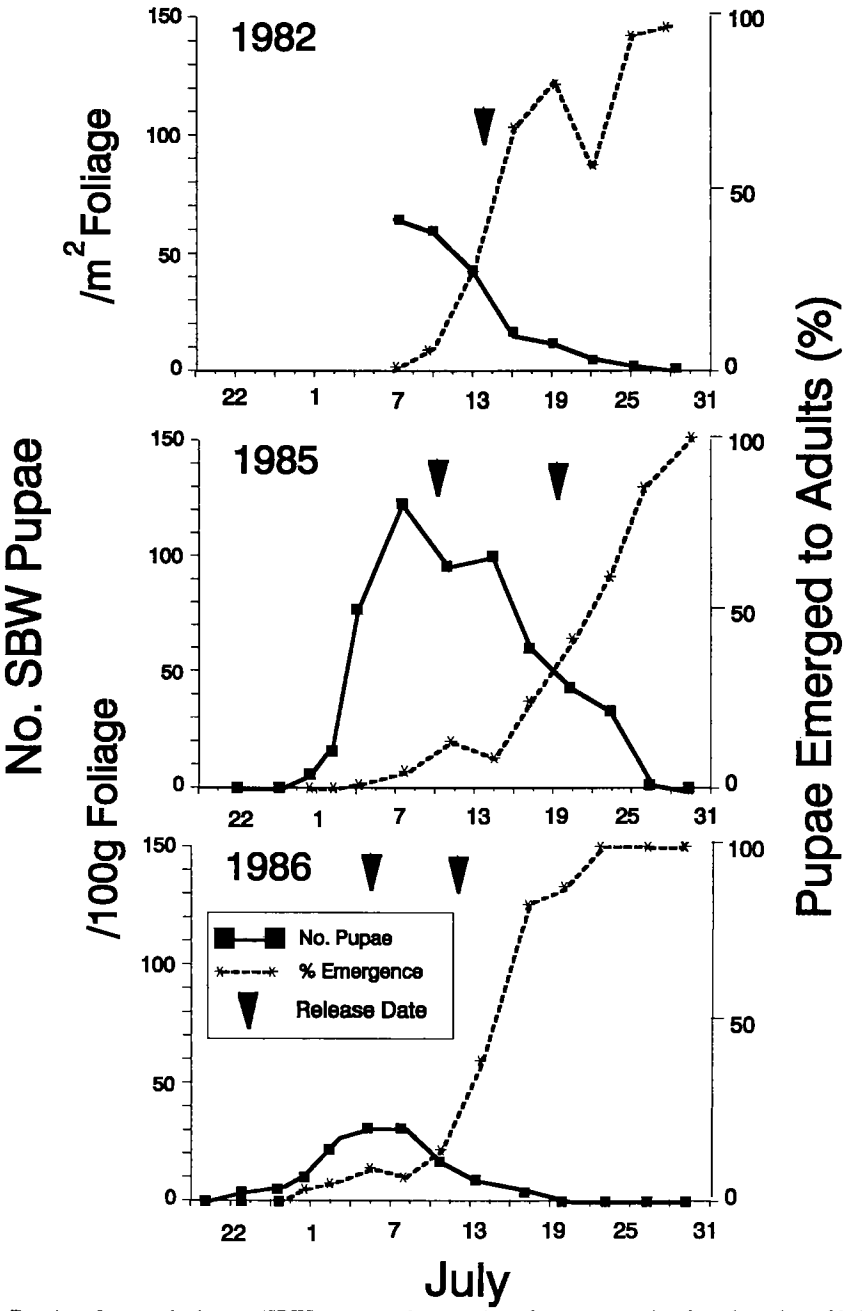


FIG. 1. Density of spruce budworm (SBW) pupae and proportion of pupae emerging from branches of balsam fir and white spruce near Hearst, Ont., in 1982, 1985, and 1986. Samples were not collected in 1983 and 1984.

were first laid on 16 July (Fig. 3) with peak oviposition occurring 9 days after egg-laying started on 25 July. Eggs began to hatch on 16 July and further releases of *Trichogramma* were not warranted.

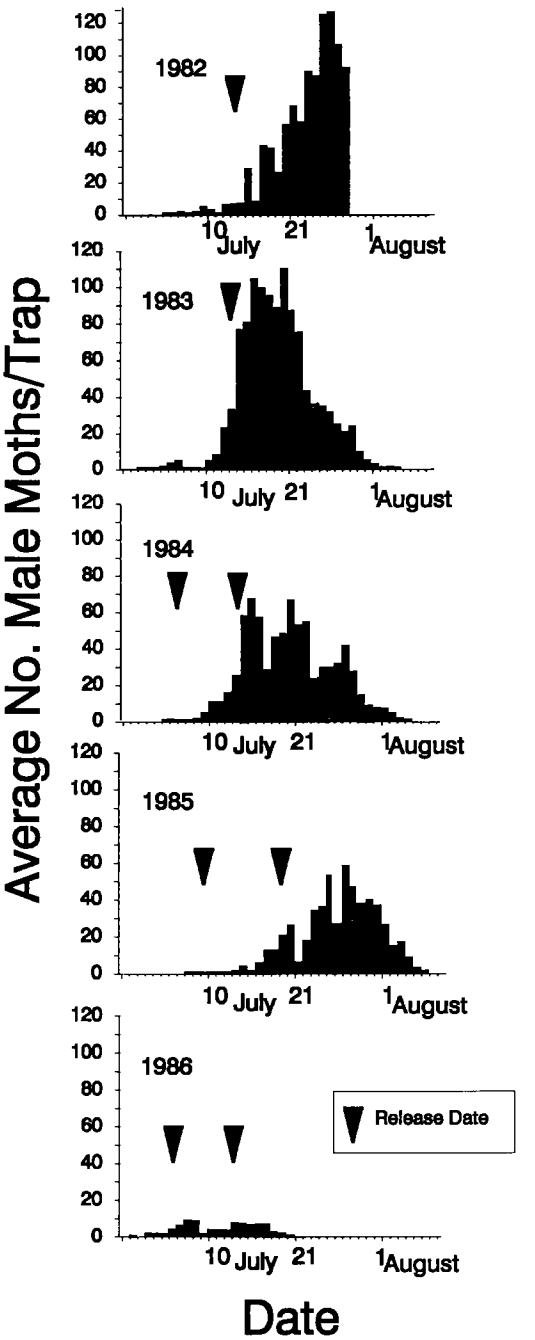


FIG. 2. Mean number of male spruce budworm (SBW) moths collected in pheromone traps near Hearst, Ont., from 1982 to 1986.

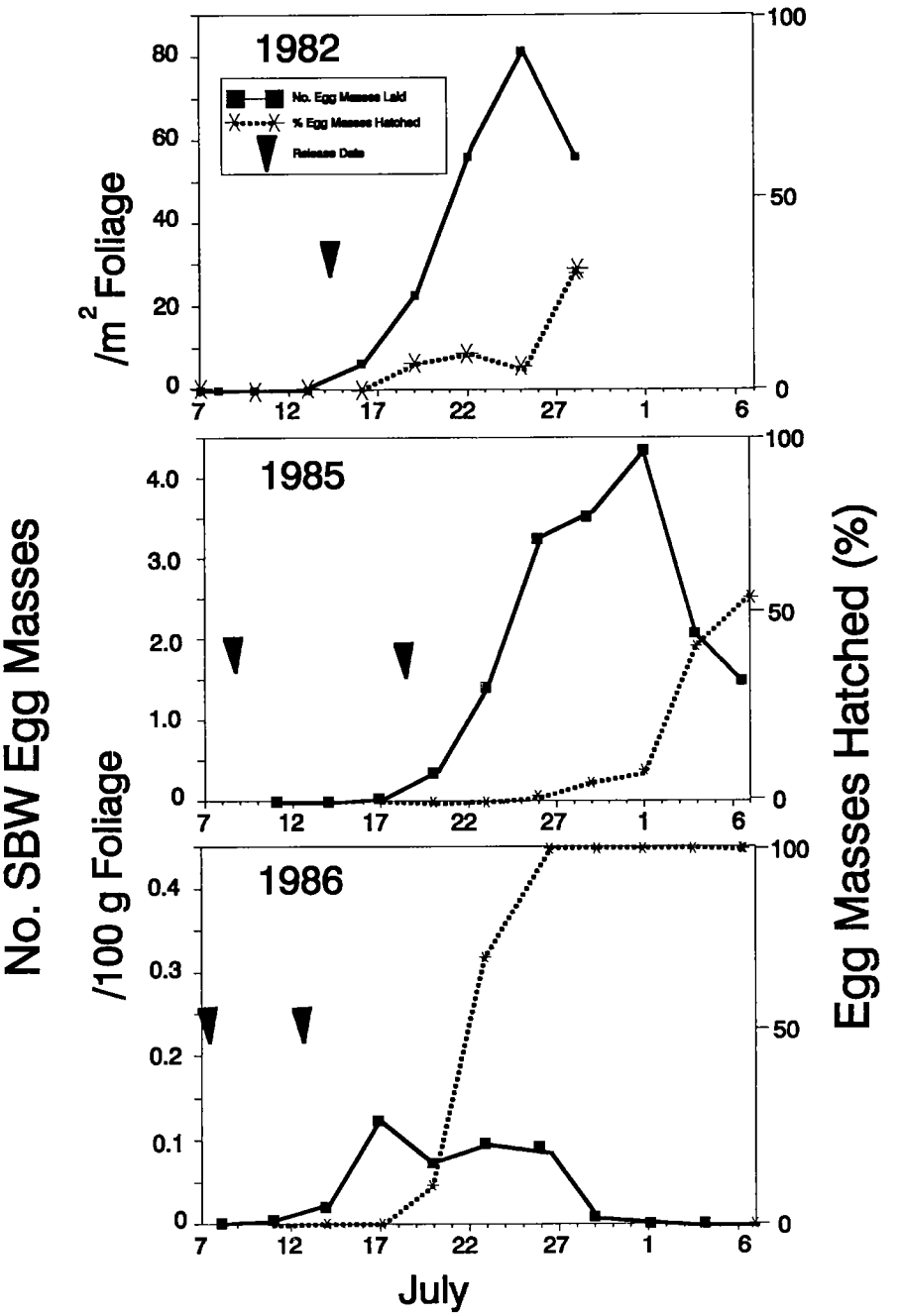


FIG. 3. Density of spruce budworm (SBW) egg masses and proportion of egg masses hatching on branches of balsam fir and white spruce near Hearst, Ont., in 1982, 1985, and 1986. Samples were not collected in 1983 and 1984.

Table 3. Emergence, longevity, and fecundity of *Trichogramma minutum* released near Hearst, Ont., from 1982 to 1986 inclusive

| | | | Emergence | | | | | Fecundity | |
|---|---|---|---|---|---|---|---|---|---|
| Year | Source | Release date | % | Duration (days) | % in first 3 days | Sex ratio (% ♀♀) | Female longevity (days) | No. SBW* eggs parasitized per ♀ | No. progeny per parasitized SBW egg |
| 1982† | BCL‡ and Biogenesis | 14 July | 98 | 8 | 53 | 54 | 3.0 | 33.1‖ | — |
| 1983§ | Rincon Vitova | 14 July | 80 | — | — | 51 | 1.6‖ | 4.0‖ | — |
| 1984§ | BCL | 10 July | 74 | 4 | 94 | 58 | 2.5 | 5.9 | 1.2 |
| | | 16 July | 85 | 6 | 85 | 58 | 2.3 | 5.8 | 1.0 |
| | Rincon Vitova | 10 July | 63 | — | — | 51 | 2.0 | 0.6 | 0.2 |
| | | 16 July | 71 | — | — | 67 | 1.6 | 4.1 | 0.7 |
| 1985§ | BCL | 9 July | 91 | 10 | 45 | 62 | 1.3 | 4.5 | 2.2 |
| | | 19 July | 53 | 16 | 30 | 60 | 1.5 | 4.3 | 1.7 |
| 1986§ | BCL | 5 July | 75 | 10 | 75 | 46 | 1.8 | 5.4 | 0.6 |
| | | 12 July | 87 | 8 | 79 | 52 | 1.6 | 5.3 | 0.6 |

*SBW = spruce budworm.
†Measurements based on a pre-release sample.
‡BCL = Biological Control Laboratory, University of Guelph, Guelph, Ont.
§Measurements based on a post-release sample.
‖Values based on 15 females from ground releases made on the same date using the same source of material sampled before release where emergence was 82% and the sex ratio was 53% females (Smith 1985).

Parasitoids were released on 14 July in three overlapping swaths across the release plot (1.3 ha) at a rate of $0.6 \times 10^6$ ♀♀ *T. minutum* per hectare. Deposit cards (see Section 3.4) were placed in groups of five at each of three stations along the length of the release plot; five cards were located ca. 20 m on each side of the release plot (20 cards total) to monitor horizontal drift of parasitized eggs. Within the release plot, on average, $0.28 \times 10^6$ parasitized eggs per card (625 $cm^2$) were deposited and no parasitized eggs were found on cards outside the plot. Pre-release sampling showed that the parasitoids had high percentage emergence, and normal sex ratio, longevity, and fecundity (Table 3). Parasitoids emerged over 8 days in the middle of the ovipositional period.

Sentinel egg masses were placed on 15 sample trees within the release area, two egg masses at the upper and mid-crown on each tree for each sampling date. Similarly, on each sample date, two egg masses on each of 75 sample trees were used to monitor parasitism in the control plot. Extremely low parasitism (1.0% of viable eggs) was observed on the release plot in 1982 (Table 4), probably due to the low application rate and poor weather conditions following release (Table 1). Maximum parasitism of sentinel egg masses in this plot was 2.3% ($N$ = 43 egg masses) on 20 July compared with a maximum of 0.5% ($N$ = 5413 egg masses) in the control plot. A second peak in parasitism (ca. 1.0%) of sentinel egg masses was observed on 6 August, suggesting the appearance of a second generation of *Trichogramma*.

Parasitism of egg masses laid naturally was assessed on 16 August by collecting 45-cm branch tips of balsam fir and spruce: 36 branches on the release plot and 43 branches on the control plot (divided among balsam fir, white spruce, and black spruce). All egg masses found on these branches were recorded. Of 684 egg masses collected from the release plot, only 5.1% were parasitized compared with 2.6% on the control plot ($N$ = 648 egg masses). In terms of viable eggs, only 0.3% were parasitized on the control plot versus 1.0% on the release plot (Table 4). Because of the low level of parasitism, larval density and, thus, population reduction were not assessed the following spring.

**1983 Releases**

For the 1983 release, a 2.0-ha plot was established directly south of plot 1, because parasitism was low in plot 1 during 1982. The control plot used in 1982 was retained in 1983 (plot C1). During the releases in 1983, the weather was warmer and sunnier than in any other study year (Table 1).

As in 1982, larval development was assessed by taking a pre-release sample of ten 45-cm branch tips, equally divided between balsam fir and white spruce, on 16 June (Table 2). In contrast with 1982, only one 45-cm branch tip was collected from the upper mid-crown of each tree. From the proportion of fifth-instar or older larvae in the sample, the predicted date of emergence was 6 July. Pupal collections were not made in 1983. Nine sticky pheromone traps (as in 1982) placed in the field between 26 June and 4 August were used to assess moth emergence. The first male moth was captured on 2 July (Fig. 2). Moth densities peaked on 20 July with no activity observed after 3 August.

A single release of $8.2 \times 10^6$ ♀♀ *T. minutum* per hectare was applied just prior to peak moth activity on 14 July (Table 3). The date and rate of application were selected to coincide with ground releases made on other plots in the same area (Smith *et al.* 1987). Twenty deposit cards placed randomly in a 0.5-ha area at the centre of the plot indicated that the parasitoids were deposited relatively uniformly ($X = 37.0 \pm 11.6$ parasitized eggs per card). No parasitized eggs were observed on cards placed outside the release plot. Based on the number of parasitized eggs released, an emergence of 80%, and a sex ratio of 1:1 (Table 3), ca. $8.8 \times 10^6$ ♀♀ per hectare were applied in 1983. The progeny of these parasitoids started to emerge in the field laboratory 12 days later, on 26 July, with emergence continuing for 25 days.

Table 4. Parasitism of natural spruce budworm (SBW) eggs and reductions in mature larval populations on plots receiving various rates of *Trichogramma minutum* near Hearst, Ont., from 1982 to 1985 inclusive

| Year | Treatment | Plot | Parasite source | Parasitoid release: Date | Parasitoid release: Total ($10^6$ ♀♀ per hectare)* | No. SBW eggs per 45-cm branch† | Viable eggs parasitized (%) | No. SBW larvae per 45-cm branch | Population reduction (%)‡ | Expected defoliation (%)§ |
|---|---|---|---|---|---|---|---|---|---|---|
| 1982 | Control | C1 | — | — | — | 240.5a¶ | 0.3 | — | — | — |
| | Release | 1 | BCL and Biogenesis | 14 July | 0.6 (1) | 228.6a | 1.0 | — | — | — |
| 1983‖ | Control | C1 | — | — | — | 247.7a | 0.1 | 22.4a¶ | — | 80 |
| | Release | 1 | Rincon Vitova | 14 July | 8.8 (1) | 168.2b | 15.9 | 7.6b | 50 | 45 |
| 1984 | Control | C2 | — | — | — | 78.0a | 1.1 | 34.9a | — | 94 |
| | Release | 2 | BCL | 10 and 16 July | 22.7 (2) | 75.4a | 79.5 | 6.1b | 82 | 41 |
| | | 3** | BCL | 10 and 16 July | 22.7 (2) | 80.5a | 83.4 | 2.4c | 93 | 20 |
| | | 4 | Rincon Vitova | 10 and 16 July | 30.9 (2) | 12.0b | 14.1 | — | — | — |
| 1985 | Control | C3and C4 | — | — | — | 100.7a | 0.2 | 11.9a | — | 60 |
| | Release | 5 and 6 | BCL | 9 and 19 July | 4.2 (2) | 94.9a | 15.6 | 12.0a | 0 | 60 |
| | | 7 and 8 | BCL | 9 and 19 July | 8.4 (2) | 119.7a | 22.2 | 8.2b | 42 | 47 |
| | | 9 | BCL | 9 and 19 July | 16.9 (2) | 87.6a | 30.4 | 5.7b | 45 | 40 |

*Total number of female *T. minutum* released; number of releases is indicated in parentheses.

†Based on unpublished data from the site, includes conversion for a mean of 14.6 eggs in each SBW egg mass (Smith 1985).

‡Reduction $= 1 - \left(\frac{\text{post-release density in treatment}}{\text{pre-release density in treatment}}\right) \times \left(\frac{\text{pre-release density in control}}{\text{post-release density in control}}\right) \times 100$ (from Fleming and Retnakaran 1985).

§Predicted level of defoliation based on 130 ± 23 buds per branch (field data) and the relationship between the number of fourth-instar spruce budworm larvae per bud per 45-cm branch described by Dorais and Kettela (1982).

‖Data provided by the Forest Insect and Disease Survey, Forestry Canada, Sault Ste. Marie, Ont.

¶Means followed by the same letter within each year and column are not significantly different at the $p \leq 0.05$ level (Duncan's new multiple range test).

**B.t.* used in this plot against third and fourth larval instars in the spring following release of parasitoids.

Sentinel egg masses were not used to assess parasitism in 1983, but instead, parasitism was examined in egg masses laid naturally. A sample of twenty-five 45-cm branch tips of balsam fir and white spruce was taken on 10–11 August in each of the release and control plots. All egg masses on a minimum of 10 mid-crown branches from each area were examined and those egg masses parasitized were categorized into four groups by the proportion of eggs parasitized: 0–25%, 26–50%, 51–75%, and 76–100%. On balsam fir, 28% of 204 egg masses were parasitized on the release plot compared with 0% of 347 egg masses on the control plot (significantly different; $\chi^2 = 16.17$; df = 1; $p = 0.05$). Similarly, on white spruce, 22% of 228 egg masses were parasitized on the release plot versus 0.6% of 336 egg masses on the control plot (significantly different; $\chi^2 = 9.04$; df = 1; $p = 0.05$). Of those egg masses parasitized, over 65% had greater than 50% of the eggs within each egg mass parasitized providing an estimated total level of viable egg parasitism of 15.9% (Table 4).

Spring larval populations in the release and control plots were assessed on 10 June 1984; >90% of the larvae were in the fifth or sixth instars. Twenty-five 45-cm branch tips of balsam fir and white spruce were taken from each plot. Significantly fewer larvae were found on the release plot ($X$ = 7.6 larvae per branch) compared with the control plot ($X$ = 22.4 larvae per branch) (Table 4). Based on the previous level of egg masses per 45-cm branch tip in these two plots (control = 247.7 and release = 168.2), this represented a 50% reduction in spruce budworm populations. Projected defoliation for the release plot was 45% versus 80% for the control plot. Suppression measures using *Trichogramma*, therefore, reduced populations of spruce budworm below the currently accepted economic threshold of 50% defoliation (Dorais and Kettela 1982).

**1984 Releases**

During 1984, three plots (plots 2, 3, and 4), each 1.0 ha in size, were established at least 3 km from the 1982–1983 study sites. A control plot (plot C2), ca. 0.25 ha, was located 2 km from the nearest release plot. Plots 2 and C2 had never been treated with insecticides for suppression of spruce budworm. Plot 3 was located in a 80-ha block sprayed at 30 BIU per hectare with *Bacillus thuringiensis* (*B.t.*) in the spring of 1984, and plot 4 was established in a 100-ha block which had been sprayed each spring with aminocarb (Matacil®) from 1981 to 1984. Intermediate weather conditions were recorded in 1984 (Table 1).

To assess larval development, a pre-release sample of 20 branches was taken, as in 1983. One 45-cm branch tip was taken from the upper mid-crown of 10 balsam fir and 10 white spruce in each release plot on 27 June. Due to the larvicide treatments in early June, development and population levels of spruce budworm varied among the plots. On plots 2, 3, and 4, there were 19, 12, and 2 per 1000 $cm^2$ foliage, respectively, with corresponding developmental indices (Dorais and Kettela 1982) of 5.9, 5.4, and 5.1, respectively. As in 1983, no pupal sample was taken. Using the proportion of each larval instar in the regression model, the predicted date of first moth emergence was 12 July (Table 2).

The first male moth was collected on 8 July in Uni-trap pheromone traps (Sanders 1986), three of which were placed in each plot (plots C2, 2, 3, and 4). The traps were in the field from 1 July to 5 August with maximum trap catches on 15 and 20 July (Fig. 2). Moth flight was completed by 4 August after three distinct peaks in activity.

Studies using ground releases of *T. minutum* against the spruce budworm indicated that two releases, 1 week apart, would significantly improve parasitism (Smith *et al.* 1987). In 1984, therefore, with more parasitized material available, two aerial releases were made at the beginning of the ovipositional period of the spruce budworm. The material was deposited relatively uniformly over each plot with essentially no drift beyond 25 m (see fig. 2 and table 4 in Section 3.4).

Release rates for *T. minutum* reared at Rincon Vitova were relatively high because the parasitoids were not cooled properly during shipment and emergence had begun by the time of release. The quality of these parasitoids was comparatively poor, with lower emergence, longevity, and fecundity than *T. minutum* produced at the Biological Control Laboratory, Guelph, Ont. (Table 3). On 10 and 16 July, 10.2 and $12.5 \times 10^6$ ♀♀ per hectare, respectively, reared at Guelph, were applied on each of plot 2 and plot 3, and 7.9 and $23.0 \times 10^6$ ♀♀ per hectare, respectively, reared at Rincon Vitova, were released on plot 4 (Table 4). In the field laboratory, the parasitoids did not emerge simultaneously, but over a 4- to 6-day period. Parasitoids reared at Guelph, Ont., parasitized 5.9 spruce budworm eggs per female and produced 1.2 progeny per parasitized budworm egg (Table 3). These progeny produced offspring with a sex ratio of 1.6 ♀♀:1.0 ♂♂, emerging over a period of 25 days beginning on 22 July. A continuous supply of *T. minutum*, therefore, was present in the field throughout the ovipositional period of spruce budworm.

To monitor temporal parasitism, three sentinel budworm egg masses per tree were placed in the upper, mid-, and lower canopy on 15 trees in each plot (divided between balsam fir and white spruce). Sentinel parasitism was monitored from 29 June to 1 August by replacing these egg masses every 3 days. Parasitism was very low in the control plot (1.1%), but parasitism was observed in all three release plots (Fig. 4). Over a period of ca. 15 days, the rate of egg mass parasitism increased sharply to a maximum of 50% on plot 4 (Fig. 4*a*) and 89% (SE = 4%) on plots 2 and 3 (Fig. 4*b*). Parasitism of sentinel egg masses by the next generation of *Trichogramma* appeared at the beginning of August (57% ± 21%), 20–23 days after the first release.

On plot 4, parasitism continued to decline after the first release, becoming indistinguishable from the control plot by 24 July. Overall parasitism was significantly lower on plot 4 than plots 2 and 3 (Fig. 4; Table 4). This low parasitism may have resulted from several factors: (1) plot 4 had relatively low larval populations of spruce budworm due to the aminocarb treatment in the spring: (2) development of spruce budworm on plot 4 was comparatively delayed and protracted due to insecticide treatment; and (3) the emergence of parasitoids reared at Rincon Vitova (released on plot 4 only) was lower because of improper shipping.

Parasitism in plots 2 and 3 (parasitoids reared at the Biological Control Laboratory, University of Guelph) was similar (i.e. small standard errors) except for the last sample date, 1 August (Fig. 4). This variability may be associated with the difference in development of spruce budworm between plots: larval development in plot 3 was behind that in plot 2 because of a *B.t.* application in the spring. This, in turn, delayed moth emergence (plot 2: <10 ♂ moths per trap on 10 July versus plot 3: <10 ♂ moths per trap on 15 July) and, thus, oviposition. Parasitism observed on the last sampling date was attributable to the progeny of *T. minutum* released on 10 July. This second generation parasitism was more apparent in plot 2 ($X$ = 92.7% egg masses parasitized) than plot 3 ($X$ = 21.4% egg masses parasitized), likely because of the more rapid development of spruce budworm and, thus, greater abundance of eggs at the time of the first release on plot 2 than on plot 3.

Parasitism of naturally laid egg masses of spruce budworm was assessed by collecting 36 whole branches per plot, 18 each of balsam fir and white spruce, on 5 August. Each branch was sequentially sampled until five egg masses were found (Dorais and Kettela 1982). The egg masses were classified as parasitized if at least one egg per egg mass turned black. In all three release plots, parasitism of viable eggs was significantly higher than in the control plot (Table 4). In general, parasitism of viable egg masses and eggs was higher on balsam fir (83–88%) than white spruce (74–84% eggs parasitized) although this was only significant on plot 3 (Table 5). Despite a parasitism rate of over 80% on plots 2 and 3 in 1984, there was no carryover of this parasitism to 1985; <1% of the 20 sentinel egg masses placed in plot C2 every 3 days during spruce budworm oviposition in 1985 were parasitized.

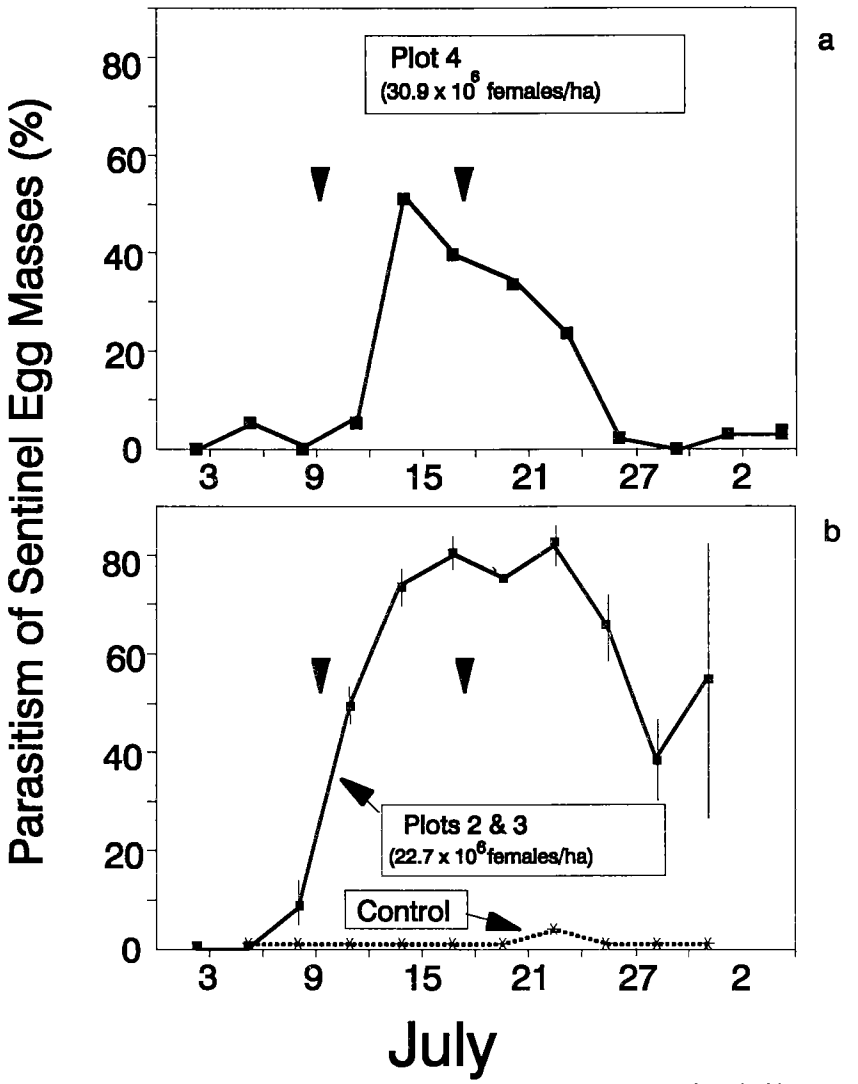


FIG. 4. Parasitism of sentinel spruce budworm (SBW) egg masses attached to balsam fir and white spruce trees on plots receiving *Trichogramma minutum* near Hearst, Ont., in 1984. Vertical lines at each sample point represent standard error for two plots.

Larval populations in the control plot and on those two plots receiving releases of *T. minutum* reared in Guelph, Ont., were measured in 1985. Thirty-six 45-cm branch tips, 18 each of balsam fir and white spruce, were taken from the upper mid-crown of trees in each plot on 18 June 1985. Plot 2 was not treated in the spring of 1985 and plot 3 was within a ca. 100-ha area which received an aerial application of *B.t.* prior to sampling, on ca. 10 June. Development of spruce budworm in plot 3, where both *T. minutum* and *B.t.* were applied, was delayed over that in plot 2 (*Trichogramma* release alone) or in the control plot (plot C2); developmental indices of spruce budworm for plots 2, 3, and C2 were 4.2, 3.7, and 4.2, respectively.

Both treatment strategies had significantly fewer larval spruce budworm than the control plot ($F = 22.51$; df = 107; $p = 0.05$) although plot 3 (*B.t.* and *Trichogramma*) had a

Table 5. Parasitism of viable spruce budworm egg masses and eggs laid naturally on balsam fir and white spruce on plots receiving releases of *Trichogramma minutum* near Hearst, Ont., in 1984 and 1985

| Year | Plot | No. × $10^6$ ♀♀ *T. minutum* released | Percentage viable* egg masses parasitized | | Percentage viable* eggs parasitized | |
|---|---|---|---|---|---|---|
| | | | Balsam fir (*n*) | White spruce (*n*) | Balsam fir | White spruce |
| 1984 | 2 | 22.7 | 95 (84) | 91 (72) | 83 | 84 |
| | 3 | 22.7 | 93 (79)† | 81 (56) | 88† | 74 |
| | 4 | 30.9 | 40 (70) | 33 (55) | 15 | 12 |
| | C2 | — | 3 (70) | 0 (66) | 0.2 | 0 |
| 1985 | 5 | 4.2 | 51 (123)† | 39 (151) | 21† | 13 |
| | 6 | 4.2 | 49 (101) | 38 (95) | 15 | 11 |
| | 7 | 8.4 | 34 (133)† | 46 (213) | 9† | 18 |
| | 8 | 8.4 | 65 (104) | 64 (138) | 41 | 42 |
| | 9 | 16.9 | 73 (80)† | 50 (136) | 40† | 24 |
| | C3 | — | 4 (229) | 3 (264) | 0.1 | 0.2 |

*Viable represents those egg masses and eggs that remained after missing, partially eaten, or infertile eggs were subtracted.
†Values between host trees that are significantly different at the $P < 0.05$ level ($\chi^2 = 6.41$; test for proportions with binomial distribution, Ostle and Mensing [1975]).

significantly greater reduction in larval populations than did plot 2 (*Trichogramma* alone) (93% vs. 82%) ($t = 7.83$; df = 71; $p = 0.05$) (Table 4). A reduction of over 90% in populations of spruce budworm was observed on both plots 2 and 3, based on the density of budworm eggs per 45-cm branch tip and subsequent larval populations. Projected defoliation for the plots was 20 and 41% versus 94% on the control plot. Both suppression measures, therefore, reduced populations of spruce budworm below the currently accepted economic threshold of 50% defoliation, with the combined strategy being most effective.

## 1985 Releases

In 1985, five release plots (1.0 ha each; plots 5–9) and two control plots (1.0 ha each, plots C3 and C4) were established. A minimum of 200 m separated all plots to reduce the chance of drift during application and dispersal by parasitoids. Releases were made when wind speeds were less than 5 km/h in the plots. The weather following each release in 1985 was the coolest and wettest of all study years with generally overcast and windy conditions (Table 1).

As in 1982, spruce budworm phenology was followed from 19 June to 6 August by taking one 45-cm branch tip every 3 days, from the upper mid-crown of each sample tree: 16 balsam fir and eight white spruce. On 29 June, the majority of spruce budworms were at the fifth larval instar and the regression equation predicted first moth emergence on 11 July (Table 2). Pupae appeared on the branch samples of both host trees on 30 June (Fig. 1). Pupal development peaked on 8 July and was completed by 29 July. The first empty pupal case was found on 8 July. Pupae maintained in the field laboratory began emerging on 10 July. Emergence in the laboratory was similar to that observed in the field, suggesting that this method was reliable for assessing emergence. The cool temperatures in 1985 delayed development of spruce budworm, including egg-laying (Fig. 3). The first egg masses were not laid until 17 July with densities peaking between 29 July and 1 August. At the time of the last sample on 6 August, 50.6% of the egg masses had hatched.

Adult moth activity was monitored with 16 pheromone traps: eight sticky traps (as in 1982 and 1983) and eight Uni-trap buckets (as in 1984). One of each type of trap was placed within a sample plot, each at least 40 m apart. The first male moth was collected in a sticky trap on 7 July, 4 days before the predicted date (Table 2). Adult activity peaked between 25 and 27 July and was completed by 7 August (Fig. 2) with populations lower

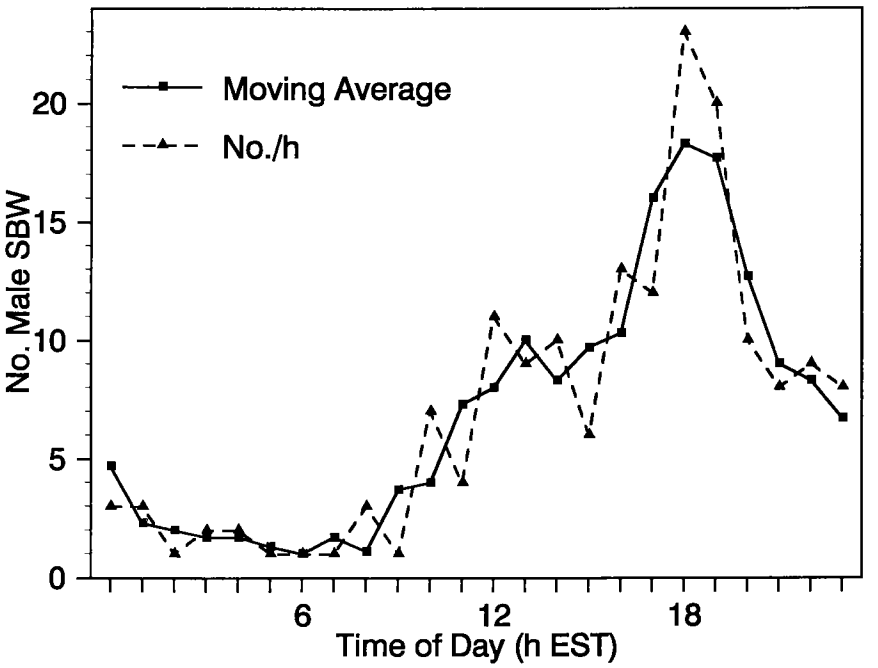


FIG. 5. Hourly catch of male spruce budworm moths in an activity meter with a pheromone lure in a forest stand near Hearst, Ont., during 1985. The moving average is a mean of three consecutive hourly catches.

than in previous years. The hourly activity of male moths was monitored between 14 July and 3 August using an activity meter with a pheromone lure identical to those in the daily traps (Fig. 5). Moth activity began after 0900 hours EST and peaked between 1700 and 1900 hours. Moths were relatively inactive between midnight and mid-morning suggesting that the best time to change pheromone traps for an accurate estimate of daily moth activity was before 0900 hours.

Parasitoids reared at the Biological Control Laboratory in Guelph were released from aircraft at three different rates in 1985 (Table 4). The two lowest rates, using double releases, were replicated twice. As in 1984, deposit of parasitized eggs on these plots was monitored (see fig. 2 and table 4 in Section 3.4). Deposit was uniform on each plot, averaging 334, 440, and 864 parasitized host eggs per card on plots 5 and 6, plots 7 and 8, and plot 9, respectively. The original design was to release 3, 6, and $12 \times 10^6$ ♀♀ per hectare per release on plots 5 and 6, plots 7 and 8, and plot 9, respectively; however, because of the cool wet weather, particularly following the release on 9 July, emergence of parasitoids in the field was reduced and the actual rates of release were 2.7, 5.4, and $10.9 \times 10^6$ ♀♀ per hectare on 9 July and 1.5, 3.0, and $6.0 \times 10^6$ ♀♀ per hectare, respectively, on 19 July.

The weather extended and delayed parasitoid emergence (Table 3). Emergence of *T. minutum* occurred over 10–16 days with only 30–45% of the sample emerging within the first 3 days of each release. This was considerably fewer than in other years; however, those parasitoids that did emerge had normal sex ratio, longevity, and fecundity. The progeny from the releases began emerging on 23 July, peaked 28 and 31 July, and continued until 20 August. These females produced, on average, 1.9 progeny per parasitized host egg with a sex ratio of 2 ♀♀:1 ♂. A continual, relatively low supply of *T. minutum* was thus provided in the field during oviposition of spruce budworm.

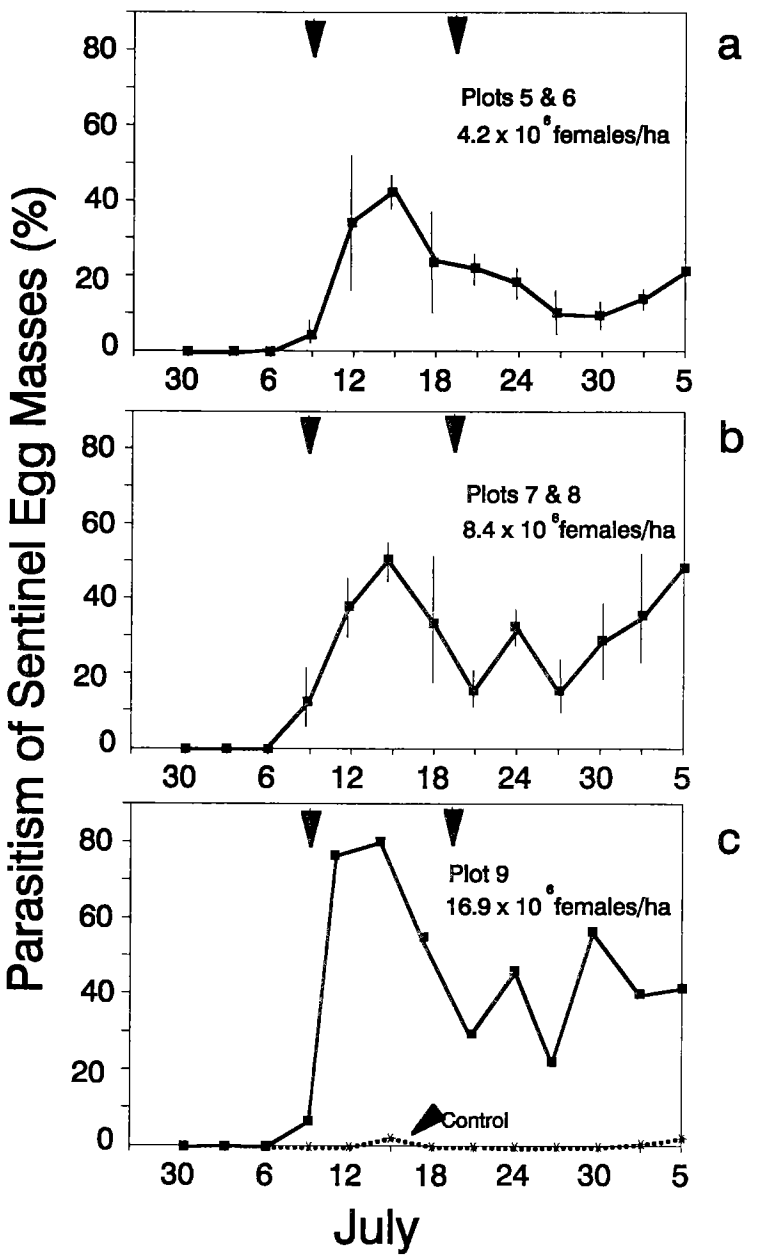


FIG. 6. Parasitism of sentinel egg masses attached to balsam fir and white spruce trees on plots receiving releases of *Trichogramma minutum* near Hearst, Ont., in 1985. Vertical lines at each sample point represent standard errors for two plots.

Fifteen trees were selected on each plot to monitor temporal parasitism. Three sentinel egg masses were attached at upper, mid-, and lower crown level to each tree between 1 July and 4 August. Immediately following the first release, parasitoid activity was evident on all the release plots (Fig. 6). The level of parasitism increased with the release rate, and parasitoid activity persisted throughout the sampling period. Over a period of 8

days, the rate of parasitism increased sharply to a maximum of 42, 58, and 80% on plots receiving 4.2, 8.4, and $16.9 \times 10^6$ ♀♀ per hectare, respectively. Parasitism by the next generation of *Trichogramma* appeared around 24 July on the plots receiving the highest two rates and continued at 20–40% throughout the remainder of the season.

Parasitism was lower following the second release than the first, due to the extension of parasitoid emergence and the low numbers emerging (Table 3). Trends in parasitism were similar on replicated plots. On the two control plots, very low parasitoid activity (<4%) was observed. The carryover effect to the following year was assessed by placing 10 sentinel egg masses on five sample trees every 3 days (two egg masses per tree) in plot 9 between 25 June and 4 August 1986. Essentially no carryover was observed on this plot (receiving the highest release rate in 1985); <3.1% of the sentinel egg masses were parasitized.

Parasitism of egg masses laid naturally was assessed in 1985 by taking thirty-six 45-cm branch tips from each release plot and one control plot on 8 August. At the time of sampling, at least 50% of the egg masses had hatched (Fig. 3) and the remainder, based on mean daily temperatures in the field, would have been unacceptable to parasitism by *T. minutum* (Houseweart *et al.* 1982). The average density was seven egg masses per branch. Parasitism of viable spruce budworm eggs in every release plot was significantly higher than in the control plots ($F = 14.67$; df = 1511; $p = 0.05$) (Table 4). In two of five release plots, parasitism of natural viable eggs was higher on balsam fir (21 and 40%) than on white spruce (13 and 24%) regardless of the release rate (Table 5). On plot 7, however, significantly more parasitism was evident on white spruce than on balsam fir.

On 11 June 1986, larval populations were assessed by taking thirty 45-cm branch tips, 15 each of balsam fir and white spruce, in each release plot and one control plot. Significant reductions in larval populations were found in plots receiving the two higher release rates, 8.4 and $16.9 \times 10^6$ ♀♀ per hectare, and no difference was apparent at the lowest rate (Table 4). Based on egg densities per 45-cm branch tip, the releases resulted in 87.4–93.5% reductions in populations. This was equivalent to 0, 42, and 45% control of budworm populations following releases of 4.2, 8.4, and $16.9 \times 10^6$ ♀♀ per hectare, respectively. Projected defoliation for the three release plots was 40, 47, and 60% (at decreasing release rates) versus 60% for the control plot. Although the natural populations of spruce budworm were generally low (plots C3 and C4), the two highest rates of *Trichogramma* release reduced the population of spruce budworm slightly below the economic threshold of 50% defoliation.

### 1986 Releases

The annual studies to 1986 showed that parasitism by *T. minutum* was not carried over beyond the year of release. Similar conclusions were reached by Smith *et al.* (1987) for ground releases of *T. minutum*. Due to this lack of carryover, the limited supply of parasitoid material, and the fact that the aerial releases did not reduce parasitoid quality (Section 3.4), three plots from 1985 (plots 5, 6, and 7) were used for ground release of *T. minutum* in 1986. Four smaller plots (25 by 25 m) were established within each plot (each separated by 50 m). Seven of the 12 plots were used to examine the effect of releasing various densities of *T. minutum* from the ground and the remaining five were used as non-release control plots. Weather conditions were average with no rainfall reported following the releases and relatively low wind speeds (Table 1).

From 16 June to 3 August, the phenology of spruce budworm was monitored by taking 45-cm branch tips from the upper mid-crown (of 22 balsam fir and 29 white spruce) within the 12 plots. Development of spruce budworm was accelerated by warm temperatures in the spring of 1986. On 20 June, over 95% of the larvae were fifth instar or older and the regression equation predicted moth emergence on 3 July (Table 2). Pupae appeared on 23 June with the first empty pupal case present 6–9 days later. Pupal populations peaked

on 5 and 8 July and by 23 July, all pupae had emerged (Fig. 1). Similar emergence was observed in the field laboratory although it was extended more toward the end of July.

Twelve sticky pheromone traps (Pherocon 1CP®), one in each plot, were used to monitor adult activity from 28 June to 3 August. The first male moth was collected on 30 June, 3 days before the predicted date (Table 2). Throughout 1986, very low numbers of males were trapped with the mean never exceeding seven moths per trap (Fig. 2). Two distinct peaks were observed, one on 8 July and one on 14 July. Because of these low populations, the number of egg masses present in 1986 was extremely low with the first egg mass found on 11 July (Fig. 3; note change in vertical scale). All fresh egg masses had been laid by 23 July with the majority hatched by 30 July.

Parasitoids were released by ground application (see Section 3.2) on each plot at rates of 2.1, 4.3, 8.6, and $12.9 \times 10^6$ ♀♀ per hectare on 5 July and 2.7, 5.3, 10.6, and $15.9 \times 10^6$ ♀♀ per hectare on 12 July, for a total of 4.8, 9.6, 19.2, and $28.8 \times 10^6$ ♀♀ per hectare. The lowest two release rates were replicated twice and the highest two were applied only on single plots. One plot received a single rate of $12.9 \times 10^6$ ♀♀ per hectare on 5 July. Parasitoids emerged over a period of 8–10 days with 75% having emerged 3 days after release (Table 3). The parasitoids had average longevity and fecundity but produced a low number of progeny per parasitized host egg. The progeny of these parasitoids began emerging in the field laboratory on 18 July. Emergence of this second generation peaked on 23 and 25 July, thereby providing a continual supply of *T. minutum* in the field during oviposition.

Two sentinel egg masses were attached in the upper and mid-crown of 96 sample trees, eight trees in each plot (seven release plots and five control plots) between 22 June and 4 August. Parasitism of these egg masses on the control plots was always less than 2.1%. In the release areas, parasitism increased slowly after the first release (over 11 days) possibly due to the low number of egg masses on these plots (Fig. 7). Maximum parasitism was observed after the second release, even on the plot receiving the single release at the highest rate ($12.9 \times 10^6$ ♀♀ per hectare) (Fig. 7*d*). This delay was not evident in parasitoid emergence (Table 3); therefore, it probably reflected an actual delay in parasitoid oviposition. On these plots, maximum egg mass parasitism of 52.8, 66.7, 53.3, 84.2, and 85.7% was achieved with total releases of 4.8, 9.6, 12.9, 19.2, and $28.8 \times 10^6$ ♀♀ per hectare, respectively. Parasitism by the second generation of *Trichogramma* peaked on 24–25 July, 10–14 days after the initial peak in sentinel parasitism.

The curvilinear relationship between maximum parasitism of sentinel egg masses and the rate of application for releases made in 1985 and 1986 is shown in Figure 8. This relationship was similar for both years and suggests that increasing rates of application above $12–16 \times 10^6$ ♀♀ per hectare will not significantly improve parasitism of sentinel egg masses. It also shows that the maximum parasitism of acceptable spruce budworm egg masses that can be expected with *Trichogramma* releases alone is 80–85%.

Parasitism of natural egg masses was assessed by collecting one whole branch from the upper mid-crown of 100 sample trees on each of the seven release plots and one control plot. Collections were made on 6 August when all egg masses had hatched (Fig. 3). Only 14 egg masses, laid in 1986, could be found. This provided an inadequate sample size for further analysis of parasitism: less than two egg masses per plot. The low populations of spruce budworm at the end of the field season in 1986 precluded the collection of larval samples on these plots the following spring to determine the level of population reduction.

## Discussion and Conclusions

We consider that timing of inundative releases with *Trichogramma* relative to oviposition by spruce budworm is one of the most important components affecting efficacy of the parasitoids in our study. A similar conclusion was reached by King *et al.* (1985) using releases of *T. pretiosum* against *Heliothis* spp. In our study, both the first release in

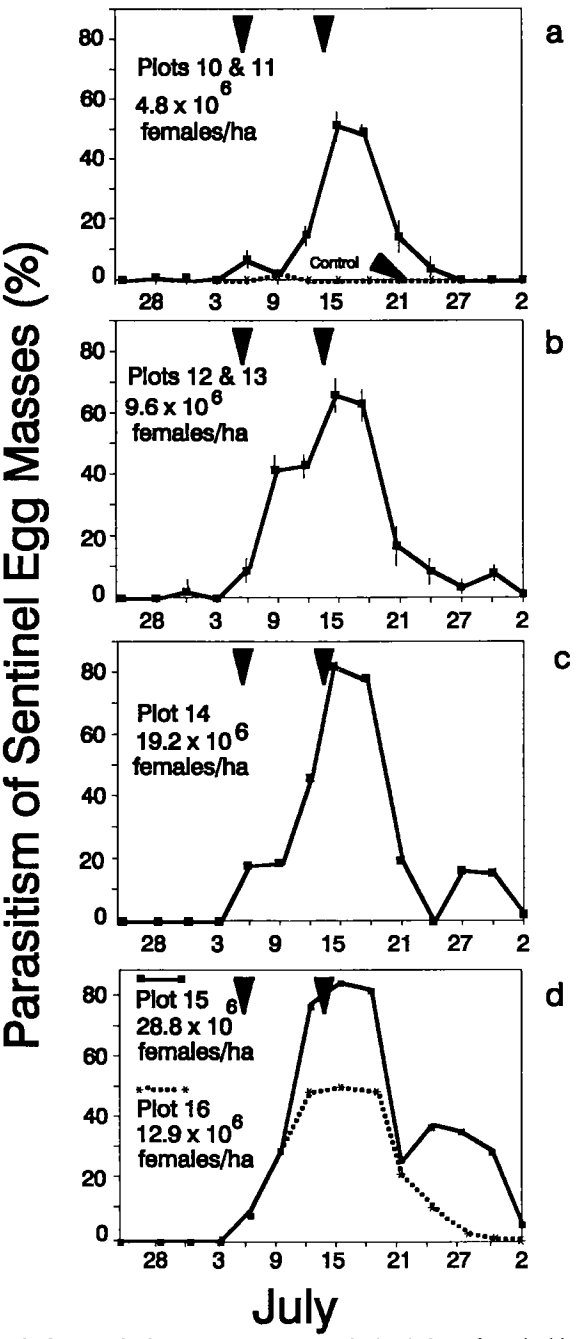


FIG. 7. Parasitism of sentinel spruce budworm egg masses attached to balsam fir and white spruce trees on plots receiving different rates of *Trichogramma minutum* near Hearst, Ont., in 1986. Vertical lines at each sample point represent standard errors for two plots.

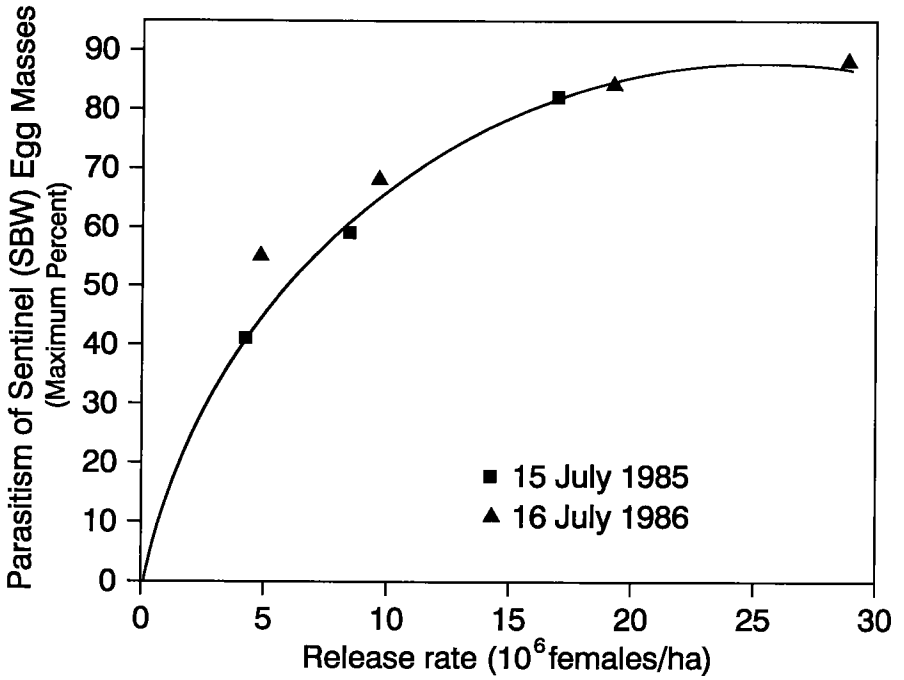


FIG. 8. The effect of release rate of *Trichogramma minutum* on the maximum parasitism level achieved on sentinel egg masses of spruce budworm (SBW) attached to balsam fir and white spruce trees near Hearst, Ont., in 1985 and 1986.

1985 and those releases made using parasitoids produced at Rincon Vitova in 1984 occurred before oviposition by spruce budworm. Although *T. minutum* were active in the field, they had little effect on parasitism of eggs laid naturally. For a univoltine species such as spruce budworm, eggs are present in the field at only one time each year. These eggs are laid over a period of about 3 weeks and are only acceptable to parasitism by *T. minutum* for 3–6 days following oviposition (Houseweart *et al.* 1982). From the present study and work by Smith *et al.* (1987), we suggest two parasitoid releases between the beginning and peak of egg-laying will provide optimal results. Obviously, reliable information on the temporal pattern of budworm oviposition is required for such a strategy.

The regression model, incorporating the proportion of spruce budworm at fifth larval instar or older to estimate moth emergence, generally provided a reliable method for predicting the onset of egg-laying. Larval samples taken at least 2 weeks before the expected onset of oviposition predicted moth activity in the pheromone traps within 3–4 days (except in 1982 where exceptionally cool weather during June delayed oviposition by 9 days). In all cases, this predictive regression allowed the rearing facility sufficient time to produce large numbers of parasitoids programmed for a specific date of emergence. As suggested by Lawrence *et al.* (1985) and Witz *et al.* (1985), pheromone traps examined before 0900 hours were good indicators of moth activity and, thus, oviposition. Although some variability will always be present in estimating moth emergence with the regression model because of the uncertainty of weather conditions, further studies quantifying the relationship between larval development and moth emergence could improve the model's accuracy.

Two releases of *T. minutum* during the oviposition period of spruce budworm provided better control than a single release. Unless the timing of a single release is exact and emergence of these parasitoids is extended over at least 10 days, insufficient *T. minutum*

will be continuously present to ensure high parasitism of freshly laid host eggs. In 1983, the single application of $14.1 \times 10^6$ ♀ ♀ per hectare was well synchronized with oviposition and yet parasitism of naturally laid eggs was only 15.9%. In 1984, however, following two releases at ca. $11.3 \times 10^6$ ♀ ♀ per hectare per release, mean parasitism of naturally laid eggs was 81.5%. Because female fecundity for *Trichogramma* is greatest during the first days of emergence (Houseweart *et al.* 1982; Smith and Hubbes 1985), two releases, 1 week apart, will be most effective if the parasitoids are programmed to emerge over a 4- to 7-day period. This will provide highly fecund females each day of oviposition by spruce budworm. As suggested by Lawrence *et al.* (1985) and confirmed by Smith *et al.* (1987), using three releases, 1 week apart, would be redundant and costly because the progeny of parasitoids from the first release would be active in the field at the same time as parasitoids from a third release. In each year of our study, the second generation of parasitoids began emerging before *T. minutum* from the second release had stopped parasitizing host eggs. When emergence from the release was not extended beyond 7 days, two releases maintained high levels of parasitism (>80%) throughout the ovipositional period of the spruce budworm.

Abundance of host eggs in the release area is thought to influence the level of parasitism following inundative release (Ridgway *et al.* 1981). Alternate hosts used by polyphagous *T. minutum* may be present on forest stands prior to spruce budworm and used as reservoirs (Houseweart *et al.* 1984*a*). In the young spruce–fir stands of our study, this effect was never observed; there was no detectable parasitism by *T. minutum* on the control plots after oviposition by spruce budworm. Any sustained parasitism in the ovipositional period of spruce budworm, therefore, was due solely to development of parasitoids on populations of spruce budworm. As observed by Houseweart *et al.* (1984*a*), we also found no evidence that densities of host eggs affected parasitism by *T. minutum*. The supply of naturally laid spruce budworm eggs declined significantly during 1984 and 1986 (see Section 3.1; Table 2) for Rogers Township, yet the level of maximum parasitism observed on sentinel egg masses following the release of about $12 \times 10^6$ ♀ ♀ per hectare each year remained similar. An independent functional response for *T. minutum* on egg masses of spruce budworm has been observed by Smith *et al.* (1986) and Smith and Hubbes (1985). Although further information is required on the functional response of *T. minutum* to spruce budworm under varying stand conditions, the abundance of host eggs appears to be less important to the success of inundative releases than their temporal distribution.

Weather had a significant impact on parasitoid activity. Cool wet conditions during the second release in 1985 reduced and extended parasitoid emergence, thus lowering the effectiveness of the releases. Smith *et al.* (1986) observed a similar effect with ground releases in 1982. It is suggested, then, that *T. minutum* produced in eggs of *S. cerealella* not be released during cool rainy conditions. When such conditions exist, it may be best to hold the parasitoids at cool temperatures until weather conditions improve. Before such a strategy can be implemented, however, information is needed as to the effect of such holding on later parasitoid emergence, longevity, and fecundity. In this way, losses in parasitoid quality due to holding can be weighed against expected losses in the field under rainy conditions.

In all years of our study, parasitoid quality was identified as an important factor in the success of the releases. Lopez and Morrison (1985) came to a similar conclusion when examining parasitoid activity on eggs of *Heliothis* species. The components previously discussed were influential because of their effect on parasitoid emergence, longevity, ability to locate hosts, and fecundity. The production, programming, and shipment systems for *T. minutum* must ensure a parasitoid with high percentage emergence, proportion of females, longevity, and fecundity. If any of these three steps is mishandled, then parasitoid quality will be lowered and the proportion of runts (brachypterous adults) increased. Such an effect was observed in 1984 when *T. minutum* were shipped from California without

proper cooling; parasitoid emergence began before release, resulting in extremely low parasitism. Future studies must ensure that these steps are carefully controlled to achieve consistently high results following release.

Of prime consideration in parasitoid quality is the continual rearing of parasitoid colonies for release. After 1983, the parasitoid material in the present study was not infused with new strains of *T. minutum* from the field but only with other strains reared equally as long in the laboratory. With ca. one generation completed every 2 weeks, the *T. minutum* strain released in 1986 had been reared for more than 100 generations in the laboratory. Although emergence and sex ratio remained relatively unchanged over this period, studies in the field showed that longevity and the number of progeny surviving per parasitized host egg declined. In contrast, maximum parasitism of sentinel egg masses at similar application rates remained the same over this period. The effect of continual mass production on efficacy has been seldom reported in the literature; if the use of *Trichogramma* is to become feasible, future research must address this potential problem.

The number of parasitoids required for release will depend naturally on many factors, but principally cost and the desired level of spruce budworm reduction (i.e. stand protection). Application rates over $12 \times 10^6$ ♀♀ per hectare per release do not justify the increased cost because the increase in parasitism is only marginal. In our study, the reduction in larval populations was proportional to the rate of parasitoid release; a rate of $4.2 \times 10^6$ ♀♀ per hectare did not lower budworm populations and, therefore, was too low to provide stand protection. Higher rates of $8.4–22.7 \times 10^6$ ♀♀ per hectare, however, did substantially reduce larval populations (42–82%) below the level that results in 50% defoliation and at which suppression measures are implemented. This is the first time that aerial releases of *T. minutum* have been shown to increase parasitism of spruce budworm eggs significantly and cause a corresponding reduction in larval populations. Unfortunately, the natural collapse of budworm populations in the study area during 1986 prevented us from obtaining another year of data to confirm this relationship between egg parasitism and larval reduction.

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# 4.0 SUMMARY AND PROSPECTS FOR THE FUTURE

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## SUMMARY

We report the results of a 5-year project to develop the technology of mass-production and inundative release of *Trichogramma minutum* Riley against the eastern spruce budworm, *Choristoneura fumiferana* (Clemens).

Techniques were developed to produce $30 \times 10^6$ *T. minutum* per week on the factitious host *Sitotroga cerealella* (Olivier). To facilitate shipment and aerial release in spruce budworm-infested forests, *Trichogramma* development was programmed so that parasitoids emerged from their hosts within 24 h of exposure after release.

Test sites for the release of *T. minutum* were selected on the basis of suitably high densities of spruce budworm, projected stability of the pest population, uniform size of host trees (especially white spruce), low levels of parasitism by natural populations of *T. minutum*, and ease of access. An area of Rogers Township, north of Hearst, Ont., fulfilled these requirements.

Aerial release of parasitized host eggs was successfully achieved with a Bell® 47 helicopter, equipped with a Brohm aerial seeder, modified to deliver parasitized eggs over an effective swath width of ca. 10 m. During the 5 years of releases, application rates varied between $0.6 \times 10^6$ and $25.0 \times 10^6$ ♀♀ parasitoids per hectare. Sticky cards were used to measure distribution of the parasitized host eggs within the stand. More than 50% of the released material reached ground level. Horizontal distribution within the plots was uneven, with more parasitoids deposited in the centre than at the edges. Horizontal drift was usually less than 25 m from the flight path, and never exceeded 100 m; maximum wind speed during the applications was >5 km/h. Characteristics of the parasitoids, measured as percentage emergence, sex ratio, longevity, and fecundity, were not affected by aerial release.

Two systems for ground release of *Trichogramma* were developed for use on small plots: a gridded point source system, using parasitized host eggs attached to cards; and a hand-held leafblower for distributing eggs in bulk. Neither system affected the quality of the parasitoids and both resulted in rates of parasitism of sentinel egg masses similar to those obtained with aerial release. Parasitism was higher in the mid- to upper crown than in the lower crown of the trees.

The ability of varying rates of *T. minutum* releases to suppress an epidemic population of spruce budworm was assessed from 1982 to 1986. Parasitism was monitored using both naturally occurring and sentinel (laboratory-reared) egg masses of spruce budworm. The best results were achieved in 1984, with two releases, 6–7 days apart, at a total release rate of $22.7 \times 10^6$ ♀♀ parasitoids per hectare. These releases produced egg parasitism levels of over 80% on both balsam fir and spruce and subsequent reductions in larval populations of over 80%. Lower release rates and single releases were not as effective, although rates of ca. $8 \times 10^6$ ♀♀ parasitoids per hectare usually resulted in more than 60% egg parasitism.

The onset of oviposition by spruce budworm was predicted using the following: a regression model incorporating larval development; pheromone trap catches of male moths; sampling of egg masses on branches; and emergence of adult spruce budworm in a field laboratory. This was essential for the correct timing of parasitoid releases and subsequent optimization of parasitism.

By varying the release rate of *Trichogramma* from $4 \times 10^6$ to $29 \times 10^6$ ♀♀ per hectare, a curvilinear relationship between release rate and parasitism level was developed. This relationship indicates that parasitism levels of 60% can be achieved with a double release of $4 \times 10^6$ ♀♀ per hectare, and that over 80% parasitism will result from a double release of $10 \times 10^6$ ♀♀ per hectare. Higher application rates provided very little incremental benefit. Previous studies using double releases of 60 000 and 120 000 ♀♀ per hectare (Smith 1985) and $24 \times 10^6$ ♀♀ per hectare (Smith *et al.* 1987) support this relationship.

The present study has shown that spruce budworm egg parasitism can result in a subsequent reduction in mature larval populations. Larval reductions were proportional to parasitoid release rates, with rates of $8–23 \times 10^6$ ♀♀ per hectare resulting in a 42–83% reduction in larval densities the following year. A parasitoid release of $23 \times 10^6$ ♀♀ per hectare, followed by one application of *Bacillus thuringiensis kurstaki* Berliner (*B.t.k.*) the following spring, reduced larval populations by 93%. To develop recommendations for the use of *Trichogramma* against the spruce budworm, however, further research is needed to determine more precisely the relationship between egg mass parasitism and larval reduction.

In general, stand species composition had little effect on the percentage parasitism of spruce budworm eggs, but parasitism was somewhat higher on balsam fir than on white spruce. No carryover effect in parasitism from year to year was detected during the study. The density of spruce budworm eggs in the plots did not affect levels of parasitism by *T. minutum*; the abundance of eggs appeared to be less important than their temporal availability.

Weather conditions immediately following release affected the efficacy of parasitoids; cool, wet weather reduced and extended parasitoid emergence, with subsequent reductions in levels of parasitism.

Most important to the success of the releases was parasitoid quality. Proper methods of production, programming, and shipment were essential to obtain parasitoids of high quality and minimize the number of runted, ineffective individuals released. Efficacy in the field was directly proportional to parasitoid quality, as indicated by percentage emergence, longevity, and fecundity.

## Prospects for the Future

### Potential for Use in Forest Management

In the past decade, forest pest control technology in Canada has been steadily weakened through the loss of registered chemical pesticides and the stagnation of development and registration of new control products. These trends are unlikely to change in the near future. Public opposition to the use of chemical pesticides in the forest environment is now well established (Paul 1988); it will likely continue and expand to include the newer generations of pesticides, e.g. insect growth regulators, pheromones, and perhaps even commercial formulations of *B.t.k.* Yet the need for cost-effective control technology is increasing as investment in forest renewal continues to rise and insect-caused losses to industrial wood supplies become less tolerable. New insect control agents are urgently needed to fill the widening technology gap, and for many reasons, scientific and social, the tactic of inundative release of insect parasitoids, such as *T. minutum*, has considerable promise.

Operational control programs against forest defoliators are undertaken for one of three purposes: control of outbreaks; containment of outbreaks; or foliage protection. *Trichogramma minutum* has the potential to serve an important role in all three. This study has shown that double applications of *Trichogramma* (when used alone or sequentially with *B.t.k.*) at the rate of $10–12 \times 10^6$ ♀♀ per hectare can reduce larval population levels by

over 80% and that population levels could be reduced sufficiently to control or contain an outbreak of eastern spruce budworm.

Current foliage protection objectives for operational spraying against spruce budworm require that 50% of the current year's growth be preserved on spruce, and 60% on balsam fir. Defoliation levels greater than 50% can be expected if the population density exceeds 377 eggs per square metre of foliage during the previous year (Dorais and Kettela 1982). Thus, any technology that can consistently reduce egg densities below 377 per square metre has the potential to provide adequate foliage protection against spruce budworm, by current standards. Clearly, the use of *T. minutum* has that potential and, furthermore, adequate protection may be achievable with release rates lower than $10–12 \times 10^6$ ♀♀ per hectare.

Apart from the potential of this technology against eastern spruce budworm, there are other forests and other pests for which *T. minutum* may be useful. Because of government regulations and public opposition, there are many forest situations in which the use of chemical insecticides is not possible. These include "no spray" zones around human habitation, drinking water supplies, aquatic habitat, and public recreation areas. In some provinces, these zones affect large areas of productive forest land, and effectively exclude them from protection. In such zones, the use of *T. minutum* would be an acceptable option. As well, there are categories of high value forests that are intensively managed and require a high level of protection, because of product value or social value — seed orchards, tree nurseries, Christmas tree plantations, urban forests, and parks. Because chemicals may be difficult or impossible to use in these situations, *Trichogramma* could serve as an effective and acceptable option.

The present research has been restricted to eastern spruce budworm, but there are other forest pests that are parasitized naturally by *Trichogramma* and may be good candidates for this technology: spruce budmoth, *Zeiraphara canadensis* Mutuura and Freeman; western spruce budworm, *C. occidentalis* Freeman; jack pine budworm, *C. pinus pinus* Freeman; eastern hemlock looper, *Lambdina fiscellaria* (Guenée); black army cutworm, *Actebia fennica* (Tauscher); and several insect pests of coniferous seeds and cones.

## Future Research

*Trichogramma minutum* are biologically active control agents capable of searching out and killing host eggs, and when used in inundative releases against spruce budworm, they can suppress outbreak populations. From the present study, it is clear that the release of *Trichogramma* against forest insect pests is biologically and technologically feasible. The future direction of this biological approach to forest pest management, however, lies in its commercialization.

Commercialization means developing the technology to optimize the mass-rearing of *Trichogramma* and expanding its use against several insect pests. In this context, three key areas for future research can be identified:

(1) the development of a cost-effective rearing system;
(2) the characterization and improvement of parasitoid quality; and
(3) the refinement of a release strategy for optimal efficacy in the field.

**Cost-effective rearing**: Operational use of *Trichogramma* will require the technology to consistently mass-rear large numbers of high quality parasitoids on a continual basis. Many of the natural hosts of *Trichogramma*, including the spruce budworm, the bollworm, *Heliothis zea* (Boddie), and the European corn borer, *Ostrinia nubilalis* (Hubner), are difficult or expensive to rear and, as a result, alternative hosts have been found for cheaper, easier mass-rearing (Morrison 1976). Lepidopterous species infesting stored products have proven to be very suitable hosts, e.g. the Angoumois grain moth, *Sitotroga cerealella*

(Olivier), and are currently the sole means of commerical production in other countries. Although research is now underway in the United States, Canada, and China to investigate the potential for mass-rearing *Trichogramma* on artificial diets, this approach is not yet commercially feasible.

The development of efficient mass-rearing technology is the key factor for commercial success. To make *Trichogramma* commercially viable and competitive with current control agents (*B.t.k.*), production costs will have to be in the order of \$10–\$20.00 per hectare. The experimental production unit developed in the present study, at the University of Guelph, currently uses the Angoumois grain moth as the rearing host and has a capacity of $35 \times 10^6$ parasitoids per week. To achieve the desired economy of scale, production would have to be increased up to $>200 \times 10^6$ parasitoids per week (or $>100 \times 10^6$ ♀♀ parasitoids per week).

Increased output and reduced costs can be achieved by automating the production system in two ways. First, the amount of manual labour in the rearing system will have to be reduced by introducing mechanically engineered designs into the system. The relatively high amount of handling in the current system is the primary cost factor in production. Second, the rearing of the parasitoid system will have to be disassociated eventually from its dependence on the rearing of the host system. This will serve to improve human health and safety standards by reducing the amount of moth scales during mass-rearing as well as enhance the reliability of the system when host eggs can either be stored in a suspended state for long periods of time or completely replaced with artificial eggs. This will eventually lead to increased outputs and reduced costs due to an improved economy of scale.

**Parasitoid quality**: To ensure consistent field efficacy and, thus, cost-effectiveness, any automated rearing system will have to be capable of producing *Trichogramma* of high quality. In China, a rearing technique has been developed to ensure that the parasitoids must fly to the host eggs to oviposit (Li 1983). Similarly, Bigler *et al.* (1987) maintained a high quality parasitoid by ensuring flight during mass-rearing. Although this approach contributes to higher parasitoid quality, it is extremely labour intensive and not suited to commercialization. Recently, in the southern United States, Morrison (1976, 1986) developed a continuous rearing system on Angoumois grain moth eggs; however, to date, no continuous quality control of parasitoids reared in this system has been implemented.

The maintenance of high quality *Trichogramma* during mass-rearing will require research to determine the optimal rearing conditions for both the parasitoid and host, as well as the effect of varying conditions of programming, cold storage, and shipping. Clearly, to be cost-efficient in Canada, large numbers of *Trichogramma* must be stored for extended periods to stockpile quantities during the winter for release in the active field season (April to September). It is important, therefore, that we develop techniques for cold storage (or other conditions of mass-rearing) that do not significantly reduce the quality of the parasitoids.

Parasitoids of "high quality" are those with biological attributes that contribute to high levels of parasitism in the field (Manweiler 1986; Pak 1986; Pak *et al.* 1986; Vinson 1976). Intuitively, for Canada, this means *Trichogramma* with the following characteristics: (1) good flying ability; (2) good host searching ability; (3) selective host specificity (host discrimination); (4) high intrinsic rate of increase; (5) good tolerance to low temperatures; and (6) acceptability of small hosts for rearing.

Of considerable importance to the success of future commercialization will be the development of a technique for identifying high quality or superior strains of *Trichogramma*. Previous experiments have shown that isozyme patterns are well suited to detecting differences in strains of *T. minutum* (Smith and Hubbes 1986*a*, 1986*b*). Studies should be continued in this area to identify biochemical markers that are characteristic of parasitoid strains and indicative of changes in parasitic biology during rearing.

To improve parasitoid quality, it will first be necessary to identify those biological attributes that are correlated with and can predict efficacy in the field (Boller 1979). Once these have been established, continued research can determine whether such attributes are linked to genetic components and, thus, are capable of being improved through selective breeding and/or biotechnological manipulation (Whitten 1979, 1986; Roush 1979).

**Optimal field efficacy**: To optimize the field efficacy of *Trichogramma*, two different research directions must be taken. The first will improve our understanding of the relationship and impact of *Trichogramma* on spruce budworm populations. The best release regimen or tactics, in terms of the number of releases, the number of parasitoids per release, and the timing of single or double releases, must be established. As well, we should improve our understanding of the effect of host density and distribution on the functional response of the parasitoid, as well as the effect of stand attributes on egg parasitism. Finally, it is important that we obtain baseline information on the potential for integrating *Trichogramma* releases with other control options such as *B.t.k.*

This proposed research could be advanced effectively through the development and use of simulation models similar to those utilized in agriculture (Knipling and McGuire 1968). Modelling, although no substitute for field releases, would reduce the relatively high costs of such field assessments by quickly identifying the most promising strategies. In this way, experimental studies could be targeted more precisely to critical questions.

The second avenue of research should be the identification of other insect pests and situations for which *Trichogramma* will be well suited as a management technique. We have noted that a number of forest insect pests in Canada can be attacked by species of *Trichogramma*. Investigations should address the potential for using inundative releases of such parasitoids to suppress these forest pests. This will entail studies to determine acceptability and availability of the various host species as well as the impact of parasitoid releases on pest populations.

*Trichogramma minutum* is the one North American species of this genus with proven effectiveness against the spruce budworm and the one currently being mass-reared at Guelph. There are a number of other species of *Trichogramma*, however, that attack forest defoliators in the United States, Europe, and China, including *T. embryophagum* (Htg.), *T. cacoecia* Marchal, *T. dendrolimi* (Matsumura), and *T. zeirapherae* Walter on forest pests such as *Dendrolimus* spp., *Zeiraphera diniana* Guenée, *Dioryctria* spp., and *Rhycionia* spp. (Mills *et al.* 1986; Walter 1985; Belmont and Habeck 1983; Hsiao 1981; Tsankov *et al.* 1980). The potential of these parasitoids, as well as any unknown species or possible ecological strains within each species, should be explored in the prospect that they will be of better "quality" than *T. minutum* for release.

## Conclusion

Successful research on cost-effective rearing, parasitoid quality, and field efficacy will expedite the commercialization of *Trichogramma* for the control of insect pests. Although this research has been directed at spruce budworm, a much broader application is anticipated with continued research and development of the technology. If we are successful in commercially producing and utilizing mass-reared parasitoids, this will have a significant impact on the way we manage insect pests in the forest environment. This technology has great potential for use as a replacement for conventional pesticides and as an agent that is compatible with microbial insecticides and silvicultural techniques. Clearly, it can play a key role in the integrated management of forest pests.

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