FORMULARIO B-II

INFORME TECNICO FINAL

SUBPROGRAMA CONTRATACON CONSULTORES CALIFICADOS

Propuesta

Consultor para multiplicación de Trichogramma para el control biológico de plagas.

FORMULARIO B-II
INFORME TECNICO FINAL
SUBPROGRAMA CONTRATACION CONSULTORES CALIFICADOS

1. IDENTIFICACIÓN DE LA PROPUESTA

1.1- TITULO DE LA PROPUESTA

Consultor para multiplicación masiva de Trichogramma para el control biológico de plagas

1.2.- ESPECIALIDAD

Entomología, Control Biológico

1.3.- IDENTIFICACION DEL CONSULTOR

Nombre Dr. Sherif Hassan
Institución : Federal Biological Research Centre for Agriculture and Forestry (BBA). Institute for Biological Control
Dirección : Heinrichstrassen 243. D-64287
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e-mail biocontrol.bba@t-online.de
País Alemania

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País Alemania

1.4.- PATROCINANTE

Instituto de Investigaciones Agropecuarias, INIA

1.5.- CONTRAPARTE NACIONAL

Marcos Gerding Paris , Entomólogo CRI Quilamapu, INIA

2. ASPECTOS TECNICOS

2.1 Itinerario desarrollado por el consultor

Fecha: 26 febrero
Lugar (ciudad e institución Chillán- CRI Quilamapu
Actividad. Arribo a la ciudad

Fecha 27 de febrero
Lugar (ciudad e institución): Chillán CRI Quilamapu
Actividad Visita al CRI Quilamapu

Fecha 1° de marzo
Lugar (ciudad e institución) Chillán CRI Quilamapu
Actividad: Reunión con directivos del CRI, Inicio de las actividades de conocimiento y análisis del proyecto de Trichogramma

Fecha 2-3 de marzo
Lugar (ciudad e institución) Chillán, CRI Quilamapu
Actividad: Evaluación del proyecto Trichogramma

Fecha 4 de marzo
Lugar (ciudad e institución) Concepción, Mininco S.A
Actividad Visita al laboratorio de producción masiva de Trichogramma que tiene Mininco, y visita de terreno a los lugares de liberación.

Fecha 5 de marzo
Lugar (ciudad e institución) Chillán CRI Quilamapu
Actividad: Control de los ácaros (Pyomotes sp), ensayos de control químico de los ácaros en presencia de huevos de Sitotroga cerealella. Evaluar mortalidad de ácaros, tiempo de exposición , productos y emergencia de larvas

Fecha 8 de marzo
Lugar (ciudad e institución) Chillán, CRI Quilampu
Actividad, continuación de las pruebas de control de ácaros y preparción de seminario

Fecha 9 de marzo
Lugar (ciudad e institución) Los Angeles CPF S.A. (Controladora de plagas Forestales)
Actividad Seminario de "Avances en controles alternativos y biológico de polilla del Brote del Pino en Chile"

Fecha 10 de marzo
Lugar (ciudad e institución) Chillán, CRI Quilamapu
Actividad: Preparción de seminario e informe técnico de la visita

Fecha 11 de marzo
Lugar (ciudad e institución) Chillán CRI Quilampu

Actividad Seminario Avances en el uso de Trichogramma par la agricultura orgánica, viaje a Santiago.

12 de marzo viaje a Alemania

2.2 Cumplimiento del o los objetivos

Los objetivos planteados para esta consultoría fueron:
Los objetivos de la propuesta son contar en Chile con el Dr Sherif Hassan para que colabore con la puesta a punto de los equipos de producción de *Sitotroga cerealella* y adaptar la tecnología de parasitación, multiplicación liberación y evaluación de Trichogramma, disponibles en Alemania, a nuestras condiciones y así poder cumplir con los objetivos del proyecto FIA.

Estos objetivos se cumplieron con creces.

2.3 Descripción detallada de la tecnología capturada, capacidades adquiridas, productos etc.

Métodos recomendados para controlar y prevenir el ataque de ácaros en la crianza masiva de *Sitotroga cerealella*.
Las salas de crianza deben ser cerradas, completamente selladas las ventanas el techo y piso deben estar limpios. Restringir el ingreso a personas que no participan en la crianza de la polilla. Las salas, equipos y granos utilizados en la crianza deben ser intensamente aseados y tratados exteriormente por cortos tiempos con acaricidas.
El control de ácaros en plantas industriales de crianza de *S. cerealella* es algo muy fácil en comparación con lo que sucede en un Instituto de Investigaciones. Se creará un prototipo para asistir a futuras industrias de crianza. La experiencia de otros países será transferida y se adaptará a las condiciones locales.
Normalmente para detener el ataque de ácaros en producciones industriales (extranjeras) se elimina el grano infestado, las salas y equipos son fumigados, usualmente usando bromuro de etilo, y se reinicia la producción usando huevos nuevos.
La solución mencionada anteriormente es más dificultosa para el INIA CRI Quilamapu, ya que la Institución tiene diferentes organismos que debe evaluar al mismo tiempo y esto hace disminuir en ciertos periodos la crianza de *S. cerealella,* dificulta la fumigación, ya que afecta a los otros organismos reproducidos y a las personas que deben laborar diariamente.

Acciones para limitar el ataque de ácaros.

1) Los huevos usados para la producción masiva de *S. cerealella* deben ser tratados en soluciones de acaricidas. Se realizaron experimentos con Dicofol 25 WP (0,36/300cc), Formalina al 10% y otros químicos.

2) Usar salas pequeñas para crianza de larvas y mejorar la protección para posibles infestaciones. Usando y rotando diferentes tratamientos de limpieza. La producción de una semana se pone en una sala y se mantiene por cuatro semanas, luego se obtienen adultos. Se dispondrá de cinco salas de desarrollo larvario (sala de crianza) para permitir que siempre una sala este en limpieza.
3) Se debe iniciar la crianza en forma limpia, usando soluciones de acaricida.
4) Usar pequeños ventiladores en las salas de crianza para reducir la temperatura de granos, después de 4 semanas de infestación con huevos de *Sitotroga,* los granos se cambian desde la sala de crianza a salas de emergencia. El cambio de salas es propicio para un fuerte ataque de ácaros.
5) Limpiar y desinfectar las cajas de emergencia que se usarán.
6) Antes de utilizar el grano debe hornearse a 100ºC por 60 minutos.
7) La producción de la Polilla Mediterránea de la Harina *Anagasta kuehniella* podría ser mantenida en otro edificio y con personas no relacionadas o que no tengan ingreso a la producción de *S. cerealella*. Este insecto es conocido por llevar ácaros.
8) Durante el tiempo de transición y hasta que la producción de *S. cerealella* está reestablecida, la producción de *Trichogramma* es reemplazada temporalmente utilizando como hospedero a *Anagasta kuehniella*. La razón para este cambio es que los huevos usados usualmente (*S. cerealella*) están infestados con ácaros y serán limpiados y para lo cual ya se estableció una nueva crianza de *S. cerealella*.

2.4 Aplicabilidad en Chile

Toda la información adquirida de la visita del Doctor Sherif Hassan es de aplicabilidad inmediata en la crianza masiva de *Sitotroga cerealella y Trichogramma spp.* Las recomendaciones aportadas por el Dr Hassan fueron puestas de inmediato en práctica de este modo no habrá retrasos en la producción masiva en septiembre.

2.5 Evaluación del consultor por la contra parte nacional

La venida del dr Hassan fue muy positiva para el desarrollo del proyecto sobre masificación de Trichogramma que se esta realizando en el CRI Quilamapu. Los aportes del consultor fueron siempre muy aterrizados y de orden práctico, tuvo muy buen acercamiento al personal que labora en el proyecto a pesar del problema idiomático.

2.6 Sugerencias

Esta consultoría podría repetirse durante el último año del proyecto de masificación de Trichogramma, año 2001, siempre habrá novedades en la producción masiva de Trichogramma.

3. ASPECTOS ADMINISTRATIVOS

3.1 Organización antes de la visita del consultor

a. Contacto inicial con consultor realizado por:

Investigador

b. Apoyo de la Institución patrocinante

Bueno.

c. Recomendaciones

Continuar con programas de consultores internacionales, son un gran aporte para la investigación en el país

3.2 Organización durante la visita

La organización de la visita no tuvo problemas y puede ser calificada como Buena

Recomendaciones

Mantener las facilidades que se dieron para el desarrollo de esta visita, los fondos llegaron oportunamente, y los pasajes se hicieron llegar sin problemas al consultor al país de origen.

Fecha: 12 de abril de 1999

Firma del responsable de la ejecución:

Marcos Gerding Paris

Controladora de Plagas Forestales S.A.
Centro de Transferencia Tecnológica
UN PROYECTO CPF S.A. - FONTEC

SEMINARIO

AVANCES EN CONTROLES ALTERNATIVOS Y BIOLÓGICO DE POLILLA DEL BROTE DEL PINO EN CHILE

HORA	TEMA	EXPOSITOR
09:30 – 09:50	Inscripciones	
09:50 – 10:00	Apertura Seminario	Sr. O. Ramírez
10:00 – 10:30	**Avances en el Control Biológico de Polilla con Orgilus**	Ms. Cs. Biológicas C. Goycoolea
10:30 – 10:40	Consultas	
10:40 – 10:55	Café	
10:55 – 11:40	***Trichogramma* una Alternativa de Control de Polilla del Brote**	Dr. S. Hassan Dr. M. Gerding
11:40 – 11:50	Consultas	
11:50 – 12:20	**Avances en el Control de Polilla del Brote con Feromona.**	Dr. G. González
12:20 – 12:30	Consultas	
12:30 – 13:00	**Hongos Entomopatógenos como una Alternativa de Control de Polilla del Brote**	Dr. A. France
13:00 – 13:10	Consultas	
13:10 – 13:30	Cierre seminario	Sr. O. Ramírez

Nota: El seminario no incluye almuerzo.

MASS PRODUCTION AND UTILISATION OF *TRICHOGRAMMA*: 15. A NEW DEVICE FOR FIELD APPLICATION.

SHERIF A. HASSAN
Institute for Biological Pest Control, Heinrichstr. 243, 64287 Darmstadt, Germany.

A new device to release *Trichogramma* in the field is being successfully used in practice in Germany since 1994. The cardboard device is hung on a plant or tree and it protects the parasitoids from rain and from predators. This protection is very important because it makes it possible to use *Trichogramma* in different developmental stages. With this, the effect of the treatment is significantly prolong.

Trichogramma brassicae releases to control the European corn borer *Ostrinia nubilalis* showed that the effect of one application lasted from three to four weeks depending on the temperature. Mass reared *Sitotroga cerealella* eggs were daily parasitized by *Trichogramma*. About 7 different developmental stages of the parasitoid were thoroughly mixed, glued on to the inner surface of the device and a roof to protect the eggs was automatically placed.

Introduction

The release of mass produced egg parasites of the genus *Trichogramma* to control lepidopterous pests has gained increasing attention world-wide in the last few years. *Trichogramma* is being used to control pests on corn, apple, plums and cabbage in Germany since1980. Field experiments to control the European corn borer in Germany were started in 1977. The release of *Trichogramma* at the rate of 200,000 parasites / ha resulted in an increase in egg parasitism of 72 - 92 % and a reduction in larval numbers between 61 and 93 % (Hassan 1981and Hassan et al 1994).

Two companies started to produce the parasite, one in 1980 and one in 1983. The mass-rearing and releasing techniques improved with experience from year to year. Close co-operation was observed between research institutions, producers, distributors, agricultural associations, plant protection services and growers.

Every year, the companies in Germany start to produce *Sitotroga cerealella* in January and *Trichogramma brassicae* Bezdenko in Marsh. The quality of the parasitoid was regularly tested at the Institute in Darmstadt. To maintain high quality at the institute, the parasitoid was reared under fluctuating conditions (26 +/- 2°C, 70 % RH, 18 h: 90 % RH, 6 h) and the rearing was made on eggs of the natural host (*O. nubilalis*) at intervals of about 6 months. Apart from that, the beneficial arthropod was exposed to high and low temperatures at intervals (32 °C and 10 °C). To prevent bottle-neck situations, about 10 units, each with about 10,000 parasites, were maintained permanently. This widens the genetic spectrum of the parasite in the rearing.

To determine the right time to release the parasite in the field, the extension service in the releasing regions monitored the flight of the pest using light traps. After the first moth was captured, the first parasitoids release was made at the earliest opportunity. The second release was carried out about two weeks later, depending on region and weather. The distribution of the parasite to the growers was carried out mainly by agricultural associations. The growers, informed by letter, collected the parasitoid at a given time and place, and released it mostly on the same day. In case of rain, the natural enemy was stored at 10 – 15 °C for 2 or 3 days without affecting vitality. Care was taken not to expose the organism to excessive

between the first and second applications. The overlap was partly intentional and desirable because of the high pest occurrence at that time. In future, the period of overlapping should be reduced by delaying the second release for a few days. The emergence pattern of the single treatment carried out by AMW was similar to the first release of the two application method, covering the period from June 22 to July 24, 1998.

Field observation showed that the new device provided excellent protection from rain as well as a fairly good protection from attacks by large predators such as *Chrysoperla carnea* in the field. However, earwigs and ants were sometime observed to enter the device and feed on the eggs. This can be reduced by optimising the size of the exit openings of the device. The near complete rain protection greatly increase survival of the immature stages, compared to the open releasing cards previously used.

The release of mass reared *Trichogramma* is carried out in different ways. In some Eastern European countries, adults are released from glass containers that are opened at each releasing site. A plant leaf of a crop is insert in the glass so that adults of *Trichogramma* can jump on it and then released. In other countries i.e. South America, parasitised host eggs in one age are placed in paper bags or glowed on cardboard that are brought out to the field. In both cases, releases at intervals of about 4 to 7 days are necessary. Growers are often reluctant to release the parasitoid at such short intervals.

The main advantage of using the new devise described in the present work is that it makes it possible to include *Trichogramma* in different development stages, including younger stages that need longer periods of time to emerge in the field. This way the effect of the treatment is prolonged and the intervals between releases increased.

Trichogramma brassicae releases carried out to control the European corn borer *Ostrinia nubilalis* in 1998 showed that the effect of one treatment lasted from three to four weeks depending on the temperature. This can be compared to 4 – 5 days when adults are released or 7 – 10 days when one development stage is applied (personal experience).

References

HASSAN, S.A. - 1981. Massenproduktion und Anwendung von *Trichogramma* : 1. Produktion des Wirtes *Sitotroga cerealella* Entomophaga, 26 (4), 339-348.

HASSAN, S.A. – 1981a. Mass production and utilisation of *Trichogramma*: 2. Four years successful Biological Control of the European corn borer. Med. Fac. Landbouww. Rijksuniv. Gent, 46/2, 417-427.

HASSAN, S.A, KOCH, F. and NEUFFER, G. - 1984. Maiszüslerbekäpfung mit *Trichogramma*. Schriftenreihe des Bundesministers für Ernährung, Landwirtschaft und Forsten, Reihe A: Angewandte Wissenschaft, Heft 299, 35 S. Landwirtschaftsverlag GmbH Münster-Hiltrup.

Figure 1: Emergence pattern and longevity of *Trichogramma brassicae* from two applications to control the European corn borer *Ostrinia nubilalis*, 1998, Biocare.

Figure 2: Emergence pattern and longevity of *Trichogramma brassicae* from a single and two applications to control the European corn borer *Ostrinia nubilalis*, 1998, AMW Nuetzlinge GmbH.

Information on the use of egg parasitoids of the genus *Trichogramma* to control the European Pine Shoot Moth *Rhyacionia buoliana*

Dr. Sherif A. Hassan, Institute for Biological Pest Control,
Federal Biological Research Center for Agriculture and Forestry,
Heinrichstr. 243, D-64287 Darmstadt, Germany.

Abstract:
The use of *Trichogramma* to control European Pine Shoot Moth *Rhyacionia buoliana* is feasible and the establishment of a prototype mass rearing is recommended. The experiments conducted in Chile the last few years show that several species can be effectively used but the trails conducted to assess the efficacy of the parasitoid under estimate expectations based on results gained in other countries. This report includes description of mass rearing of the natural enemy, methods to select effective species for use in biological control and suggestions for field methods to assess efficacy.

Priority for the use of *Trichogramma* in Pine plantations:
Priority should be given to pine trees of three to five years of age. This is because the amount of vegetation of the large trees will provide ample space for the parasitoid compared to younger plantations. *Trichogramma* is more effective where suitable microclimate is available that provide humidity. This plantations also has weeds of high size that improves the climate in hot days and provide alternative hosts that will increase the spread of the natural enemy. These locations, because of the size of the tree, are more difficult to spray with chemical pesticides.

Asses the efficacy of *Trichogramma*
The field experiments conducted in Chile in 1996 and 1998 under estimate the efficacy of the parasitoid. The following suggestions are made to improve methods for preparing the *Trichogramma* for release as well as for designing the experiments.

1. Plot size :
Trichogramma is shown to fly in the field and cover a distance of about 30 m in less than one month, therefore the plot size should be large (at least half a ha) and the distance between the plots should be at lease 30 m. To insure homogeneity between treated and untreated control plots the number of plots in a given field should be reduced to a minimum. Experience in corn fields in Germany has shown that an experiment with one treated plot and one untreated control plot (each of half a ha) in one field would give useful results. Such an experiment can easily be repeated in different fields, three replicates are recommended.

2. Age structure of released parasitoid :
To insure high efficacy, total parasitism of the pest eggs should be achieved during the first two weeks of egg laying. If this is done without gaps, the parasitoid should be able to multiply in the field and high level of control in obtained. The age structure of the released *Trichogramma* is therefore of great importance. The mixing of parasitoids in different development stages is recommended. *Trichogramma* of at least three different development stages should be used in each releasing point. The releasing devise should provide protection from predators and from rain. Gaps in the first two weeks are specially important because the negative effect (lake of parasitoid) is greatly magnified later in the season.

3. Monitoring of experiments:
Due to the large fluctuations in the distribution of the pest, the sampling of side shoots from only three trees per plot dose not reflect the actual level of damage. It is therefore recommended to monitor damage directly in the field rather than cutting shoots to be examined in the laboratory. In autumn, the damage of shoots is clearly seen and the counting of the number of damaged and undamaged shoots can easily be done. Large number of trees can be monitored without too much effort. The damage to the top growing point of trees ought to be monitored separately, because of its special value.

The advantages of using *Sitotroga*:
The advantages over *Corcyra cephalonica* and *Ephestia kuehniella* is that: (1) *Sitotroga* is disease resistant, no problems in mass rearings were recorded, (2) The larvae are not cannibalistic, sterilization of eggs not needed,(3) the adults are easily collected making use of its natural behavior to descend to the bottom of cage, no need for anaesthesia (4) no eggs are laid during the first day after emergence, no losses before collection. *Sitotroga* rearings are however affected by the itch mite *Pymotes* spp. to a higher degree than the other two hosts and care must be given to private attacks. *Corcyra* is reared on rice bran which is cheaper and more available in several countries compared to wheat but the collection of the adults in the mass rearing is more time consuming, requiring more equipment an working space compared to *Sitotroga*. This limits its suitability for large scale operations.

Remarks to Location and Building
A suitable location for *Trichogramma* rearing is near the area where the parasite is to be used and close to principal roads, public transportation,or an airport. This will speed transportation of insects and enlarge the area of use.

Proper housing and adequate equipment are needed for establishing a successful *Trichogramma* mass rearing facility. The building should be large enough to accommodate current production needs and, if possible, sufficient space for future expansion. A building that is well isolated is required and thick walls and high ceilings are desirable to save energy for cooling and heating. Small tight glass windows that are also fitted with insect proof screens to prevent infestation are needed. Floors and walls should have washable surfaces. It is also desirable that rooms that lead to the outside have double doors.

The room requirements for rearing *Trichogramma* and its host are approximately as follows: (1) Media preparation and infestation of grain (medium size), (2) Larval development (small size), (3) Adult emergence (large hall, or several rooms), (4) Oviposition (small size), (5) Rearing of parasite (small size), (6) Administration. The rooms (1), (2) as well as the rooms (3) and (4) can be combined without technical problems. A grain storage room outside the building is needed.

Preparation and infestation of grain
One large wheat grain provides optimum media for one *Sitotroga* larva. With small size grain, the larva is forced to feed on more than one grain losing time and valuable rearing space. Although wheat is by far the best *Sitotroga* larval food, small corn grain can be used. The use of large corn grain is not economical because each grain is mostly only infested by one *Sitotroga* larva wasting space and food material.

Six kg of wheat and one litre of water are mixed thoroughly in a 10 litre metal pot. A wet cloth is spread on the surface of the wheat, the pot is covered and placed in a dry chamber at 100 °C and heated for 6 hours with stirring every 2 hours. It is of critical importance that the wheat is well mixed and the cloth on the top is rewetted every 2 hours. Heating both decontaminates the wheat by killing all unwanted organisms and also softens the grain and creates cracks on the surface which enhances infestation and development of the *Sitotroga* larvae. The hot wheat and water can be thoroughly mixed by a gloved arm and a long wooden spoon. Mixing is improved by pouring the hot wheat from one pot to another. Heating the grain should be carried out by placing the pot in an oven and not by putting it on direct flame. After 6 hours of heating, the grain should be mixed once more, and spread on trays or a table, to a depth of about 5cm and covered with a dry cloth and left overnight. The next day, the grain is loaded into the holding cribs and *Sitotroga* eggs are applied (1 g eggs per kg wheat).

Careful preparation of the grain is critical because it directly affects the performance of the rearing. If the grain is not properly mixed with water, the egg production could drop up to 70%. If not well heated, unwanted organisms will often multiply faster than *Sitotroga* and destroy the rearing. The cost

Adult emergence

Most of the moth emerges between week 3 and 10 after placing the cribs in the cage (5 - 12 weeks from infestation of the grain). The ventilator should be swished on as soon as the new cribs are placed in the cage and swished of 3 to 4 weeks later as the temperature reduces. When *Sitotroga* moths emerge from the grain they walk through the wide mesh of the crib and spend from a few hours to one day in the upper part of the cage where copulation also occurs, few or no eggs are laid during this time. Then, they walk down through the space between the cribs and enter the funnel and the adult collecting bottle below it. The size of the adult collecting bottle can vary between 1000 and 2000 ml depending on the number of adult emerging. It is particularly agreeable that *Sitotroga* adults do not lay eggs during the first day of life. The adults are collected every day by empting the bottles into an egg laying drum.

The optimum conditions for the adult emergence room, is 24°C, 50-60% RH and the critical grain temperature is 35°C.. With six emerging cages each with 16 cribs and a production rate of 50 kg wheat per week (8 cribs of 6 kg each), the daily egg production should be about 70 g.. The cribs can be kept for a total period of 14 weeks from infestation (2 weeks in the larval development room and 12 weeks in the emergence room). This allows one generation of moths to emerge and still leaves sufficient time for cleaning the cages. This period can be shortened to 10 weeks at top production time.

Rapid utilization of grain (i.e. within one generation of *Sitotroga*) has proven most economical in our hands.. The use of wheat heavily infested with *Sitotroga* eggs at one time insures rapid turnover and optimum utilization of equipment. Provided that grain's well prepared, and adequately infested, and that larval development occur under optimal conditions, most of the grain was utilized in one generation and the duration of adult emergence was minimized. Prolonging adult collection over more than one generation encourages the build up of unwanted insects and mites, greater variability in host egg size, and generally less predictable results through time.

Egg laying

The Sitotroga adults in the plastic bottles of the emerging cages are emptied into an egg laying drum every day. The drums (51cm long and 28cm diameter) have wire gauze walls and are fitted into a semi-automatic egg collection unit especially developed for this purpose. Moths are introduced into the drum through a 65 mm diameter hole in one end, which is then sealed with a rubber stopper. Each batch of moths covers the bottom of the cylinder to a depth of 20 to 30 mm. In the egg collection unit, the cylindrical cages are rotated for 15 minutes every 3 hours at intervals. During rotation the moth scales, which have accumulated over the preceding 2.75 hours, are sucked off by stationary air suction metal tubes. The eggs, which are too heavy for the vacuum to remove, fall through the mesh openings in the drums onto shallow trays in the bottom of the unit.

The collection unit, which contains three or six cylinders, rotates them at about 0.5 rpm. This is too slow to disturb the moths (they lay most of their eggs through the screen of the drum but also under each other's wings) but fast enough to tumble the eggs out through the mesh and onto the collecting trays below. From here they are collected daily, sieved to remove rubbish, then stored in a refrigerator. The egg laying unit can be placed in the preparation room, in the emergence room or in a separate room depending on the size of the project. The optimum conditions for egg laying are 26°C and 60% humidity.

Control of mites

There are several species of mites that occur in *Sitotroga* rearing. Most of them feed on dust and debris, but a few are predators. The following control strategy for these mites aims to keep their number low in the rearing rather than to eradicate them: (1) *Sitotroga* eggs used for infesting the grain must be free from mites. (2) The grain in the holding cribs should be removed from the rearing room within 14 weeks from its infestation day. (3) The grain should be adequately heated before use and again before it is discarded.

between strains of the same *Trichogramma* species and stressed the importance of choosing an appropriate strain for use in biological control. Others have indicated particular attributes that would increase the biological abilities of the parasite and its effectiveness in the field (van Lenteren 1986, Pak 1988, Pak et al. 1991, Bigler 1989). In recent years there has been an increasing awareness of this problem among *Trichogramma* research workers.

When a suitable host egg is encountered, the female *Trichogramma* examines the egg by antennal drumming, drills into it with her ovipositor and lays one or more eggs within the host egg, depending on its size. When a *Trichogramma* female finds "preferred" host eggs, it will usually stay on or near them for a long period of time until all or most of them are parasitized. Less preferable host eggs may be totally rejected or the parasite might lay a few eggs before leaving the location to search for more suitable hosts. Diversity in host preference of *Trichogramma* species has now been fully recognized as an important factor with regard to biological control.

For the parasite to be successful in the field it must locate food and shelter before moving in search of its host. The presence and distribution of food differ from crop to crop and from year to year. Adult parasites that do not find food and moisture in the first day have a much shorter life span and less chance of locating hosts. If food is scarce and adults expend more time and energy finding it, the choice of parasites with high searching capacity and energy reserve is crucial. The abundance and distribution of the host is equally important. If an adult has to fly from one tree to an other in search of a host, it would need high flying and walking abilities to find host before exhaustion. These attributes are important both for the survival of the parasite and for its usefulness as a biological control agent.

Judging by the numerous attempts of biological control with *Trichogramma* over the past 80 years, it is remarkable how little basic research has been conducted on these insects. The entomophages searching behavior in the field following its release is a key process in the successful parasitization of hosts. Unfortunately this is an area about which there is little information available (Noldus 1989). Salt (1940, 1958), one of the first to realize the importance of the study of behavior of parasitoids for their utilization as biological control agents, made a detailed study of behavioral and physiological aspects of parasitism by *Trichogramma*. His work has been continued in recent years by, among others, Klomp et al. (1980); Pak (1988) and Schmidt & Smith (1989). Their studies have focused on the processes that occur after a wasp has contacted a host, namely host acceptance and host suitability. Searching behavior preceding host contact has received less detailed attention. *Trichogramma* exhibits a strong preference for Lepidoptera species, including a large number of important agricultural pests. Adult *Trichogramma* require both water and food (honey) for maximum fecundity. They spend most of the their time seeking this food, host eggs, or looking for a mate. The searching activity of the adult parasite is generally believed to be stimulated by chemical substances, Kairomones, produced and left by its hosts.

A common practice among the users of *Trichogramma* in many parts of the world is to release a strain that was collected from one pest to control another. Evaluation in commercial releasing areas is seldom undertaken. For practical reasons, attempts to use an available, but less effective species, to control a particular pest are often knowingly undertaken. The purity of the parasite in mass rearing is also important to ensure effectiveness. Ways to select appropriate strains and secure methods of rearing them are needed to ensure success of the augmentative approach.

Potential of egg parasites as agents for biological control:
A survey of the natural abundance of egg parasites and their potential for use in biological control was reported by *Trichogramma* News in 1990. The results from 20 different countries showed that 11 genera of egg parasites, include 54 species, occurred regularly in fields on 34 crops involving 69 pests. The advantage of using egg parasites over larval parasites in biological control is that the former prevents hatching. Moreover, egg parasites can be mass reared more easily in large numbers. Success or failure in the use of egg parasites in biological control will depend, among other factors, on the choice of species.

	3000	cabbage	*T.evanescens*
	6000	vineyards	*T.cacoeciae*
	6000	plum, apple, forest	*T.dendrolimi*
China	1200000	corn, cabbage, tomato, pepper	*T.dendrolimi*
	20000	sugarcane	*T.chilonis*
Colombia	241000	cotton, soybean, sugarcane, tomato, sorghum	*T.pretiosum*
Egypt	1800	sugarcane	*T.evanescens*
France	10120	corn	*T.brassicae*
	12	vineyard,chestnut	*T.cacoeciae*
Germany	*5100*	*corn*	T.evanescens, *T.brassicae*
	10	apple	*T.dendrolimi, T.cacoeciae*
Great Britain	10	tomato,ornamentals	*Anagrus atomus*
Honduras	2000	corn, sorhum	*Telenomus remus*
	18	banana	*T.minutum, T.pretiosum, T.platneri*
India	14000	sugarcane,cotton,corn	*T.chilonis*
	250	tomato	*T.brasiliensis*
	250	paddy	*T.japonicum*
Iran	35000	sugarcane	*Platytelenomus hyalas*
	445	corn,rice,pomogranite	*Trichogramma* sp.
Italy	5	sweet corn	*T.maidis*
	5	vineyards	*T.cacoeciae*
Malaysia	163	cocoa	*Trichogrammatoidea bactrae fumata*
Peru	130000	cotton	*T.pretiosum, T.brasiliensis*
	81000	sugarcane	*T.exiguum, T.brasiliensis*
	4000	orange	*T.perkinsi*
	2000	apple	*T.euproctidis*
	1700	olive	*T.euproctidis*
Philippines	200000	sugarcane	*T.chilonis, T.nana, T.chilotraeae, T'toidea bactrae bactrae*
Portugal	500	pasture	*T.cordubensis*
Romania	40	corn	*T.maidis*
Russia	5100000	corn & other	*T.evanescens*
South Africa	2500	citrus	*T'toidea cryptophlebiae*
Switzerland	4000	corn	*T.evanescens*
Taiwan	14000	corn	*T.ostriniae*
	55800	sugarcane	*T.chilonis*
Uruguay	300	sugarcane	*T.galloi*
USA	354000	cotton,corn,soybeans	*T.pretiosum*

Table 2: The use of eight *Trichogramma* species to control pests on different crops and countries.

Trichogramma species	treated area (ha)	crops	pests	countries
T.brassicae	41440	corn	*Ostrinia nubilalis*	Austria, Bulgaria, France, Germany, Romania,

crop range. Until recently, pre-introductory evaluation or screening of relevant strains was seldom carried out.

It is a valid practice to choose an indigenous *Trichogramma* strain to mass produce and release on the same crop. A local strain is preferable to an imported one if efficacy is adequate. Although local strains are more adapted to the environment, if the efficacy of the stain is not satisfactory, efforts to explore for better parasites should be made.

The use of imported strains:
Strains from different agroecosystems may differ in their potential. A useful strategy, therefore, would be to compare all relevant species according to a literature study. Indigenous and imported strains of these species from different localities may also be compared. The strains should first be screened in laboratory experiments, with promising ones further tested in semi-field and field experiments. Furthermore, the efficacy of commercial parasite releases should be regularly assessed.

As mentioned above, a local *Trichogramma* strain is often chosen for use in biological control because the parasite is expected to be well adapted to the local environmental conditions. However, a local strain might not be the most effective one for the particular purpose. More effective parasites with better adaptation to the crop and/ or the host may be found elsewhere in areas were the crop has been grown for a longer time. Evolution or adaptation of the parasite in different ecosystems are unlikely to be identical. In the course of centuries, the genetic structure of certain populations and the host searching behavior on a particular type of plant might have evolved to make the parasite more effective than in other similar agroecosystems. For biological control to compete effectively with chemical pesticides, increased efficacy is important. Efforts to acquire more effective strains is therefore justified.

An "FAO Code of conduct" has recently been drafted to ensure the safe use biological control agents and prevent the accidental introduction of unwanted organisms into importing countries. In particular, the code aims to harmonize cooperation between research scientists and authorization officers.

The simultaneous release of more than one strain:
To increase efficacy, a combination of more than one *Trichogramma* species may be released to control one pest. The choice for such a combination should be based on the results of laboratory, semi-field and field experiments. One advantage of such practice would be that the two species may be complementary to each others in aspects such as time of emergence, searching behavior, area of activity (upper or lower part of the plant), longevity and tolerance to extreme weather conditions.

Field experiments to optimize the use of *Trichogramma* to control the codling moth and the summer fruit tortrix moth were conducted between 1988 and 1990 (Hassan et al. 1988). In three field experiments, a combination of two egg parasite *Trichogramma dendrolimi* and *T. embryophagum* was found to increase effectiveness by about 10% compared with the release of the same total number of *T. dendrolimi* alone. Four releases, each at 2.5 million parasites ha, were conducted in each case. With the mixture of species, the reduction in codling moth damage was 52.9, 84.3 and 74.1 % compared with 42.0, 78.0 and 66.7 % respectively for the release of *T. dendrolimi* alone. The damage of the summer fruit tortrix moth in the same experiment was reduced by 39.2 and 85.4 % when the mixture was used compared with 23.5 and 70.7 % when only *T. dendrolimi* was released. In further trials, a combination of *T.dendrolimi* and *T.cacoeciae* was used to control the codling moth. The effectiveness increased by 7.0 or 11.1 % compared with each of the two strains alone. The increase with summer fruit tortrix moth in the same experiments was 9.1 or 14.1 % in comparison to the other strains alone (Hassan, Rost 1993). The commercial use of this combination of species commenced in Germany in 1992.

Tolerance to weather conditions:
Tolerance of prospective *Trichogramma* strains to extreme weather conditions in the relevant area is important. However, with an augmentative release strategy, only the weather conditions during the

nubilalis egg masses in releasing fields in Germany. No contacts were observed in plots were other *Trichogramma* species were released. The parasite leaves the eggs to search for a more "preferred" host.

Results of experiments (Hassan and Guo, 1991) to compare the host preference of 15 candidate *Trichogramma* strains, to contact and parasitize *O.nubilalis* eggs showed that when the parasite was offered the choice between eggs of the natural host *O . nubilalis* and eggs of the mass rearing host *S.cerealella*, *T.ostrinae* from China P.R. (strain number 62, collection at the Institute in Darmstadt) and *T.brassicae (evanescens)* from Moldavia (10) showed strong preference for the eggs of the European corn borer *O.nubilalis*. The data clearly indicated that *T.ostrinae* had the strongest preference for corn borer eggs compared with all the other *Trichogramma* strains tested. The *Trichogramma* adult females had much more contacts with and parasitized higher number of *O.nubilalis* eggs than *S.cerealella* eggs. It was therefore unexpected to find that *T.ostrinae* from Taiwan (61) exhibited no preference for the pest eggs and had comparatively low egg laying capacity. This strain has apparently lost its preference for the natural host, possibly due to the continuous laboratory rearing on alternative hosts. All the other 13 *Trichogramma* strains tested preferred to contact and parasitize *S.cerealella* over *O.nubilalis*. Among the 15 strains tested, *T.dendrolimi* (strain 20) and *T. chilonis* (32) were the most fecund, parasitizing the highest number of *Sitotroga* eggs.

Zhang Zhili (1986) showed that *T. ostriniae* was the dominant species of parasitoid attacking corn borer eggs of all generations and is a key factor affecting populations of the Asian corn borer in the Beijing suburbs. It accounted for over 95 % of the *Trichogramma* recorded, whereas *T. dendrolimi, T. chilonis, T. evanescens* and *T. closterae* together for less than 5 %. He showed in laboratory and field experiments that *T. ostriniae* was much more effective than *T. dendrolimi* which, according to Wang Cheng-Lun (1986), is being annually released in practice on an area of about 5.000 000 ha in 5 provinces of China.

The preference of *T. brassicae (evanescens)* from Moldavia (10) and the local *T.evanescens* from Germany F.R. (105) for corn borer eggs was significantly lower than *T. ostriniae* but higher than all the other strains. The difference between these two strains was also significant. The level of significance among these three strains was 5 % for contacts and at 1 % for number of eggs lay per female. The results of this test agree with those of a field experiment that was carried out by Hassan (1985) near Gross-Gerau to compare the effectiveness of different *Trichogramma* strains. Single field releases at 150 000 parasites per ha resulted in 86.1 % egg parasitism with *T. brassicae (evanescens)* Moldavia (10) and 81.3 % reduction in the number of *Ostrinia* larvae compared with 64.5 % parasitism and 65.4 % larval reduction for *T. evanescens* Darmstadt (105). *T. brassicae (evanescens)* (10) in Moldavia and *T. ostrinia* (62) in China may have had better conditions to adapt to the corn borer than the strain *T. evanescens* (105) in Germany. In Germany this species occur mainly in cabbage fields, not in corn. These experiments showed that two imported *Trichogramma* strains were more effective than the local one. That these two species are known in practice to be effective against the corn borer indicates the usefulness of the screening method. *T. ostriniae* has being successfully used in Asia for a long period time and *T. brassicae (evanescens)* in Europe since 1978.

Results confirm that the choice *T.brassicae (evanescens)* to control the European corn borer (*O.nubilalis*) in western Europe is justified. However, the better performance of *T. ostrinia* in the host preference test suggests its possible value as an alternative. About 15.000 ha of corn are being treated annually with the Moldavia strain of *T. brassicae (evanescens)* or the very similar species *T. brassicae*in France, Switzerland and the Federal Republic of Germany. In Germany, the reduction in the field number of *O.nubilalis* larvae obtained by releases, compared with untreated control plots, varied between 70 and 93 %. Two treatments each at the rate of 75.000 per ha are being carried out starting at the beginning of the *Ostrinia* adult flight, as indicated by light traps (Hassan 1985, Hassan et al.1978, 1986).

The contact and parasitism method, was also used by Hassan (1989) to select suitable *Trichogramma* strains to control the Codling moth *Cydia pomonella*, the two summer fruit tortrix moths *Adoxophyes*

against the European corn borer. The number of corn borer eggs parasitized by the two species was not significantly different in all the experiments conducted.

The searching capacity of six *Trichogramma* species on cabbage plants with naturaly laid *P. xylostella* eggs was tested by Wührer & Hassan (1993) . The release of *T'oidea bactrae, T. ostriniae, T. chilonis, T. pretiosum, T. pintoi* and *T. cacoeciae* in a ratio of 1 female to 20 eggs lead to a parasitism of 55.8, 40.3, 28.7, 16.7, 4.9 and 4.2% and a larval reduction of 66.7, 54.1, 42.6, 20.1, 14.2 and 20.8% respectively. When releasing *T. ostriniae, T'oidea bactrae* and *T. chilonis* in a ratio of 1:1, larval reduction was 89.3, 84.6 and 74.7%.

Both the corn and cabbage experiments confirmed the findings of the laboratory trails. Species that were effective in the laboratory also had the highest efficacy in the semi-field test.

Field test to asses the efficacy:
The release of the parasite in the field should be done as close as possible to methods used in practice. These aspects include, the releasing device, the number of parasites per unit, the distance between releasing points and the number of releases. In field crops, the plot should be square in shape and include at least 25 releasing points, monitoring should be restricted to the center part. The distance between plots should be as large as possible, i.e. a minimum of about 30 m in a corn field. The size of the plot in fruit orchards will depend on the type of trees, about 25 larger trees in a square shaped plot will allow monitoring of the 9 central ones.

The increase in egg parasitism as well as of the reduction in larval infestation should be monitored. Voegelé et al. (1975) developed a method to asses parasitism of the European corn borer eggs in *Trichogramma* releasing experiments and used by Hassan (1981). Eggs laid on 21 selected corn plants surrounding releasing points are examined at curtain time intervals and the number of parasitized *O. nubilalis* eggs as well as emerged pest larvae is counted. The advantage of this method is that the eggs are observed at intervals directly in the field until they turn black or the host larva hatches. With this they are exposed to the parasite during all its development time in the field.

The reduction in larval infestation in corn fields was carried out by Hassan et al. (1978) by dissecting about 8 x 25 plant per plot. Half of these plants were taken at the releasing point, the other half between releasing point. In fruit orchards, the number of damage fruits related to the total number of fruit per certain number of trees is counted.

Facilities and requirements needed for the test
Certain facilities and requirement are needed to conduct laboratory and semi field experiments to select *Trichogramma* strains suitable for use in biological control.

(1) A continous mass rearing of a suitable replacement host such as the Angoumois grain moth *Sitotroga cerealella* (Oliv.), the flauer moth *Ephestia kuehniella* or the rice moth. The replacement host in the experimental laboratory should be free of *Trichogramma* at all times. To prevent contamination, the rearing of the replacement host should be located away from any *Trichogramma* rearing and should be operated early in the day before any *Trichogramma* rearing has been dealt with.

(2) Rearing of the candidate parasite strains. Experience has shown that different *Trichogramma* strains could easily be reared fairly close to each others without the risk of being mixed. Rearing of a large number of strains in an environmental cabinet or in a small room were established in many laboratories in several countries without major problems.

About 50 species or strains were kept at the institute in Darmstadt in small glass tubes for many years without contamination. Each strain was kept in several glass tubes (145 mm long and 26 mm in diameter) with a cloth cover. The tubes were confined in a plastic container with one side darkened by black paper to keep the photopositive parasites away from the cover of the tubes. The tubes were kept in an environmental chamber at 18^0C, 80 % RLF and 8 h light. Adult *Trichogramma* emerging in the

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Report of visit to Chillan, Chile in February 25 to March 12, 1999

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This is a report of the second visit to INIA CRI Quilamapu in February 25 to March 12, 1999. Report of the first visit was submitted in 1997. The present report includes information on the mass rearing of *Trichogramma* with especial emphasis on mite control. Laboratory and field methods to select effective species for use in biological control are discussed. A new device to release *Trichogramma* in the field that was developed in Germany is described and possibilities for use in Chile are discussed.

The pests dealt with are primary the European Pine Shoot Moth *Rhyacionia buoliana,* the codling moth to *Cydia pomonella* and the corn earworm *Helicoverpa zea.*

Achievements since last visit in 1997

1) Building and equipment to mass rear the Angoumois grain moth *Sitotroga cerealella* of excellent quality installed.
2) Laboratory experiments to select effective *Trichogramma* species to control *C. pomonella* and *H. zea* successfully conducted.
3) Field experiments to control *R. buoliana* and *H. zea* successfully conducted.

Actions recommended

1) Take steps to control the itch mite in the mass rearing of *Sitotroga.*
2) Conduct semi field and field experiments to compare selected *Trichogramma* species to control *R. buoliana* and *C. pomonella.*
3) Develop a device to release *Trichogramma* in the field that protect the parasitoid from rain and predators in the field.

1) Mass rearing of the Angoumois grain moth *Sitotroga cerealella* as a host for egg parasitoids of the genus *Trichogramma.*

A mass rearing of the Angoumois grain moth *Sitotroga cerealella* was established in 1998 at the INIA CRI Quilamapu, Chillan. A description of the mass rearing method was given in an earlier report. Examination of the rearing in March 1999 showed that it is infested by the itch mite. The present report discusses the problem of itch mite attacks and recommends methods for prevention and control.

The itch mite is very dangerous; it multiplies rapidly and can destroy the *Sitotroga* rearing in a very short time. It is usually carried into the rearing by other unwanted insects and

other organisms such as beetles, cockroaches and mice. The *Sitotroga* breeding room and the adult emerging rooms should particularly be protected from this mite. These rooms should have tight insect proof windows and all cracks in roofs, floors and walls should be completely closed. Restricting the use of the rooms should prevent the entering of unwanted organisms. Unwanted insects usually infest grain purchased at the market. This should be heated in the preparation room before it is brought into the breeding room. The breeding and emerging rooms (including equipment) should be intensively cleaned and treated from the outside with a short-lived acaricide.

The itch mite can be controlled much easier in industrial plants for rear *Sitotroga* and *Trichogramma*, compared to Research Institutions. In a commercial mass rearing for egg parasitoids, no other species that transmits the mite are tolerated. Most parasitoid producers are specialized and are in position to deal with any source of attack much easier than Research Institution.

The aim of establishing the mass rearing at INIA Quilamapu is to create a prototype to assist industrial companies to build. Experience in other countries is transferred and the system is adapted to local conditions.

Possible solutions

The normal procedures at industrial level upon itch mite attack in most countries and cases is that the rearing is stopped, the infested grain is discarded, the building and equipment are fumigated, usually using ethyl bromide, and the clean rearing is reestablished using new eggs.

The above mentioned solution is more difficult for most Research Institutions compared to Industry. Large number of projects involving different organisms is going on at the same time. This does not only increase the chances of infestation but also make it difficult to fumigate without disrupting the work of many. Therefore as alternative several Research Institutions have tried to develop methods to limit the number of mites in the rearing and keep it at a tolerable level. Till now very little progress was achieved.

Action to limit itch mite attack

(1) Egg used for the mass rearing of *Sitotroga* should be dipping in a suitable acaricide before using them. Experiments with Dicofol 25 WP (0,36/300cc) and Formalin 10% and other chemicals are suggested.
(2) Use of small rooms for larval breeding that are better protected from infestation. These may be used in rotation to be cleaned thereafter. The production of one week may be put in one room and is kept for 4 weeks till the fist adult hatch.
(3) Treat breeding cribs by dipping them with suitable acaricide solution.
(4) Use small ventilator in breeding room to reduce temperature of grain after 2 weeks of infestation with *Sitotroga* eggs. Transfer grain cribs from breeding to emergence room only after the beginning of the moth emergence. Chance of mite attack is higher in emergence room.
(5) Clean and desinfect emergence cages before use.

(6) Before discarding used grain the cribs should be heated at 100°C for at least 60 minutes.
(7) The rearing of the Mediterranean flour moth *Ephestia kuehniella* should be replaced to another building and persons working with it should not enter the *Sitotroga* rearing. This insect is known to carry itch mites.
(8) During the transition time and until the *Sitotroga* rearing is reestablished, the rearing of *Trichogramma* strains should be replaced to another building and *Ephestia* eggs should be used as host. The reason for this is that the host eggs used at this time are infested with itch mite.

2) Laboratory experiments to select *Trichogramma* for use in the biological control of the codling moth *Cydia pomonella*

The comparison of several species in the laboratory by TORRES and GERDING (in preparation) showed that *T. cacoeciae* had much more preference to *Cydia pomonella* eggs that *Sitotroga* eggs, followed by *Trichogramma* sp."Cato". *Trichogramma* sp. "Remehue" had the highest fecundity on both *C. pomonella* and Anagasta kuehniella eggs, *T. dendrolimi* and *T. platneri* were less fecund and parasitised more A. kuehniella eggs than C. pomonella eggs. The results indicated that *T. cacoeciae* and *Trichogramma* sp."Cato" are good candidates for the control of *C. pomonella*, but because the last one is an indigenous species it should have priority.

Action: A semi-field test in cages each with a small apple tree could be conducted to compare the searching behavior of different *Trichogramma* species. Placing *C. pomonella* eggs and monitoring for parasitism could assess the searching performance of the parasitoid.

3) Laboratory and field experiments to select *Trichogramma* for use in the biological control of the corn earworm *Helicoverpa zea*

VELASQUEZ and GERDING assessed the suitability of 6 *Trichogramma* species to attack *H. zea* eggs. In no choice trails, the capacity of parasitism was assessed by offering the adult parasitoids eggs of either *H. zea, S.cerealella or A.kuehiella.* Monitoring was conducted after 7 days. *Trichogramma* spp. "Remehue" and *T.pretiosum* (strain 1) were found to be more effective than the remaining 4 species.

The indigenous species *Trichogramma* "Remehue" was further tested in the field with 4 different doses (200.000, 400.000, 800.000 and 1.600.000 eggs per ha). The damage to corn ears was reduced by 78% ($p = 0,05$) at the release rate of 1.600.000 eggs per ha. The lower rates also reduced damage significantly from the control plots but to lower degrees. The use of *Trichogramma* to control this pest was therefore recommended.

The use of *Trichogramma* on corn is successful in a large number of countries. One of the reasons is the dens coverage of the plants that provide suitable environment to the parasitoide, higher humidity.

Action: Conduct larger field experiments in commercial growing corn to demonstrate the effect to growers. The chances of success are high.

4) Field experiments to control European Pine Shoot Moth *Rhyacionia buoliana*

Results of field experiments carried out in 1997 were included in a previous report. GERDING conducted an experiment in 1999 in co-operation with the company "Forestal Mininco S.A.". Releases were carried out by either spraying the parasitised eggs or by hanging paper bags or capsule that included the eggs.

Counting infested top and lateral buds separately assessed the reduction in the number of infested buds in the treated plots compared to untreated areas. Two release rates "spray" were used 300.000 and 900,000 eggs per ha. The reduction of the apical buds was estimated to be 80 and 66% for the two dose rats respectively. Similarly, the reduction of the lateral buds was found to be about 50% for both the two dose rats.

The reduction of damage in the plots treated by hanging bag or capsule that included *Trichogramma* ranged from 15 to 25% and was much lower.

The field experiments conducted in Chile from 1997 to 1999 showed that the use of *Trichogramma* to control European Pine Shoot Moth *Rhyacionia buoliana* is feasible.

Action: A semi-field test in cages each with a small pine tree could be conducted to compare the searching behavior of different *Trichogramma* species. If eggs of *R. buoliana* are not available, *C. pomonella* eggs could be used to assess the searching performance of the parasitoid.

5) A new device for the application of *Trichogramma* in the field

A new releasing device is being successfully used in practice in Germany since 1994. The cardboard device is hung on a plant or tree and it protects the parasitoids from rain and from predators. This protection is very important because it makes it possible to use *Trichogramma* in different developmental stages. With this, the effect of the treatment is significantly prolonged.

Trichogramma brassicae releases to control the European corn borer *Ostrinia nubilalis* showed that the effect of one application lasted from three to four weeks depending on the temperature. Mass reared *Sitotroga cerealella* eggs, were daily parasitized by *Trichogramma*. About 7 different developmental stages of the parasitoid were thoroughly mixed, glued on to the inner surface of the device and a roof to protect the eggs was automatically placed.

General

The release of mass produced egg parasites of the genus *Trichogramma* to control lepidopterous pests has gained increasing attention world-wide in the last few years. *Trichogramma* is being used to control pests on corn, apple, plums and cabbage in

Germany since1980. Field experiments to control the European corn borer in Germany were started in 1977. The release of *Trichogramma* at the rate of 200,000 parasites / ha resulted in an increase in egg parasitism of 72 - 92 % and a reduction in larval numbers between 61 and 93 % (Hassan 1981and Hassan et al 1994).

Two companies started to produce the parasite, one in 1980 and one in 1983. The mass-rearing and releasing techniques improved with experience from year to year. Close co-operation was observed between research institutions, producers, distributors, agricultural associations, plant protection services and growers.

Every year, the companies in Germany start to produce *Sitotroga cerealella* in January and *Trichogramma brassicae* Bezdenko in Marsh. The quality of the parasitoid was regularly tested at the Institute in Darmstadt. To maintain high quality at the institute, the parasitoid was reared under fluctuating conditions (26 +/- 2°C, 70 % RH, 18 h: 90 % RH, 6 h) and the rearing was made on eggs of the natural host (*O. nubilalis*) at intervals of about 6 months. Apart from that, the beneficial arthropod was exposed to high and low temperatures at intervals (32 °C and 10 °C). To prevent bottle-neck situations, about 10 units, each with about 10,000 parasites, were maintained permanently. This widens the genetic spectrum of the parasite in the rearing.

To determine the right time to release the parasite in the field, the extension service in the releasing regions monitored the flight of the pest using light traps. After the first moth was captured, the first parasitoids release was made at the earliest opportunity. The second release was carried out about two weeks later, depending on region and weather. Mainly agricultural associations carried out the distribution of the parasite to the growers. The growers, informed by letter, collected the parasitoid at a given time and place, and released it mostly on the same day. In case of rain, the natural enemy was stored at 10 – 15 °C for 2 or 3 days without affecting vitality. Care was taken not to expose the organism to excessive heat or cigarette smoke. The release was carried out by hand and took 20 min. per ha. Releasing cards (50 ha) each with 2000 parasites (= 100000 ha/ treatment) were used.

In the present work, experiments were carried out to test the new releasing device using a mixture of *Trichogramma* in different developmental stages. To estimate the duration of treatments to control the European corn borer *O. nubilalis*, the emergence of the *Trichogramma* adults from releasing devises used in practice was assessed.

Preparation of host and parasitoide

The *Sitotroga* rearing involves (1) the preparation and infestation of grain with *Sitotroga* eggs, (2) the maturation of larvae at optimal conditions for about two weeks, (3) emergence of adults, (4) the collection of eggs, and (5) the cleaning and storing of the eggs.

Parasitism by *Trichogramma* was carried out daily by introducing host eggs into cages with large numbers of *Trichogramma* adults. The ratio of parasitoid to host was 1 : 3. Rearing temperature was 27 °C with 70% RLF and continues week defused light. A mixture including immature stages of *Trichogramma* from 1 to 6.5 days of age was used for the field applications.

After mixing the parasitised host eggs, these were introduced to a machine that automatically glued them on the inner surface of the card board device. By the same machine, the card board is folded and formed to include a closed section that include the eggs and a hanger. Small openings are left for *Trichogramma* to leave the devise.

Description of device

The device used by AMW Nuetzlinge GmbH is 11.5 cm long and 5.5 cm wide. The hanger part is 7.00 cm long and 5.5 cm wide, it has a triangle hole in the centre opened from one side to place the device on the plant. This triangle hole is 3.5 x 3.5 x 3.5 cm situated in the centre leaving a wide margin of 1.3 cm from the left and the upper sides. The right edge is 0.8 cm wide and has the cut to inserting the devise around the plant, i.e. corn stomp. The eggs are glowed on an area of 1.5 x 5.5-cm of the inner surface. The total length of the device is 15.5 cm and the lower 4.2-cm part of it, is bent to form the roof over the eggs. The parasitoids leave the device from openings on both the right and left sides. The device used by Biocare is different in form but provide the same advantages.

The duration of a treatment depends on the ratio of the different ages of the *Trichogramma* development stages used in the mixture. To judge on the quality of the mixture produced for practical use and the degree of protection provided by the device, the emergence of the adults from samples of commercially used *Trichogramma* was tested. The eggs from releasing devices were placed in glass tubes, provided with honey as food, closed with gauss and placed at outside temperature. The adults in the tubes were counted every day until all the parasitoids died.

Time of effectiveness of treatment

The results of the experiments carried out to test releasing cards of the companies Biocare and AMW Nuetzlinge GmbH was given. The *Trichogramma* adults of the first release were available from June 22 to July 24, 1998 (4 weeks), the second release from July 4 to August 3 (4 weeks), (AMW) first release from 22 June to July 24 (4 weeks), second release from July 8 to August 2 (3 weeks). Adult parasitoids emerged nearly every day from the devices of both companies. The emergence patterns showed strong overlap of adult occurrence between the first and second applications. The overlap was partly intentional and desirable because of the high pest occurrence at that time. In future, delaying the second release for a few days should reduce the period of overlapping. The emergence pattern of the single treatment carried out by AMW was similar to the first release of the two application method, covering the period from June 22 to July 24, 1998.

Protection from rain and predators

Field observation showed that the new device provided excellent protection from rain as well as a fairly good protection from attacks by large predators such as *Chrysoperla carnea* in the field. However, earwigs and ants were sometime observed to enter the device and feed on the eggs. This can be reduced by optimising the size of the exit openings of the

device. The near complete rain protection greatly increases survival of the immature stages, compared to the open releasing cards previously used.

Remarks and advantages

The release of mass reared *Trichogramma* is carried out in different ways. In some Eastern European countries, adults are released from glass containers that are opened at each releasing site. A plant leaf of a crop is insert in the glass so those adults of *Trichogramma* can jump on it and then released. In other countries i.e. South America, parasitised host eggs in one age are placed in paper bags or glowed on cardboard that are brought out to the field. In both cases, releases at intervals of about 4 to 7 days are necessary. Growers are often reluctant to release the parasitoid at such short intervals.

The main advantage of using the new devise described in the present work is that it makes it possible to include *Trichogramma* in different development stages, including younger stages that need longer periods of time to emerge in the field. This way the effect of the treatment is prolonged and the intervals between releases increased.

Trichogramma brassicae releases carried out to control the European corn borer *Ostrinia nubilalis* in 1998 showed that the effect of one treatment lasted from three to four weeks depending on the temperature. This can be compared to 4 – 5 days when adults are released or 7 – 10 days when one development stage is applied (personal experience).

Action – releasing device

It is suggested that a device be produced in Chile for releasing *Trichogramma* that will provide protection to the parasitoide from rain and predators in the field. Ideal would be that the device can be suitable for used for hand or for air distribution.

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AVANCES DE CONTROL BIOLOGICO CON TRICHOGRAMMA EN EL AMBITO FORESTAL EN CHILE

Dr. Marcos Gerding

La historia de los Trichogramma se remonta al año 1883 cuando fue determinado el género Trichogramma y ubicado en el orden Hymenoptera, familia Trichogrammatidae. Hasta hoy se han identificado en el mundo, más de 145 especies de Trichogramma, que están parasitando huevos de más de 400 especies de insectos, destacándose el orden lepidoptera como huésped de este género. *Rhyacionia buoliana* es también parte de los lepidopteros susceptibles al control con Trichogramma. La primera confirmación a este hecho es que en la zona de Angol en 1994, el entomólogo Ernesto Cisternas, encontró huevos de *R. buoliana* parasitados por una especie de Trichogramma, esta resultó ser nueva especie para la ciencia y está en proceso de publicación, su nombre será *Trichogramma nerudai*. Esta detección de la especie nueva, realizada tanto por INIA como por MININCO, en huevos de la polilla del brote se repitió por tres años consecutivos,. Existen además otras detecciones de Trichogramma en rodales de pino, capturados por los profesionales de BIOFOREST, utilizando huevos centinelas (trampas), esta colección viva se encuentra en los laboratorios del CRI Quilamapu-INIA, a la espera de ser identificadas. La mayoría de estas especies capturadas son solamente hembras, lo que impide su identificación, pues la taxonomía de los Trichogramma se basa en la genitalia masculina.

En 1994 se introdujeron dos especies de Trichogramma, *T. dendrolimi y T. telengai*, con miras a liberarse en el bosque. Estudios de laboratorio de demostraron que la especie nativa era igual o mejor que las dos especies que se habían importado.

La utilización de Trichogramma en gran escala como agente de control biológico está ampliamente desarrollada en el mundo, en los últimos 20 años mas de 30 países se han visto involucrados en la utilización de Trichogramma para el control de diferentes plagas en cultivos y explotaciones forestales. En el presente trabajo se pretende informar de los avances y tecnología que están siendo desarrolladas en el mundo para el uso de Trichogramma y las posibilidades que se tienen en Chile para adoptar esta estrategia de control como parte del manejo de plagas.

Los Trichogramma son pequeños insectos de 0,2 a 1,5 mm de tamaño, que se distínguen por tener tres segmentos en el tarso, su color es variable entre amarillo y café obscuro y presentan una pequeña vena sigmoidal en el ala. Son insectos solitarios o gregarios, endoparásitos de huevos de varios ordenes de insectos, siendo los lepidópteros los más comunes. La reproducción puede ser telitoquica en algunas especies sólo se encuentran hembras y en otras especies arenotoquica se presentan ambos sexos.

En Chile se ha estado experimentando con Trichogramma en el control de la polilla del brote del pino, en la selección de la especie, dosis de liberación y método práctico para la liberación en el bosque. En liberaciones se ha estado desarrollando métodos de aspersión con pulverizadores de espalda, con buenos resultados y cápsulas de celulosa que pueden ser distribuidas desde el aire.

RESULTADOS

En estudios realizados en laboratorio se definió que especie tenía mayor preferencia por los huevos de *R. buoliana*. Las observaciones realizadas durante los primeros 30 minutos el mayor porcentaje de encuentros lo presenta *T. dendrolimi* (strain D4) en las tres edades del huevo (Figura 1), demostrando una mayor eficiencia de encuentro con el huésped, en relación con la edad del huevo no se apreciaron diferencias. De la misma forma en las observaciones realizadas luego de terminada la parasitación, no hubo diferencias en el parasitismo de huevos de una edad determinada. En relación con la especie y strain más eficiente parasitando *R. buoliana*, *T. nerudai* fue significativamente mejor que las otras dos especies y sus strains (Cuadro 1 y Figura 2).

Cuadro 1. Parasitación de huevos de *R. buoliana* por tres especies de *Trichogramma*.

Edad de huevos		Encuentros durante 30 minutos (%) 1 día		2 días		3 días		Parasitismo a los 5 días (%) 1 día		2 días		3 días	
T.	D 4	24.0	a	19.3	a	17.3	a	70.7	ab	70.0	ab	54.7	b
Dendrolimi	D 9	13.3	b	8.7	bc	8.7	bc	64.0	b	56.7	bc	47.3	bc
	D 12	5.3	cd	8.7	bc	11.3	abc	49.3	bc	45.3	bcd	34.7	bc
T. telengai	T 177	4.7	d	4.7	c	6.0	c	16.7	d	29.3	cde	38.7	bc
	T 180	15.3	ab	7.3	bc	7.3	c	38.0	c	26.7	de	24.7	c
	T 183	15.3	ab	5.3	c	9.3	abc	47.3	bc	14.0	e	20.7	c
	T 189	8.0	bcd	6.0	c	5.3	c	48.7	bc	45.3	bcd	44.7	bc
T. nerudai		12.0	bc	14.7	ab	15.3	ab	92.0	a	84.0	a	90.7	a

En cada columna letras iguales indica que no existen diferencias significativas Prueba de Rango Múltiple de Duncan (PRMD) ($P \leq 0.05$)

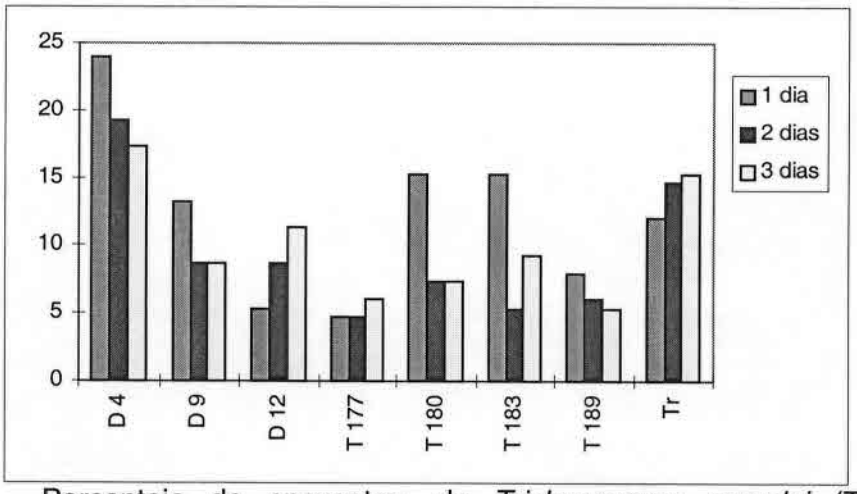

Figura 1. Porcentaje de encuentros de *Trichogramma nerudai* (Tr), *T.dendrolimi* (D) *y T.telengai* (T) con huevos de *R. buoliana* de 1, 2 y 3 días de edad, a los 30 minutos de observación.

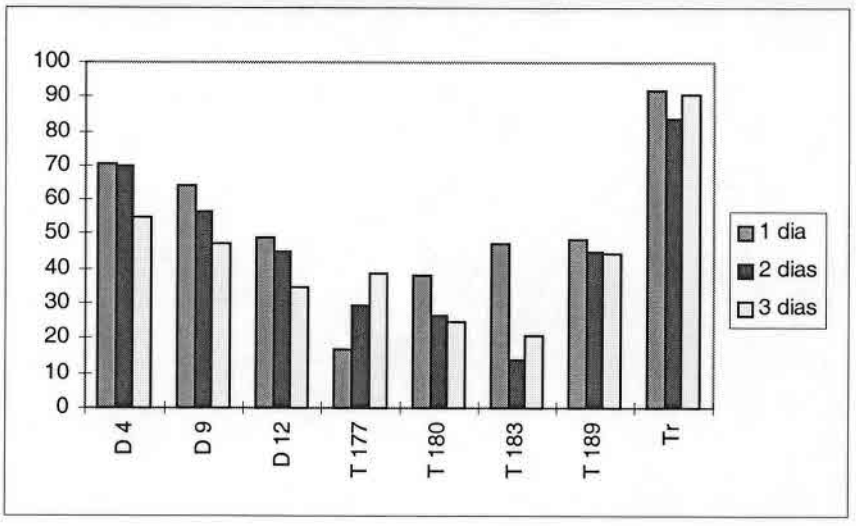

Figura 2. Porcentaje de parasitación de *Trichogramma nerudai* (Tr), *T.dendrolimi* (D) *y T.telengai* (T) en huevos de R. buoliana de 1, 2 y 3 días de edad.

Pruebas de preferencia entre el hospedero *natural R. buoliana* y alternativo *Sitotroga cerealella*

En los primeros treinta minutos de observación no existió una diferencia en el número de contactos de los Trichogramma con el huevo de los distintos hospederos ya que la hembra al verse expuesta ante huevos alternativos, inicialmente no mostró una clara preferencia, sino hasta que ha palpado con sus antenas y reconocido al hospedero preferido. Sin embargo, en las observaciones al término del proceso de parasitación, *T. dendrolimi* (D9), *T. telengai* (T177, T189) y *T. nerudai* presentaron una mayor aceptación por huevos de *R. buoliana*, como se aprecia en el Cuadro 2, en que las diferencias entre el parasitismo en *R. buoliana* y *S. cerealella* fue altamente significativo (Figuras 3 y4). Sólo en el caso de *T. nerudai* se observó que las hembras ovipusieron más de un huevo en cada huésped, un 93% de los huevos parasitados por esta especie presentaban doble embrión del parasitoide.

Cuadro 2. Porcentaje de parasitación de *Trichogramma* sobre *R. buoliana* (Rb) y *S. cerealella* (Sc) .

ESPECIE	STRAIN	Contactos durante 30 minutos (%)				Parasitismo a los 5 días (%)				Diferencia *R. b. - S. c.*	
		R. b.		S. c.		R. b.		S. c.			
T. dendrolimi	D 4	12.7	A	19.3	a	30.0	c	42.0	a	-12.0	ns
	D 9	9.3	A	10.0	ab	52.0	ab	22.7	b	29.3	**
	D 12	6.0	A	5.3	bc	10.0	d	9.3	cd	0.7	ns
T. telengai	T 177	12.0	A	9.3	bc	57.3	a	22.7	bc	34.6	**
	T 180	8.7	A	0.7	c	14.0	d	6.0	d	8.0	ns
	T 183	16.0	A	4.7	bc	40.7	bc	13.3	bcd	27.4	**
	T 189	8.7	A	5.3	bc	44.7	abc	16.0	bc	28.7	**
T. nerudai		11.3	A	8.7	bc	62.0	a	19.3	bc	42.7	**

En cada columna letras iguales indica que no existen diferencias significativas PRMD ($P \leq 0.05$)
ns no existen diferencias significativas
* diferencias significativas PRMD ($P \leq 0.05$)
** diferencias significativas PRMD ($P \leq 0.01$)

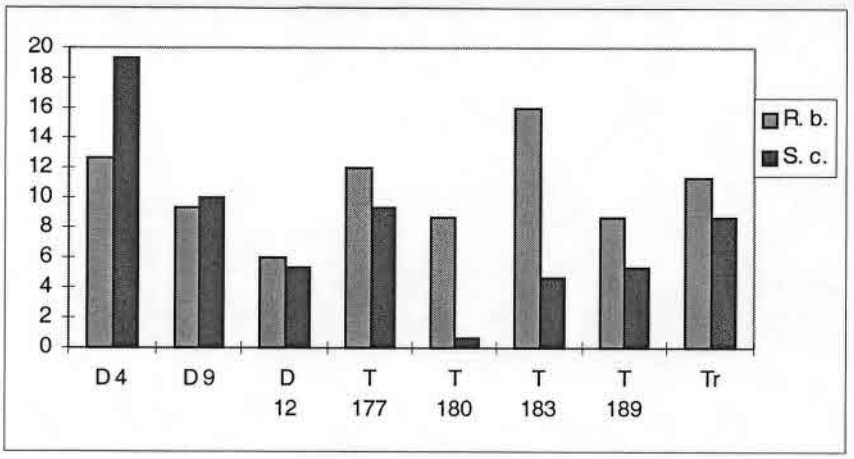

Figura 3. Porcentaje de encuentro de *Trichogramma nerudai* (Tr), *T.dendrolimi* (D) *y T.telengai* (T) con huevos de *S. cerealella* y *R. buoliana* a los 30 minutos de observación.

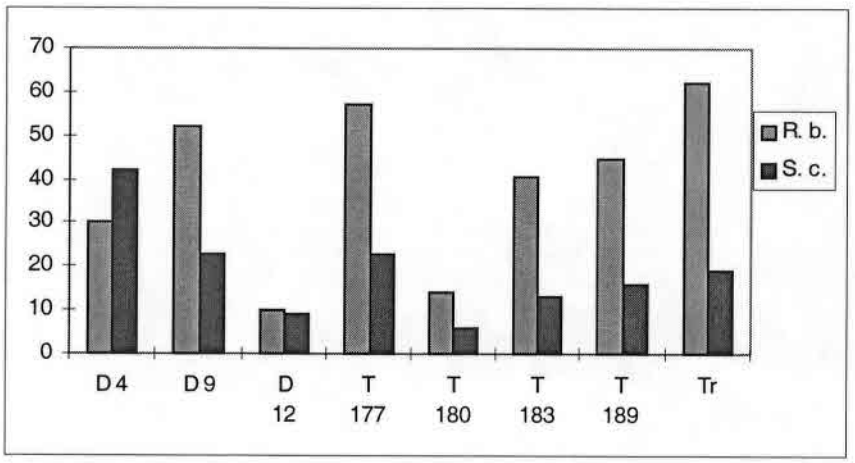

Figura 4. Porcentaje de parasitación de *Trichogramma nerudai* (Tr), *T.dendrolimi* (D) *y T.telengai* (T)sobre huevos de *S. cerealella* y *R. buoliana* a los 5 días de observación.

Con estos resultados se ha definido no utilizar los otros parasitoides en las liberaciones de Trichogramma en el control de la polilla del brote del pino. Con los resultados de terreno, no publicados, se han obtenido reducciones en el nivel de daño, utilizando dosis de liberación que permitan el manejo de los Trichogramma en laboratorio..