Developing Trichogramma (Hymenoptera: Trichogrammatidae) as a pest management tool

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Abstract
The egg parasitoid, Trichogramma (Hymenoptera: Trichogrammatidae), is used extensively around the world as a biological control agent for the control of lepidopterous pests. Wasps are either released to augment an existing population “inoculative release,” or they are released in large numbers to coincide with maximum pest pressure “inundative release.” Field releases however, have had variable success. This has been attributed to wasp quality and issues relating to the release and integration of wasps into an agricultural setting. Wasp quality can be split into genetic and environmental components. Here, genetic quality is discussed in terms of the identification and maintenance of species/strains most suited to the particular situation. Environmental effects that are thought to impact upon wasp quality include rearing host effects; rearing conditions i.e. under constant environmental conditions, or by acclimation; and storage conditions. Release and integration issues that are considered important for the development and maintenance of a successful IPM approach include host/parasitoid synchrony, pesticide choice and timing of application as well as weather conditions at the time of release. In this paper we focus on and discuss quality issues, both genetic and environmental, as well as consider information pertaining to optimal release conditions, in relation to the development and maintenance of Trichogramma as an effective biological control agent.

Introduction
Parasitoids can have a major impact in natural and agricultural ecosystems where they influence or regulate the population density of many of their hosts (Godfray 1994). Trichogramma and other egg parasitoids are generally part of the local ecosystem and often contribute to the control of lepidopterous pests in the absence of disruptive pesticides. There are many examples of pest control by naturally occurring Trichogramma such as the control of Helicoverpa eggs in corn in Brazil (De Sa & Parra 1994), control of the noctuids, Brusseola fasca and Jesamina calamistis, and pyraloids, Chilopartellus orichalociliellus and Eladana saccharins, which attack maize in east Africa (Bonhof et al. 1997) and control of the cranberry fruitworm, Acro basis vaccinii, in the U.S.A. (Simser 1994).

Naturally occurring predators and parasites are often not present in sufficient numbers at the right time to keep pest species within an economically sustainable limit. Trichogramma release programs can be used to overcome these limitations. There are two ways to use Trichogramma release in pest control: “inoculative” releases to maintain and augment an existing population, or “inundative” releases to introduce large numbers of insectary reared Trichogramma to coincide with maximum host presence. Both approaches aim to increase Trichogramma parasitism of the pest to reduce crop losses.

Trichogramma have been used in inundative releases more than any other natural enemy (Stinner 1977). Situations where Trichogramma are used to control lepidopterous pests include grapes (Glenn & Hoffmann 1997), tomatoes in greenhouses (Shipp & Wang 1998), tomatoes in the field (Consoli et al. 1998) and sugar cane (Greenberg et al. 1998a). Trichogramma are also used against Helicoverpa armigera on a variety of crops in India (Romeis & Shanower 1996) and on sweet corn in Australia (Scholz et al. 1998). Trichogramma is an effective biological control agent against the European corn borer, Ostrinia nubilalis (Lepidoptera: Pyralidae), throughout Europe (e.g. Mertz et al. 1995) and North America (Andow et al. 1995). They are used in nonfood crops such as cotton (Naranjo 1993) and to provide foliage protection in forests (Bai et al. 1995). Trichogramma are even used against Lepidoptera in stored grain, where Trichogramma evanescans and T. embryophaga attack Ephestia kuehniella and E. elutella (Scholler et al. 1996).

Despite the widespread use of Trichogramma, there are relatively few cases where the successful control of a pest can be unequivocally ascribed to releases of these parasitoids. There are many documented failures of Trichogramma releases despite a few notable successes (Twine & Lloyd 1982, Smith et al. 1987, Li 1994).
The variable success of Trichogramma field releases can be attributed to a number of factors (Smith 1996). These include pesticide applications, weather conditions at the time of release, lack of host parasitoid synchrony and the quality of the release material. This last area is complex and encompasses diverse issues such as technical aspects related to rearing, storage and shipping of the release material as well as the characteristics of the Trichogramma chosen for release programs.

In this paper we focus on quality issues and consider three main questions. Firstly, how do we ensure that the Trichogramma strain or species is suited to release conditions? This requires effective screening of genetic variability. Once suitable strains are identified, mass reared populations need to be monitored to ensure that undesirable characteristics are not selected. Secondly, which environmental factors can influence quality and how do we manipulate these factors to ensure that the quality of released wasps is maximised? Most environmental factors relate to the nature of the rearing host, which can influence the size and behaviour of the adult wasps. Finally, what release conditions influence the success of Trichogramma? Releases should ideally be integrated into all aspects of the target pest environment using a system approach (Lewis et al. 1997).

**Quality issues: genetic and environmental components**

Phenotypic variation can have genetic and environmental components. Environmental variation is often mediated via the size of the host egg, which in turn affects the size of the Trichogramma (e.g. Bennett & Hoffmann 1998, Glenn & Hoffmann 1997). In addition, there are genetic factors that can act independent of size. For example, Bigler et al. (1988) and Dutton et al. (1996) found that walking speed was affected by genetic factors independent of size.

**Genetic quality: identification and maintenance of appropriate strains**

Successful commercial release programs require Trichogramma that are effective against target hosts under field conditions. The overall release quality of Trichogramma will be influenced by many traits. Identifying which traits are related to optimal release quality is a major problem when screening for suitable strains. It depends on the specific field situation in which the parasitoids are being used as to which trait(s) will be important. Once an effective strain has been identified, quality needs to be maintained, avoiding selection for undesirable traits in mass rearing conditions.

There are many examples where Trichogramma species and strains of the same species differ in traits that may be related to field performance. For instance, the ability of foraging females to discover hosts has been found to vary among strains. Foraging ability is likely to be related to the surface area that females prospect per unit time (Bigler et al. 1993) and walking speed (Bigler et al. 1988, Dutton et al. 1996). Strain variation has also been found for longevity (Bigler et al. 1993, Dutton et al. 1996), emergence rates (Dutton et al. 1996, Frei & Bigler 1993), sex ratio (Bigler et al. 1993) and fecundity on both natural and factitious hosts (Bigler et al. 1993, Dutton et al. 1996, Frei & Bigler 1993).

Trichogramma strains can also differ in the extent to which they respond to environmental change. Variation has been found between species and strains for their ability to survive and parasitise under different temperatures (Pak & Van Heiningen 1985). The variation in Trichogramma strains for cold tolerance and adaptability to low temperatures makes this a useful criterion for evaluation of candidate strains for inundative releases in cold conditions (Voegele et al. 1988). Flight propensity (Forsse et al. 1992, Prasad et al. 1999), walking speed and activity (Suverkropp et al. 2001) and rates of travel (Boldt 1974) are also affected by temperature and may represent useful traits for screening.

While it is clear that variation exists among Trichogramma strains and species for a number of traits that may contribute to the success of field releases, there are three questions that need to be addressed when assessing the potential impact of this variability.

**What is the relative contribution of genetic versus environmental factors to the phenotypic variation?**

Identifying strain differences within one generation represents a first step in a genetic analysis, but provides no information as to whether variation persists across generations particularly if environmental conditions change. Genetic studies need to encompass more than one generation and should ideally consider the heritability of traits across different environments. Examples of genetic studies on parasitoids encompassing more than one generation include Wajnberg and Colazzo (1998) and Bennett and Hoffmann (1998).
What are the effects of mass rearing on phenotypic variation?

Once useful strains have been identified, phenotypes can still change due to commercial conditions. Mass rearing facilities usually have constant temperature and regulated periods of dark and light, with high parasitoid host densities and limited need for host searching behaviours such as walking or flying. The presence of genetic variation within species means that Trichogramma may readily adapt to conditions used in mass rearing facilities (Sorati et al. 1996). This appears to be one of the major problems encountered in quality control of Trichogramma for mass release (van Bergelijck et al. 1989). Inbreeding effects may also cause a decline in quality in commercial cultures. However, as Trichogramma are haplodiploid, major inbreeding effects are unlikely. Sorati et al. (1996) suggest that the absence of inbreeding effects in Trichogramma could be used in the maintenance of mass reared colonies. If the strains selected for mass release could be intensively inbred to fix useful genes and minimise their loss during adaptation to artificial environments, desirable traits could be maintained.

Which traits are relevant for field success?

There is likely to be an association between locomotion and parasitism in the field. Variation in travel speed has been used to estimate capacity for host location and the efficiency of *T. maidis* strains for inundative biological control programs (Bigler et al. 1988). There have also been attempts to combine quality parameters in devising an index for quality (Liu & Smith 2000). In particular, Dutton et al. (1996) measured four quality parameters: walking speed, lifespan and fecundity on the natural host as well as the factitious host. However, fecundity on the factitious host was found to be a better predictor of success in the field than the quality index. More recently, fluctuating asymmetry has been looked at as an additional indicator of wasp quality. Hewa-Kapuge and Hoffmann (2001) found that although there was no association between the asymmetry of individual traits and wasp fitness, the ability of *Trichogramma nr. brassicae* to successfully find host eggs could be predicted by the combination of asymmetries of nine different traits.

Environmental effects: rearing host

To rear Trichogramma on a commercial scale, it is necessary to use a factitious rearing host, such as *Ephesia kuehniella* or *Sitotroga cerealella*, rather than the natural or target host. The choice of factitious host is often dictated by the ease of rearing and not necessarily by any factors related to the likely success of the wasps being produced. Factitious hosts are selected on the simplicity of their mass production, mechanisation of rearing processes and cost of production compared with that of using the target pest (Greenberg et al. 1998b).

There are several potential effects of rearing parasitoid biocontrol agents on a non-target host. Rearing host can affect qualities such as development time (Bai et al. 1995), travel speed (Boldt 1974), longevity, percent emergence and sex ratio (Corrigan & Laing 1994). The size of the factitious host egg could alter the size of the emerged wasp, which could have effects on wasp attributes such as target host acceptance and fecundity. Often Trichogramma reared from one host will be used to target several pests, introducing many potential complications, as the same wasps may not be suitable across several different hosts.

There appear to be only minor changes in acceptance of the target host when Trichogramma are reared on a factitious host. Bjorksten and Hoffmann (1995) found that oviposition experience had a stronger effect on host preference than pre-adult experience (learning through development in rearing host). Bjorksten and Hoffmann (1998) found experience effects in *T. nr. brassicae* due to rearing host and oviposition by females, but these effects only influenced the likelihood of parasitism in low ranked hosts and not in high ranked hosts that would comprise the target pests in a mass release program.

There is evidence that rearing host can cause variation in size of emerged wasps (Bai et al. 1995). *T. carverae* reared on *E. postvittana* eggs are significantly larger than those reared on *S. cerealella* (Glenn & Hoffmann 1997) and the size of mass produced *T. pretiosum* and *T. minutum* adult females is dependent on the size of the rearing host egg in which the insect develops (Greenberg et al. 1998c). But does size matter? There are reports of increased size being associated with increased fitness in parasitoids, measured as success in locating hosts (Bennett & Hoffmann 1998, Kazmer & Luck 1995) and with increased fecundity (Bai et al. 1995, Greenberg et al. 1998c) although this is not always the case. Bigler et al. (1993) found that wasps emerged from *E. kuehniella* were bigger than those emerged from *S. cerealella*, but there was no perceived quality difference.
Because results may not be consistent across studies, it is necessary to examine factors influencing field performance in chosen species/strains. For instance, where size may be a good indicator of success of *T. carverae* released in grapevines (Bennett & Hoffmann 1998), this may not be true of other species/systems. Fecundity on the factitious host may be species/strain specific so it is necessary to compare fecundity on the rearing host to the target host before a comprehensive cost-benefit analysis of mass rearing-release system can be made (Corrigan & Laing 1994, Greenberg et al. 1998c).

**Other environmental effects**

Apart from host effects, other environmental factors may also impact on wasp quality. In particular, wasps can become acclimated to particular conditions. Mass production facilities rear the wasps at constant temperature (usually 25°C). It is possible that wasps reared under constant conditions in insectaries are of low quality because they fail to survive extreme temperature fluctuations encountered in the field (Scott et al. 1997). This may reduce the effectiveness of releases in cold weather. Scott et al. (1997) reared *T. carverae* at three temperatures (14°C, 25°C and 30°C) and found that only wasps reared at 14°C parasitised eggs at that temperature. The substantial reduction in parasitism of the sugarcane borer, *Diatraea saccharalis*, by *T. galloii* in the winter months may be related to a thermal alternation shock, as the wasps are mass reared at constant temperatures (Consoli & Parra 1995).

Responses to high temperature can also be improved via acclimation and may be useful in improving the quality of beneficial insects (Huyn & Berrigan 1996). There is some experimental evidence that heat resistance can be increased by acclimation. Scott et al. (1997) found that heat hardening *T. carverae* adult wasps at 33°C or 35°C for 1–2 hours increased survivorship at 40°C. Maisonhaute et al. (1999) and Hoffmann and Hewa-Kapuge (2000) also found that high temperatures could protect against subsequent lethal heat shocks. Unfortunately any benefits of acclimation could be offset by fitness costs of the acclimation process (Hoffmann 1995, Huey & Berrigan 1996). Gunie and Lauge (1997) found that temperature shocks affected the emergence rate and fecundity of females. Even a short low amplitude shock of 32ºC had a strong negative effect on emergence rate and fecundity under benign conditions. However, in both *T. nr. brassicae* and *T. carverae*, acclimation conditions can be set so that there are no deleterious effects on parasitism rate and longevity (Hoffmann & Hewa-Kapuge 2000, Thomson et al. 2001). Acclimation seems a promising procedure for releases when temperature extremes are unavoidable, particularly as acclimation has been shown to increase parasitism under field conditions (Thomson et al. 2001). But specific research is needed to determine the optimum temperature, stage of development and length of exposure for different species/strains to improve performance at high temperatures while minimising the costs.

**Quality control**

To ensure a high quality product is delivered to the grower, optimum conditions for rearing, storage and shipment are imperative. It is important that producers become aware of the negative effects of poor handling of Trichogramma (Dutton & Bigler 1995). Bigler et al. (1993) tested the quality of commercially available Trichogramma and concluded that more elaborate product control systems were necessary to increase reliability of the product.

Product quality is recognised as one of the most important reasons for failure of the biological control agent *T. chilonis* against *H. armigera* (Romeis et al. 1998). O’Neil et al. (1998) evaluated the quality of four commercially available natural enemies including *T. pretiosum*. The post shipment quality from ten companies was assessed for emergence rates, sex ratio, survivorship, species identity, reproduction and parasitism. Considerable differences in the number received, survivorship and emergence rates were found. Field studies using commercially available *T. brassicae* against *O. nubilalis* in sweet corn differed in emergence profiles in two different years (Mertz et al. 1995). When several commercially available species of Trichogramma used against *Plutella xylostella* were compared, inconsistent responses were observed within most of the products indicating potential problems with quality control (Vasquez et al. 1997).

The insects need to be reared on an industrial scale and cold storage makes possible the management of large quantities of living material for intensive periods of use. It is necessary for rearing facilities to have reliable systems for storing eggs, pupae or adults (Chang et al. 1996). Diapause and quiescence constitute major physiological adaptations for sustaining survival during environmental extremes and both adaptations can have practical applications in storage during mass rearing (Chang et al. 1996, Zaslavski & Umarova 1990).
Unfortunately, cold storage may impact on the success or efficiency of the organisms to be released. These effects include reduced fecundity (Chang et al. 1996, Frei & Bigler 1993), poor flight activity (Dutton & Bigler 1995), reduced longevity (Jalali & Singh 1992) and reduced emergence rate (Cerutti & Bigler 1995, Jalali & Singh 1992).

**Release and integration issues**

After selection of the optimum species or strain for the target host and ensuring the best product possible after storage and shipment to the point of release, release conditions need to be considered to ensure maximum parasitism. The grower has some control in ensuring host parasitoid synchrony, avoiding contact with harmful pesticides, controlling the stage of development at the time of release, providing protection from predation and releasing in optimal weather conditions.

**Host parasitoid synchrony**

Host availability is the key to realising the highest possible level of parasitism. For successful augmentative releases, monitoring of the pest species is essential to ensure application of the Trichogramma when host eggs are available (Hassan 1994). Large numbers of released organisms need to be synchronised closely with the start of oviposition in the pest (Smith 1994). Monitoring of the target host must be continued through the growing season of the crop and further inundative applications made when appropriate. It is also necessary to ensure that target eggs in the area at the time of release will be acceptable to the released Trichogramma. A high rate of parasitism will be achieved only if the wasps reach the host eggs when they are susceptible to parasitism (Glenn & Hoffmann 1997). Reports vary as to the acceptability of host eggs of different ages to Trichogramma species. Some eggs are acceptable for most of their development time (Glenn & Hoffmann 1997), but other studies show the age of host eggs will affect performance (Shipp & Wang 1998, Monje et al. 1999).

**Pesticides**

Whether attempts to improve levels of Trichogramma parasitism are by inoculative or inundative methods, there is a high potential for failure if there is associated pesticide use. Trichogramma is generally sensitive to pesticides (Franz et al. 1980). Chemicals can be immediately toxic and cause death (contact toxicity) and this effect can persist (toxicity of dried residue) (Hassan et al. 1998, Thomson et al. 2000). Contact with pesticides at the less susceptible life stage (parasitoids within hosts) can cause prolonged development time of immature stages and reduced rates of emergence, fecundity, parasitism capacity, adult longevity and mating likelihood (Franz et al. 1980, Consoli et al. 1998, Hassan et al. 1994, Brunner et al. 2001, Takada et al. 2001).

Exposure of Trichogramma adults to pesticides may also interfere with mating. Delpuech et al. (1999) found that pyrethroids and chlorpyrifos at sublethal doses interfered with sex pheromone communication. Male *T. brassicae* treated with a very low dose of the insecticide deltamethrin failed to respond to females and untreated male response to treated females was also significantly decreased. In Trichogramma, like other insects, sex pheromonal communication probably involves nervous transmission both for the reception and emission of the pheromone. Even if insecticides do not provoke mortality, sublethal doses may be a threat for species and population equilibria by modifying physiological or behavioural parameters such as pheromonal communication.

There is a changing perception of pesticide use as resistance and effects of chemicals are recognised, leading to ways to minimise chemical use and combine pesticide use with protection of introduction of beneficial organisms in IPM programs. As it becomes more apparent that biological control is an important component of pest management, there has been a change in consumer attitude which is leading to the development of suitable new pesticides. Research is currently identifying pesticides suitable for use in conjunction with biological control, as it is clearly preferable to use chemicals that have minimum toxicity to beneficial organisms (Franz et al. 1980). To determine which chemicals will be most suitable for inclusion in an IPM program, insecticides must be tested for their effect on beneficial species. A battery of standardised laboratory and semi field test protocols based on lethal and sublethal effects as evaluation criteria for the side effects of pesticides on beneficial organisms have been developed by the IOBC/WPRS working group ‘Pesticides and Beneficial Organisms’ (Hassan et al. 1994, Hassan et al. 1998). The results showed that preparations greatly differ in initial toxicity as well as persistence.
Reducing pesticide resistance and replacing insecticide use with inoculative or inundative releases of Trichogramma is complicated by the fact that each crop is sprayed with many chemicals. For this reason, rather than approach biological control as a replacement for chemical control, a more conservative integrated pest management (IPM) approach is often used. In such a program efforts are made to reduce the use of pesticides, to test and use less toxic pesticides to beneficial species and to remove releases of beneficial organisms as far as possible from spray applications (Thomson et al. 2000). In fact several researchers report higher parasitoid activity and better control of pests without the use of pesticides (Scholz et al. 1998, Simser 1994). For instance, in Helicoverpa armigera on sweet corn in Australia, an application of deltamethrin reduced the action of a large natural population of T. pretiosum wasps and resulted in higher larval infestation and significantly more cob damage (Scholz et al. 1998).

Weather conditions at the time of release
Weather is one of the most important factors influencing the performance of biological control agents (Naranjo 1993, Wang et al. 1997, Shipp & Wang 1998, Fournier & Boivin 2000). Climatic factors, particularly temperature extremes, must be considered in any project involving pest management with beneficial insects. In India, several species of Trichogramma have gained importance against different lepidopteran pests, but the performance of these parasitoids under some climatic conditions seems erratic (Ramesh & Baskaran 1996). Trichogramma galloi is the most common egg parasitoid of the sugar cane borer, Diatraea saccharalis, in São Paulo, Brazil. Field releases indicate a substantial reduction in parasitism capacity of T. galloi during the winter months (Consoli & Parra 1995). The likely weather conditions at the precise time of release also need to be considered particularly where Trichogramma may encounter daily extreme temperatures. Ambient air temperatures in desert cotton growing areas of California and Arizona where Trichogramma are released to control pink bollworm, Pectinophora gossypiella Saunders, frequently exceed 40°C (Naranjo 1993). T. brassicae released to control European corn borer, O. nubilalis, in south east France may be exposed to temperatures as high as 44°C during dispersal in enclosed cardboard capsules (Chihrane & Lauge 1996).

Concluding remarks
To address the inherent variability, each pest system must be studied both in the laboratory and in the field before a cost benefit analysis can be completed. Maximum fecundity on the target host, competence at the required temperature (is acclimation appropriate?) and optimum rearing host to produce wasps of high fitness and fecundity are key considerations. Different rearing procedures can result in important variation in the quality of the Trichogramma. This variation as well as genetic changes in continuous laboratory cultures may have significant effects on laboratory reared parasites to be released in the field. The economics of production may favour producing and releasing greater numbers of less efficient parasitoids to obtain the same degree of control although this may not compensate for low quality Trichogramma (Dutton et al. 1996). Low numbers of wasps should prove equally effective if large individuals with a high fitness are released (e.g. Bennett & Hoffmann 1998). The cost benefit analysis for each system must include the cost of producing the optimum Trichogramma for the situation and investigate whether the same result can be achieved more economically by producing more of a less effective wasp. Field trials are essential to confirm that laboratory tests of suitability are confirmed prior to release. Finally research is needed to understand methods of storage and dispersal of wasps to ensure the best product possible is released at the point of use.

In summary, improvement in the success of Trichogramma as a biological control agent requires optimisation of the materials used, particularly choice of rearing host for provision of a high quality product. This product cannot be affected by inappropriate cold storage or poor conditions during transit and needs to be released at the optimum time to maximise the number of Trichogramma at the time of maximum host density. Releases should be appropriately timed to minimise interaction with toxic pesticides or else relatively harmless pesticides must be chosen. Most importantly, each system must be studied with the optimal species or strain chosen and the potential of acclimation assessed. Obviously not all these parameters can be chosen to fit a specific case, but in a cost benefit analysis, the cost of each choice can be considered in terms of maximising benefit. A more sophisticated/integrated approach is required where all aspects of the host/parasitoid interaction as well as the physical conditions such as temperature or presence of pesticides need to be considered.
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The management of diamondback moth and other crucifer pests


