Improving biocontrol of *Plutella xylostella*

Edited by A.A. Kirk ■ D. Bordat

Proceedings of the international symposium

Montpellier, France
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CIRAD, the Centre de coopération internationale en recherche agronomique pour le développement, is the French agricultural research centre for international development. Its mission is to contribute to the economic development of the tropical and subtropical regions through research on agriculture, training, and dissemination of its results.
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Cover
Diadromus collaris Gravenhorst female
Diadegma mollipla (Holmgren) female
Cotesia plutellae (Kurdjumov) cocoons
Plutella xylostella (L.) female
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Contents

Preface

Plutella xylostella (DBM) is the most cosmopolitan of pests and has spread, in part naturally by wind aided movement, and by the hand of man, to all those parts of the planet where crucifers are grown as crops or exist as wild plants. It is resistant to many pesticides and some biologically based toxins. Hence biological control has been used both as a component of IPM programs designed to manage *Plutella* and on its own to reduce DBM populations to an acceptable level. The results have been varied, with good success in some areas and complete failure in others. How can the biological control of DBM be improved?

The Symposium "Improving biocontrol of *Plutella*" springs from an idea put forward by Garry Hill (CABI), and Dominique Bordat (CIRAD) in 1999. Sixty-one delegates from 25 countries attended the CIRAD/USDA International Symposium held in Montpellier from 21-24 October 2002.

Keynote speakers presented reviews on the current status of *Plutella* in different parts of the world, pathogens as biocontrol organisms, and classical systematics of parasitoids. The different topics are arranged into 8 chapters beginning with a global perspective on biological control of DBM (Chapter 1). Chapter 2 discusses Hymenoptera as biocontrol agents of DBM and reviews current parasitoid taxonomy. Chapter 3 discusses the role of entomopathogens in DBM biological control. The review covers each pathogen group, advances achieved and their contribution to the biocontrol of DBM. Chapter 4 reviews biological control of DBM in Africa where although ranked as the most destructive crucifer pest, yield loss information is lacking. Very high parasitoid diversity was recorded from South Africa and current biocontrol work in Africa is discussed. Chapter 5 reports on the biocontrol of DBM in South and Central America. DBM causes immense damage to crucifers in the region and the review highlights attempts to control it using biocontrol and selective insecticides which conserve biocontrol agents. The North America review (Chapter 6) points out that DBM belongs to a complex of pests attacking crucifers. A dynamic approach including the conservation and introduction of biocontrol agents would improve overall management of DBM. The review of biocontrol of DBM in Asia (Chapter 7) highlights the region wide approach to management of DBM. Some of the most successful IPM and classical biocontrol programs have been carried out in Asia. However continued use of ineffective insecticides is the greatest challenge to biocontrol in the area. Chapter 8 reviews biocontrol of DBM in the Oceania region. Despite good control of DBM by introduced agents in New Zealand and Australia continued use of insecticides and subsequent resistance has led to crop failures recently. A report on the workshop sessions constitutes chapter 9 in this book. Recommendations included improving taxonomic methods using on-line keys and genetic characterization, improved exchange of information and dependable methods for rearing and applying biological control agents, and faster registration of biopesticides.

In addition a further 28 proceedings contributions make up the rest of the publication. The quality of them is very high and many are from areas little represented at mainstream meetings. The editors are very grateful to all the people who have contributed to the aim of "Improving biocontrol of *Plutella xylostella*" and to several anonymous reviewers who have improved the contributions.

Alan Kirk
Dominique Bordat

1. Biological control of *Plutella xylostella:* a global perspective

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ABSTRACT

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is the major cosmopolitan pest of brassica and other crucifer crops. In many areas of the world, where such crops are grown for cash, the wide scale overuse of insecticides has created an ongoing resistance management problem. Until implementation of a more integrated (IPM) approach to the pest management of DBM and other crucifer pests is more widespread, the role of biological control will remain confined largely to forage and other low value crops.

INTRODUCTION

Three million ha of cabbages are grown worldwide and the most important pest species of these and other brassica crops is the diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Talekar and Shelton, 1993). Brassica crops are of particular importance in peri-urban environments and high farm gate prices have led to the frequent overuse of insecticides. The situation is most acute in the sub-tropics and tropics, where farmers often grow crops continuously and apply mixtures of insecticides on a weekly or sub-weekly basis (see Case history). Overuse of pesticides has led to resistance (Tabashnik *et al.*, 1987; Shelton *et al.*, 1993), crop residue problems, environmental contamination and destruction of indigenous natural enemies (NE). The frequent application of mixtures of pesticides also has a considerable impact on the profit margins of growers.

In the lowland tropics and sub-tropics, the life cycle of DBM can be 14 days or less and with a cabbage crop cycle of *ca.* 12 weeks this results in each generation of the pest being sprayed at least twice, with perhaps 25 consecutive generations exposed to pesticides p.a. This very high level of selection pressure, coupled with the high fecundity of DBM, has resulted in this species becoming one of the relatively few crop pests worldwide where resistance to a wide range of insecticides is a severe problem. Nowhere is this better illustrated than in resistance to crystal (Cry) endotoxins in *Bacillus thuringiensis (Bt)*-based spray products. While sometimes included with biological control agents, *Bt* products are in practice natural product insecticides, and as such are vulnerable to resistance if the selection pressure is great enough. Field resistance to *Bt* was first reported for DBM in Hawaii and Malaysia in 1990, and resistance now appears to be widespread in Asia and the Americas (Ferré and Van Rie, 2002). No other insect species has developed significant levels of field resistance to *Bt.*

Biological control, and more integrated methods of pest management (IPM) in general, represent more sustainable alternatives to chemical control of DBM; they are urgently required and are potentially more economic. Where brassica crops (and DBM) have been introduced, as, for example, into South East Asia, there have been a number of attempts at classical biological control. The interest in the use of biological control agents against DBM is reflected in the extensive programme assembled for the present symposium.

BIOLOGICAL CONTROL OF DBM

A variety of arthropod natural enemies (NE) and pathogens of DBM (Table 1) have been studied but interest has focussed mainly on one group, the hymenopteran parasitoids. *Diadegma* and *Cotesia* spp. are regarded as the most important primary parasitoids of DBM (Verkerk and Wright, 1996) and a number of introductions have been made, particularly in South East Asia and Australasia. *Cotesia plutellae* (Kurdjumov) is particularly well adapted to tropical conditions. Other introductions have included *Oomyzus sokolowskii* (Kurdjumov) and *Diadromus collaris* (Gravenhorst) (Verkerk and Wright, 1996; Case history).

A major problem following the introduction of parasitoids can be their lack of compatibility with pesticides (Talekar *et al.*, 1992). Susceptibility can vary markedly between species and compounds (Idris and Grafius, 1993; Furlong and Wright, 1993; Goudegnon *et al.*, 2000; Xu *et al.*, 2001). For example, *D. semiclausum* (Hellén) appears to be particularly sensitive to a wide variety of insecticides, including acylurea chitin deposition inhibitors (Furlong and Wright, 1993), compounds that are generally thought to be relatively harmless to NE. However, even *D. semiclausum* can be compatible in the field with some compounds, including *Bt* products and abamectin (Verkerk and Wright, 1997).

Biological control of DBM is most likely to succeed in low value crops, where little or no insecticide is used, providing also that climate and agronomy are favourable. For example in the highlands of Papua New Guinea, where there is almost continuous subsistence agriculture and a stable near-temperate climate, *D. semiclausum* has successfully established following its introduction in 1995, whereas *C. plutellae* didn't achieve lasting establishment in the arid lowlands (Saucke *et al.*, 2000). Similarly, introduced parasitoids (*D. semiclausum* and *D. collaris*) and pathogens (*Zoophthora radicans*) have provided adequate control of DBM on forage crucifers in North Island, New Zealand, while, in South Island, insecticides are sometimes required on crucifers grown for human consumption (Walker *et al.*, 2004).

An alternative approach is to use augmentative or indundative applications of biological control agents. Here, the long-term effects of pesticides are of less concern. Where frequent (often weekly) applications are made, biological control agents are in effect used as 'biopesticides' and little secondary cycling of the organism is expected. Augmentative and inundative applications of parasitoids to control DBM are considered at this symposium, together with the potential of baculoviruses, particularly *P. xylostella* granulosis virus (*Plxy*GV) (e.g. Grzywacz *et al.*, 2004).

Factors that have hitherto restricted the use of entomopathogens against agricultural pests have included the specificity of many pathogens, their relatively slow action, lack of persistence and high cost compared with chemical control, difficulties in mass production and supply, variable quality control, short shelf life, limited patentability and the high cost of registration for small and medium sized companies. Some of these problems are either not applicable (speed of action) or have been largely overcome (mass production and supply) for entomopathogenic nematodes and these organisms have had some degree of commercial success against a range of soil pests. The potential of nematodes as foliar applications against agromyzid leafminers and thrips on protected crops has also been reported (Willams and Walters, 2000; S.J. Piggott, personal communication). Field application of entomopathogenic nematodes against DBM has met with limited success (e.g. Bauer *et al.*, 1998). Advances in formulation technology and the development of more appropriate methods for spraying nematodes (Lello *et al.*, 1996; Mason *et al.*, 1999) may lead to more robust control of DBM, either by nematodes alone or in combination with other products such as *Bt* (Bauer *et al.*, 1998).

INSECTICIDE RESISTANCE AND BIOLOGICAL CONTROL

Conservation of indigenous or introduced species of NE requires a more rational approach to the use of pesticides, including wherever possible a switch to more selective compounds within an IPM programme. Insecticide resistance has been a mixed blessing in the development of more sustainable control methods for DBM. Resistance to broad-spectrum insecticides led to a switch in the 1980s and 1990s in many areas to less persistent, more selective compounds, thereby helping to conserve natural enemies (Case history). However, in the absence of effective resistance management programmes selective compounds have also been applied too frequently, without alternation, and resistance has developed or has started to develop to such products, including *Bt*, abamectin and spinosad (Tabashnik, 1994; Iqbal *et al.*, 1996; Zhao *et al.*, 2002). Without advice and sufficient incentives, growers tend to apply each new compound as soon as it becomes available, irrespective of whether the compound is selective or more broad-spectrum in its action. The time taken for DBM populations to develop resistance to a new product can be as little as 2 years, assuming cross-resistance mechanisms are not already present.

If we are to both understand and manipulate DBM populations, a firm understanding of *P. xylostella* population genetics will be required. In many areas, crucifers are often grown in a non-continuous and asynchronous manner in numerous small plots. This should impose a fairly strict metapopulation structure upon DBM in these localised areas, with potentially important implications for the development and spread of resistance and for biological control. Polymorphic microsatellite loci appear to be particularly promising as molecular tools for studying spatial heterogeneity in DBM populations (Butcher *et al.*, 2002; Endersby *et al.*, 2004).

Case history: The Cameron Highlands

The Cameron Highlands is the major upland vegetable-growing area in Malaysia, and cabbage crops attract a premium price in the markets of Kuala Lumpur and Singapore. This region consists of eight inter-linked valleys with over 2000 farms, typically of 1-2 ha each. DBM became a serious pest in the 1940s although reasonable control was maintained using the few, relatively low activity insecticides then available combined with cultural methods. With the introduction of the much more effective synthetic organic insecticides in the 1950s, growers in the Cameron Highlands and around the world became increasingly reliant on chemical control and the development of DBM populations resistant to a succession of products began (Iqbal *et al.*, 1996; Verkerk and Wright, 1997).

In the 1970s *C. plutellae* was found in the Cameron Highlands, while *D. semiclausum*, *D. collaris* and *O. sokolowskii* were introduced. However, these introductions were not a success, *O. sokolowski* did not establish and the other parasitoid species did not appear to exert any control of DBM. It wasn't until the late 1980s, when resistance to most broad-spectrum compounds left *Bt* and abamectin as the main insecticides being applied that greater establishment of *C. plutellae* and *D. semiclausum* occurred.

Resistance to *Bt kurstaki* was first reported in a DBM population in the Cameron Highlands in 1990 (Syed, 1992), and some resistance to *Bt aizawai* and abamectin has also developed (Iqbal *et al.*, 1996) although all of these products are still widely applied, usually in mixtures with less-selective compounds (Table 2).

In 2002, resistance to fipronil, spinosad and indoxacarb was detected in two field populations from this region (A.H. Sayyed and D.J. Wright, unpublished data). If the current usage pattern continues, with sprays of mostly mixtures on average every 6 days and with only about a quarter of growers practicing any form of resistance management (Table 2) then severe resistance and control failures are likely to occur. This could encourage the widespread reintroduction of more broad-spectrum insecticides, such as pyrethroids (to which resistance would have temporarily declined), with catastrophic effects on the NE complex.

For IPM to be adopted more widely in the Cameron Highlands and elsewhere, greater understanding and co-operation between growers, extension and research workers, governments, industry and consumers is required. A key feature for successful IPM is the use of pest monitoring or scouting (which can include the use of pheromones: Ng *et al.*, 2004) and the concurrent adoption of economic thresholds for pesticide application (e.g. Amend and Breslow, 1997; Ng *et al.*, 2004). Land can be expensive and the potential returns for growing up to four crops of cabbage p.a. are very high. Without more information and advice growers may lack the confidence to change their pest management practices: the perceived risks may be too great. In many areas other pests (aphids, thrips, other Lepidoptera) and diseases also have to be considered in control strategies (Shelton, 2004), making IPM of the primary pest DBM less straightforward.

INTEGRATED PEST MANAGEMENT (IPM)

Various other approaches to controlling DBM within an IPM programme have been investigated and in some cases adopted successfully (Ng *et al.*, 2004). The use of pheromones for mating disruption (e.g. Mitchell *et al.*, 1997) or 'Lure and Kill' (Mitchell, 2002) has shown some promise. While overhead irrigation (McHugh and Foster, 1995) or screen netting (Ng *et al.*, 2004) can be used for DBM control in some situations. Cultural methods include intercropping (Asman *et al.*, 2001) and the use of trap crops (Luther *et al.*, 1996; Charleston and Kfir, 2000; Shelton, 2004) and can help attract and maintain populations of natural enemies (Mitchell *et al.*, 1997). Intercropping crucifer crops with annuals can provide a pollen source for hover flies (syrphids) (White *et al.*, 1995; Morris and Li, 2000) and a nectar source for *Diadegma* spp. (Idris and Grafius, 1995) but such interactions can be problematic (Zhao *et al.*, 1992). Increased weed diversity can also result in greater densities of carabid and staphylinid predators (Schellhorn and Sork, 1997).

Partial host plant resistance (Way and van Emden, 2000) may also have a role in DBM management. Synergist interactions between plant resistance and chemical control can allow less pesticide to be applied (Verkerk and Wright, 1996). Some brassica varieties can also have positive effects on biological control of DBM (Verkerk and Wright, 1996; Verkerk *et al.*, 1998). However, grower acceptance of varieties is very market driven and many brassica crops that are popular with consumers, particularly Chinese cabbage, are often highly susceptible to DBM.

Transgenic brassica crops expressing *Bt* Cry toxins (e.g. Zhao *et al.*, 2000; Cao *et al.*, 2002) are of great potential value for use in IPM systems against DBM and other crucifer insect pests. They appear to be compatible with NE (Schuller *et al.*, 2003) and their use could lead to significant reductions in insecticide use (as in the case of *Bt* cotton). However, their use will require very effective resistance management strategies (Tabashnik *et al.*, 2003) given the misuse of *Bt* spray products against DBM, and the consequent background of resistance in a number of field populations, and the inherent ability of this pest to develop resistance.

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Table 1. Examples of biological control agents for DBM.

Hymenopteran parasitoids

Diadegma spp. [Ichneumonidae: larval parasitoids]
Cotesia plutellae (Kurdjumov) [Braconidae: larval parasitoid]
Oomyzus sokolowskii (Kurdjumov) [Eulophidae: larval/pupal parasitoid]
Diadromus spp. [Ichneumonidae: pupal parasitoid]
Microplitis plutellae Muesebeck [Braconidae: adult parasitoid]

Predators

Adult coccinelids and minute pirate bugs
Larval lacewings and carabids

Pathogens

Baculoviruses
GV [e.g. *PlxyGV*]
NPV [e.g. AcMNPV; *Px*MNPV]

Bacteria

Bacillus thuringiensis (Berliner) var. *kurstaki* / var. *aizawai*
Bacterial symbionts (see Nematodes)

Fungi

Zoophthora radicans Brefeld
Beauveria bassiana Vuillemin
Metarhizium anisopliae (Metscnikoff) Sorokin
Paecilomyces farinosus (Wize) Brown and Smith / *fumosoroseus* (Holm) Brown and Smith
Microsporidia [*Nosema* spp., *Vairimorpha* spp.]

Nematodes

Steinernema spp. (with *Xenorhabdus* spp. symbionts)
Heterorhabditis spp. (with *Photorhabdus* spp. symbionts)

Table 2. Pesticide usage against diamondback moth (DBM) in the Cameron Highlands, Malaysia: farm surveys conducted from October to December in 2000 and 2001 (n = number of farms) (R. Butcher, J. Cook and D. Wright, unpublished data).

	2000 (n = 195)	2001 (n = 103)
Farms (%) observed with DBM	98	96
Spray regime (% unless stated)		
Mean spray interval (days)[1]	6	6
Spray infrequently (> 14 days)	2	6
Apply mixtures of products	91	93
Alternate mixtures or products	23	24
Example of pesticide usage (%)		
Bt with non-selective products[2]	57	54
Bt products (total usage)[3]	65	60
Abamectin	75	65
Fipronil	85	74
Spinosad	75	83
Indoxacarb	3	22

[1] Minimum spray interval reported was 2-3 days.
[2] Includes: fipronil, organophosphates and pyrethroids.
[3] *Bacillus thuringiensis (Bt)* products, abamectin, indoxacarb, spinosad.

2. The taxonomic status and role of Hymenoptera in biological control of DBM, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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ABSTRACT

Cabbage is cultivated worldwide and other cruciferous crops are also widely present in various regions. Thought to be of palaearctic origin and imported into newly colonized countries by European immigrants, the plant was however accompanied by an important pest which now has a cosmopolitan distribution: the diamondback moth (DBM), *Plutella xylostella* (L.). Its economic importance can be appreciated through the number of papers consecrated to it. During the last 20 years and according to the Review of Applied Entomology (RAE), 1937 papers dealt with this moth. Oddly, while papers dealing with insecticides and *Bt (Bacillus thurengiensis)* resistance and applications are numerous, those concerning parasitoids are fewer than expected. Does this mean that there is a lack of interest in biological control over this time? The aims of this chapter are to review current Hymenopteran parasitoid taxonomy, the problems faced when using Hymenoptera as biological control agents and future perspectives.

INTRODUCTION

A review of DBM biocontrol literature including surveys for natural enemies was carried out. Two thousand specimens collected in 23 countries, mostly in Europe, Africa South of the Sahara, North and South America were examined from 1984 to 2002. Major parasitoid specimens were initially compared to reference collections or identified by specialists, in order to provide accurate identifications. The DBM biocontrol program in Montpellier is a CIRAD/USDA-European Biological Control Laboratory (EBCL) collaborative effort. CIRAD has an interest in crop protection in tropical countries, especially legume crops; Dominique Bordat was responsible for this part of the programme working on the biology of parasitoids and biochemical characterization of populations. The USDA is interested in biological control of DBM in the USA and actively collects parasitoids from many parts of the world (Delvare and Kirk, 1999).

RESULTS

Biodiversity of the parasitoid complex

Table 1 lists the parasitic Hymenoptera cited in the literature or resulting from recent collections. Names were verified for the family Ichneumonidae according to the recent updated world ichneumonid catalogue of Yu and Horstmann (1997). The chalcidoid names were checked through the data base of Noyes (2001). More than 150 species are recorded, although 15 of them were definitively rejected by Noyes (1994) through information provided by specialists of Ichneumonoidea and Chalcidoidea. The above list also provides the original sources of the citations and the known distribution of the parasitoids; it is clear that their actual distribution is probably larger, as we rely on published data, which depends on the activity of entomologists. Noyes (1994) listed more than 90 species while Fitton *et al.* (1991) listed about 65 species, mostly based on voucher specimens deposited in the British Museum of Natural History. We have on the other hand examined 61 different species. Some of them could not be precisely identified, due to lack of accurate taxonomic works or reference collections; a few species examined here are undescribed. Fitton and Walker (1992) discussed the parasitoid complex of the DBM, especially the taxonomic problems related to the species involved. Their conclusion was that care must be taken concerning the biological data, as much of it is wrong. Old data, i. e. published before 1950, are unreliable unless they can be checked through the examination of voucher specimens. Noyes (1994), from examination of the relevant specimens, estimated that about 2/3 of the published data were wrong. These mistakes have their origin in 1) misidentifications of the parasitoids; 2) wrong host-parasitoid associations; 3) misidentification of the hosts. Therefore only recent surveys followed by accurate determinations can give an appreciation of the true diversity of the parasitoid complexes.

Trophic relationships and relative abundance of the species

The complex includes primary parasitoids, facultative hyperparasitoids and obligatory parasitoids. According to the host stage attacked, the primary parasitoids can be further subdivided into egg, larval or larval-pupal and pupal parasitoids. Table 1 shows that we regularly recover a relatively limited number (n = 14) of the following primary parasitoids: *Cotesia plutellae* (Kurdjumov), *Diadegma* spp., *Diadromus* spp., *Oomyzus sokolowskii* (Kurdjumov), etc. Species such as *Apanteles piceotrichosus* Blanchard, *Diadegma mollipla* (Holmgren) or *D. leontiniae* (Brèthes) have a regional distribution, while others are almost cosmopolitan, having been accidentally introduced together with the moth, or through biological control programs. A number of primary parasitoids have marginal importance: they are rare on DBM and generally in small numbers on this pest. *Cotesia rubecula* (Marshall) and *Brachymeria femorata* (Panzer), which attack *Pieris* spp., belong to this group. They usually parasitize other lepidopterous pests present on cabbage but sometimes fail to recognize their hosts. Finally parasitoids such as *Cotesia ruficrus* (Haliday), *Tetrastichus howardi* (Olliff), *Conura albifrons* (Walsh) and *C. torvina* (Cresson), are generalists, recovered from numerous hosts.

The egg parasitoids: *Trichogramma* and *Trichogrammatoidea*

Egg parasitoids include members of the genera *Trichogramma* and *Trichogrammatoidea*, which are well known from Lepidoptera eggs. There are a very limited number of species involved. The literature may be misleading, as species concerned with laboratory studies are often quoted. It is strange that no *Telenomus* (Scelionidae) has been found on DBM, especially in tropical countries, where they are abundant and diverse and where some species play an important role in controlling major lepidopterous pests. Does this result come from a lack of surveys? Is the genus really absent on DBM? The limited number of known oophagous parasitoids might result from collecting techniques. Looking for natural DBM eggs, which are deposited singly, is difficult and tedious. Instead of this it is more efficient to proceed to artificial infestations or to put out sentinels i. e. eggs from a laboratory culture which are left in the fields. This is a good method for obtaining qualitative results.

Identification and taxonomy

The species of the above genera are difficult to identify, especially for non specialists. Their determination requires special slide-mountings, and the examination of male genitalia. Some of them are morphologically very similar and difficult to distinguish from each other. Taxonomists have therefore developed other tools such as morphometry; enzymatic characterization and now DNA analysis (see Noyes, 2001 for a complete list of references). However many of these trichogrammatids were described before these techniques appeared and their types can be used only for morphological examination. This is particularly the case for the type species of *Trichogramma*, *T. evanescens* Westwood. According to its condition, only a part of the wing around the stigmal vein can be used. The female holotype was initially collected near London, and taxonomists are now collecting Lepidoptera eggs in the same place to get fresh and reliable material for comparison. Remarkably 10 *Trichogramma* spp. have now been collected from the locality and it is still not known what the most frequently found egg parasitoid of Lepidoptera quoted in the literature is ! The same problem arises for *T. brassicae* Bezdenko, sometimes recorded from DBM, the type of which is apparently lost. Fortunately, *T. chilonis* Ishii, the commonest species recovered from eggs of the moth, is relatively well known, although there is some confusion with *T. australicum* Girault (Viggiani, 1976). Another difficulty comes from the fact that having no access to types; scientists working with enzymatic or molecular tools have their own concepts on the identity of the species. These tools can help us to distinguish species and populations but not necessarily to get the correct names: this is a nomenclature problem, which especially concerns the European fauna. On the other hand *Trichogrammatoidea brasiliensis* (Ashmead), a Neotropical species recorded from DBM, was only recently transferred to this genus (Pinto, 1997); it was previously known as a *Trichogramma*.