ENZYMATIC IDENTIFICATION OF *Glyptapanteles* sp. (INSECTA: HYMENOPTERA) FROM MADEIRA ISLAND

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During a scientific expedition carried out in Madeira Island in September 1997, *Pseudaletia (=Mythimna) unipuncta* (Lepidoptera: Noctuidae) larvae were collected in maize fields and pastures. These larvae were parasitized by a braconid belonging to *Glyptapanteles* genus. This population was characterised biochemically to identify the species. Seven enzyme systems studied by electrophoresis were analysed: aldehyde oxidase (AO), a-glycerophosphate dehydrogenase (a-GPD), tetrazolium oxidase (TO), malate dehydrogenase (MDH), glucose-6-dehydrogenase (G6PD), malic enzyme (ME), isocitrate dehydrogenase (IDH). All systems showed only one band, with two exceptions: α-GPD and ME which had two bands which corresponded to different loci. No polymorphic enzymes were detected. Comparing this results with those obtained from *G. militaris* collected in Azores Islands and in Quebec-Canada we can suggest that the population of *Glyptapanteles* collected in Madeira Island belongs to *G. militaris* species.

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INTRODUCTION

Pseudaletia (=Mythimna) unipuncta (Lepidoptera: Noctuidae) is a cosmopolitan species, considered as the most important pest in Azorean gramineous fields (TAVARES 1989). Several studies concerning the establishment of biological control programs against this pest have been carried out in our laboratory (TAVARES 1989; VIEIRA 1992; OLIVEIRA 1996). In this sense, it is important to survey the pest natural enemies and preserve their characteristics during the several steps of biological control programs, such as, the mass rearing processes and field releases. However, some problems may arise when such programs are under development such as species mis-identification (especially for sibling species) leading to a loss of effort, time and money (POWELL & WALTON 1989). Electrophoretic study, as a tool for identification, may help to overcome some of such errors (PINTUREAU & VOEGELÉ 1980; PINTUREAU 1987; POWELL & WALTON 1989).

In the Azores, *P. unipuncta* has few species of parasitoids from Hymenoptera (TAVARES 1989; OLIVEIRA 1996), with the braconid *G. militaris* being the main native biological control agent. Therefore, a larger survey for *P. unipuncta* biological control agents was expanded to other Macaronesian Islands, such as Madeira.

During a scientific expedition carried out in Madeira Island in September 1997, *P. unipuncta* larvae were collected in maize fields and pastures. These larvae were parasitized by a braconid belonging to *Glyptapanteles* genus, as in Azores Islands.

Since electrophoretic studies have been used to identify and distinguish between sibling species (PINTUREAU & VOEGELÉ 1980; PINTUREAU 1987; PINTUREAU et al. 1990) we analyzed seven enzyme systems by gel electrophoresis to compare the parasitoids from Madeira with the Azorean population of *G.*

militaris. This study allowed an increase on the records of Madeira native species and the genetic characterization of the wasp's populations, contributing to the proper development of biological control programs.

MATERIAL AND METHODS

Adult parasitoids were obtained from larvae of *P. unipuncta*, randomly collected at Madeira Island Miguel Island only in pastures. The encountered *P. unipuncta* larvae, more than 100, were individually isolated in plastic cages (4.5x3 cm), and kept in the laboratory at 22±1°C, 75±5% RH and L:D 16:8, for daily observation of parasitoid emergence. Twenty-eight of these larvae were parasitized by *G. militaris*, producing an average of 32 cocoons per host. After parasitoid adult emergence, the insects were stored frozen at -20 °C until subsequent electrophoresis (frozen period < 1 month).

Each adult was isolated and homogenized in 15 microliter of "Trudgill" solution and centrifuged for 5 minutes at 9 000 rpm, according to the methodology used by PINTUREAU (1987).

A vertical electrophoresis on polyacrilamide gel was performed on seven enzyme systems: aldehyde oxidase (AO), α–glycerophosphate dehydrogenase (α-GPD), tetrazolium oxidase (TO), malate dehydrogenase (MDH), glucose-6-dehydrogenase (G6PD), malic inzyme (ME) and isocitrate dehydrogenase (IDH). The techniques used generally followed the methodology described by PINTUREAU (1987), PINTUREAU et al. (1991) and OLIVEIRA (1996), with specific details in table 1. Eighteen individuals (each one from an individual parasitized host) collected in Madeira Island were run for each enzymatic system. As control, two positively identified specimens of *G. militaris* from São Miguel Island (OLIVEIRA 1996) were run for each enzymatic system.

RESULTS AND DISCUSSION

The electrophoretic patterns observed in *G. militaris* were similar for the populations of Madeira and Azores (Fig. 1). Aldehyde Oxidase, Tetrazolium oxidase, Malate dehydrogenase, Glucose-6-dehydrogenase and Isocitrate dehydrogenase exhibited a single band, for each of these five systems, therefore only one locus was found. However, α-Glycerophosphate dehydrogenase and Malic enzyme exhibited two bands, corresponding two different loci.

Table 1
Electrophoretic conditions used to study seven enzymes systems in *G. militaris*.

Protein	Buffers: Bridge	Buffers: Gel	Migration: time (Minutes)	Migration: voltage (Volts)	Revelation: time (Minutes)	Revelation: solution
Aldehyde oxidase (AO)	Tris/HCl 4 % pH 6.7	Tris/HCl 7 % pH 8.9	30 90	150 300	30 room temp	Tris/HCl Benzaldeide NBT PMS
α-Glycerophosphate D,L,Glycerophosphate dehydrogenase (α-GPD)	"	"	30	150	40	Tris/HCl α- NAD
Tetrazolium oxidase (TO)			120	300	37°C	NBT PMS MTT
Malate dehydrogenase (MDH)	"	"	30 90	150 300	40 37°C	Tris/HCl L-Malic acid NAD NBT PMS
Glucose-6-dehydrogenase (G-6-PD)	"	"	30 90	150 250	40 37°C	Tris/HCl Glycerol-6-phosphate NADP $MgCl_2$ NBT PMS NBT
Malic inzyme (ME)	"	"	30 90	150 300	15 37°C	Tris/HCl L-Malic acid NADP $MgCl_2$ PMS MTT Agar 2 %
Isocitrate dehydrogenase (IDH)	"	"	30 90	150 300	60 37°C	Tris/HCl D,L-Isocitric acid NADP $MgCl_2$ PMS MTT

The two populations of *G. militaris* were examined for nine allozyme loci, but all of them appeared entirely monomorphic. The enzymes used in this study are useful to characterize genetically *G. militaris*, but they constitute only a part of the species genome. Most species of Hymenoptera are reported to have low electrophoretic variation (PINTUREAU 1987; OMWEGA & OVERHOLT 1996). Our data indicated that *G. militaris* falls in the former group with less variability, as *Cotesia glomerata* (unpublished data).

The comparison of both populations suggests that the *Glyptapanteles* population collected in Madeira Island belong to *G. militaris* species. The same bands were observed for a population of *G. militaris* from Quebec-Canada (OLIVEIRA, 1996). The results of the morphological study carried on both populations confirm the enzymatic identification of the species (OLIVEIRA et al.1999).

According to BAEZ (1993) and GRAHAM (1986a, 1986b), the genus *Apanteles* (= *Glyptapanteles*, *Cotesia*) is cited from Madeira, but the species were not identified. Therefore, the species *G. militaris* is the first time recorded for this island.

The presence of this parasitoid in Madeira Island can be a good indicator of the natural control exerted by this wasp on the populations of the agricultural pest *P. unipuncta*. Furthermore, the population of *G. militaris* should be protected from the indiscriminate use of pesticides.

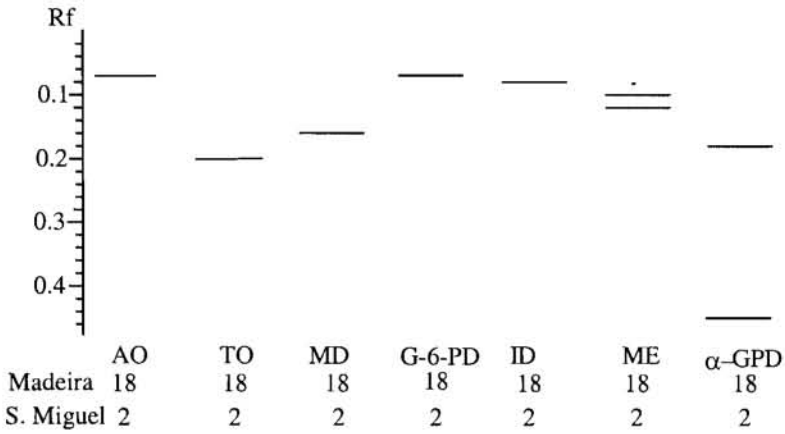

Fig. 1. Electrophoretic partterns of seven enzyme sistems observed in two populations of *G. militaris* from Madeira and Azores Islands; AO - Aldehyde oxidase; TO - Tetrazolium oxidase; MDH - Malate dehydrogenase; G-6-PD - Glucose-6-dehydrogenase; IDH - Isocitrate dehydrogenase; ME - Malic enzyme and α-GPD - Glycerophosphate dehydrogenase.

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