AN INVESTIGATION INTO THE OVERWINTERING CAPABILITY OF *Istocheta aldrichi* (MESNIL) (DIPTERA: TACHINIDAE) A PARASITOID OF *Popillia japonica* NEWMAN (COLEOPTERA: SCARABAEIDAE) ON TERCEIRA ISLAND, AZORES

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A study of diapause in the parasitic fly *Istocheta aldrichi* (Diptera: Tachinidae) was undertaken to help investigate the possibility of its establishment in Terceira as a biological control agent of the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae). It was concluded that pupae should be imported from the USA only when they are almost ready to emerge, and to optimise rearing conditions there to increase emergence percentages. Conditions are not ideal for its development in Terceira, as this univoltine parasitoid has a high pupal mortality both in laboratory cultures and outdoors during the overwintering period. These studies should continue until *I. aldrichi* has become established in the Azores.

SIMÕES, A.M. & S. GRENIER. 1999. Investigação sobre a capacidade de sobrevivência durante o inverno na ilha Terceira, Açores, de *Istocheta aldrichi* (Mesnil) (Diptera: Tachinidae) um parasitóide de *Popillia japonica* Newman (Coleoptera: Scarabaeidae). *Arquipélago*. Ciências Biológicas e Marinhas 17A: 23-26. Ponta Delgada. ISSN 0873-4704.

Foi estudada a evolução da diapausa do parasitóide *Istocheta aldrichi* (Diptera: Tachinidae) e a possibilidade de este se estabelecer na ilha Terceira como um agente de controle biológico do escaravelho japonês, *Popillia japonica* (Coleoptera: Scarabaeidae). Concluímos que as pupas devem ser importadas dos EUA perto do período de emergência a fim de aumentar a percentagem desta, uma vez que se trata de um parasitóide univoltino com uma elevada percentagem de mortalidade durante o seu estado pupal quer nas condições de laboratório e de campo durante o período de inverno. Estes estudos devem de continuar até ao estabelecimento da *I. aldrichi* nos Açores.

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INTRODUCTION

The Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae) has become a serious pest in Terceira Island following its accidental importation from the USA during the 1970's. One of the objectives for the biological control of this beetle was to use a parasitoid species from an area where the host is indigenous, i.e. Japan (SIMÕES 1993). There was expressed interest in experimental introductions of different parasitoids against *P. japonica* in the Azores.

The parasitic fly *Istocheta aldrichi* (Mesnil) (Diptera: Tachinidae) was first introduced into Terceira in 1993; it is one of the most important parasitoids of adult Japanese beetles. After a review of biological control methods against the Japanese beetle in the Azores, studies on *I. aldrichi* began in Terceira in 1994. This species was introduced into the USA in 1920 for the biological control of the Japanese beetle. A historical review of its use for this purpose, and also the subsequent changes in its distribution there, have been made by CLAUSEN et al. (1927).

The effects of temperature, light, humidity and photoperiod on their diapause are very important factors in the immature stage development of tachinid parasitoids (GRENIER 1986). A review of the knowledge of the morphological and bioecological parameters of *I. aldrichi* and its host *P. japonica,* has been compiled by SIMÕES et al. (1995). Since the first introductions were not successful (SIMÕES in press), the objective of this paper is the evaluation of diapause of *I. aldrichi* by testing the importance of low temperature on its development.

MATERIAL AND METHODS

About 2,000 *P. japonica* parasitized by *I. aldrichi* were obtained from the United States Department of Agriculture (USDA) in July 1996. It was determined that 51% of the *P. japonica* adults which showed an *I. aldrichi* egg on the thorax had a well developed fly puparium inside their abdomen. Samples each with 50 abdomens of parasitized beetles containing puparia were placed individually in Costar 24 Well Cell Culture Clusters, each sample then being kept under different conditions as defined below. Before being placed in the wells, the date and pupal weights were recorded, as were later the dates of emergence and sex ratio of the resulting adult flies.

To estimate the pupal weight at pupation time, a measure of the pupal volume could be a good approximation if we assume a specific density near 1. In this condition, Weight in mg = Volume in mm^3 (GRENIER & BONNOT 1983). The volume of a pupa could be calculated as a revolution ellipsoid using the following formula: V (volume) = PI/6 x L (length) x D $(\text{diameter})^2$ (Fig. 1). The graph shows a good correlation between the volume (i.e. the estimate of the weight) and the actual weight. The authors say that in fact, the weight of a pupa is also difficult to determine precisely because it decreases from the time of pupation until fly emergence (GRENIER & BONNOT 1983).

In the laboratory, pupae were exposed to several temperature combinations utilizing 4, 20, & 23º C, with photoperiods of 8:16, 10:14, & 16:8 L:D respectively. The relative humidity was maintained at approximately 70%. Weights of the pupae were recorded each time the temperature was changed to check if the pupae were still alive: a lighter weight indicating death followed by desiccation. The combinations used were 1 and 2 months at 20ºC (symbolized as I or II), followed by 1, 2, 3, or 4, months at 4ºC (symbolized as 1 to 4); and 3 months at 20ºC (III) followed by 5 months at 4ºC (5). The symbols for these different modalities are thus given as I-1, I-2, I-3, I-4, II-1, II-2, II-3, II-4 and III-5.

I aldrichi pupae were all held at 23ºC and 16:8 L:D until emergence following their removal from cold conditions at 4ºC. Whenever the temperature was to be lowered or raised, pupae were kept at 12ºC with 10:14 L:D for a two day transition period.

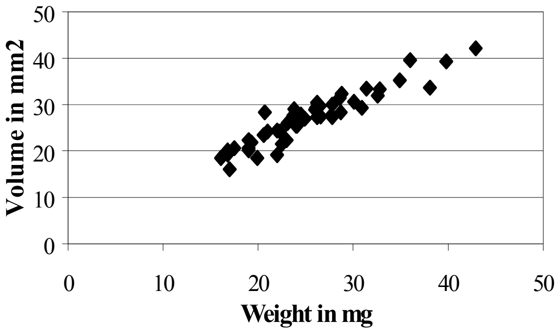

Fig. 1. Graph showing distribution of the weight of pupae in relation to volume.

In other samples pupae were kept inside an individual container in a oven, one kept at Terceira island mean monthly temperatures with soil (Ter.soil) or with wet paper (Ter.paper) and similarly in another oven at mean monthly temperatures for Connecticut, USA (USA) during the year. For both stoves, the following different photoperiods were used: corresponding to March-April and August-November 10:14 L:D, December-February 8:16 L:D and May-July 16:8 L:D.

In the field, to test different ways of releasing parasitoids, the samples of parasitized beetles were placed on the ground (on ground)*, on the ground on a paper (paper)* or under the ground (underground)* under natural conditions at the Plant Protection Laboratory, Department of Agricultural Sciences, Terra Chã, Terceira Azores.

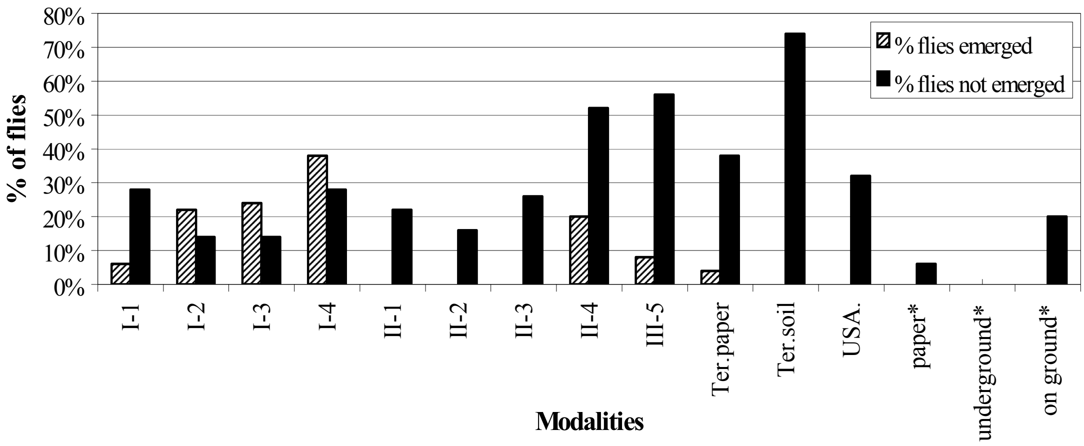

Fig. 2. Only percentage of adults formed under different modalities.

For all the different modalities, unhatched puparia were dissected and examined under a microscope after the period of emergence, to see if any adults had completely formed but not emerged.

RESULTS

Emergence of the adult parasitoids at Terceira island was observed in both natural and laboratory simulated conditions. (Fig. 2). Higher percentages of adults (more than 60) were observed in 4 modalities (I4, II4, III5, and Ter.soil). Most of the adults formed did not emerge, as in modalities II1, II2, II3, Ter. Soil, USA, paper*, and on ground*. Under natural conditions only in the underground* modality was there no formation of adults inside the pupae, which were found to contain many bacteria and fungi. The highest percentages (38%) of adult emergence were recorded in modality (I-4) in which pupae were kept inside an oven for a month at 20ºC and 10:14 D:L, then at 4ºC for four months at 8:16 D:L, and finally at 23ºC with 16:8 L:D until adult emergence.

It seems that adult fly emergence increases on modalities I (one month at 20°C at the beginning) and decreases on modalities II when the pupae are kept for two months at 20°C except in modality II-4.

CONCLUSIONS

Despite the natural conditions on Terceira Island being favorable in most respects to the successful development of *I. aldrichi*, overwintering is a problem because the pupae appear to need a cold period with temperatures around 4°C for four months to achieve their maximum emergence rate.

Pupae should not be imported from the USA until they are almost ready to emerge in order to increase emergence percentages.

Parasitized beetles should be kept in the USA until the parasitoids have pupated before shipping them to Terceira so that a greater percentage of imported beetles contain a healthy fly pupa.

Methods of rearing in the USA before importation to Terceira must be altered to increase success in development of the parasitoid only 51% of parasitized beetles in the batch obtained from USDA in 1996 contained fly pupae.

Comparative studies on development of *I. aldrichi* should be conducted in the future both USA and on Terceira.

It was observed that the weight of a pupa is a good indicator of its quality.

I. aldrichi is a univoltine parasitoid, which has a high pupal mortality both in laboratory and outdoors in Terceira during the overwintering period.

The emergence of the parasitoid must be compared by using two different ways of maintaining the pupae under natural conditions both on Terceira and in Connecticut.

Taking all these factors into consideration, it should now be possible to use rearing conditions that maximize the survival rate of overwintering pupae, either in the field or in laboratory. Studies on this, and the effect of temperature on emergence should continue until *I. aldrichi* becomes successfully established in the Azores.

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