

LOW TEMPERATURE EFFECT ON VIABILITY OF *DIADEGMA INSULARE* (HYMENOPTERA: ICHNEUMONIDAE) PUPAE AND EFFECT OF THIS PARASITOID ON FEEDING RATE OF DIAMONDBACK MOTH LARVAE (LEPIDOPTERA: PLUTELLIDAE)

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ABSTRACT

Laboratory tests with *Diadegma insulare* (Cresson), a parasitoid of the diamondback moth (*Plutella xylostella* (L.)), were conducted to evaluate the viability of cocoons stored at 4°C for varying lengths of time and also the effects of parasitism by *D. insulare* on the feeding rate of diamondback moth larvae. The percentage of adults emerging from cocoons declined steadily with time spent in storage at 4°C and was predictable ( $r^2=0.9603$ ;  $Y=98.3-2.06X$ ;  $P<0.01$ ). No emergence of *D. insulare* was observed after 49 d in storage. There was a significant difference in feeding rate of parasitized larvae and non-parasitized larvae. Consumption of collard foliage by parasitized larvae (86.25% of them were parasitized) the first day after stinging was about the same as for non-parasitized larvae; but the parasitized larvae then became sluggish and fed very little on days 2-5 when the experiment was terminated.

Key Words: Biological control, parasitoid, parasitism, *Brassica oleracea*, *Conura side*

RESUMEN

Fueron conducidos ensayos de laboratorio con *Diadegma insulare* (Cersson), un parasitoide de *Plutella xylostella* (L.). Se evaluó la viabilidad de los capullos almacenados a 4°C durante diferente tiempo y los efectos del parasitismo por *D. insulare* en la tasa de alimentación de las larvas de *P. xylostella*. El porcentaje de adultos emergidos de capullos declinó establemente con el tiempo de almacenaje a 4°C y fue predecible ( $r^2=0.9603$ ;  $Y=98.3 - 2.06X$ ;  $p<0.01$ ). No fue observada emergencia de *D. insulare*

después de 49 días de almacenaje. Hubo diferencia significativa en la tasa de alimentación de las larvas parasitadas y no parasitadas. El consumo de follaje de acelga por las larvas parasitadas (86.25% de ellas fueron parasitadas) el primer día después de ser picadas fue casi el mismo que el de las larvas no parasitadas, pero las larvas parasitadas se volvieron lentas y se alimentaron muy poco en los días 4-5 cuando el experimento estaba terminando.

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The diamondback moth, *Plutella xylostella* (L.), is the most important pest of cabbage and other cruciferous crops worldwide (Muckenfuss et al. 1990). Because the larvae attack crops from seedling to harvest stage, virtually all cabbage crops are treated with insecticides during the growing season. Larval control with chemical pesticides started failing in the 1980s and insecticide resistance has since spread to *Bacillus thuringiensis* spp. *kurstaki* Berliner (McLaughlin & Mitchell. 1993, Tabashnik et al. 1990, Shelton & Wyman 1992). Coupled with resistance, the increasing cost of pesticides and environmental awareness has encouraged the search for alternative management strategies (Ooi & Sudderuddin 1978).

Integrated pest management provides the most viable alternative to the heavy reliance on pesticides. An integrated approach to control lepidopterous pests in cabbage using multiple tactics is described by Biever et al. 1994. The system has had some success in the Rio Grande Valley of Texas and in Central America. For diamondback moth control, this involves scouting, limiting the use of conventional pesticides, more reliance on *B. thuringiensis*-based insecticides, and parasitoids. Of the parasitoids attacking the diamondback moth, *C. plutellae* Kurdjumov, *Diadegma insulare* (Cresson) and *D. semiclausum* (Hellen) show the most promise (Ooi & Lim 1989, Ooi 1990, Tabashnik et al. 1990).

*D. insulare* is the most important parasitoid of diamondback moth in North America (Latheef & Irwin 1983, Pimentel 1961, Oatman & Platner 1969, Harcourt 1960, 1963, 1986, Losata & Kok 1986, Horn 1987), with parasitism rates of >90% reported in untreated fields (Muckenfuss et al 1990). To integrate *D. insulare* into diamondback moth management systems, more information is needed on its biology, ecology and effect on diamondback moth.

The purpose of this study was to evaluate the survival of field-collected *D. insulare* pupae stored at a low temperature (4°C) for varying lengths of time, and the effect of *D. insulare* parasitism on feeding by host larvae.

#### MATERIALS AND METHODS

##### Viability of *D. insulare* Pupae Stored at Low Temperature

Pupae of *D. insulare* were collected from collard plants (*Brassica oleracea* var. *acephala* L.) serving as a trap crop between 2 commercial fields of cabbage (*Brassica oleracea* var. *capitata*) in Bunnell, Florida in mid-May 1995 (E. R. Mitchell et al., unpublished data). No attempt was made to cold-adapt parasitoids before use in experiments. High *D. insulare* parasitism levels were recorded for diamondback larvae on the collards during the growing season.

For the study, collard plants were uprooted, bagged, and transported to the laboratory where *D. insulare* cocoons were collected. The cocoons were inspected carefully to exclude empty cocoon cases, held in 250-ml waxed paper Dixie™ cups with ventilated lids and stored in a Nor-lake™ walk-in environmental chamber maintained at 4°C and

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70±10% RH with continuous fluorescent lighting. For 7 consecutive wk after the cocoons were collected, varying numbers were removed from cold storage and put into fresh Dixie™ cups with ventilated lids. The cups were then set up in the laboratory under ambient conditions of 25±2°C, 70-80% RH and under continuous fluorescent light. A water-soaked cotton ball and light smears of honey on the inside of the cup served as water and food sources for emerging parasitoids. Adult emergence was checked daily by opening the cups in a screened cage. Emerged adults were aspirated and sexed. A control group from the same initial collection of pupae not subjected to cold storage also was set up under ambient laboratory conditions and followed for emergence.

Adult emergence was recorded for 25 d after initial set up of each batch of pupae. Unemerged cocoons were examined for evidence of parasitism using a dissecting microscope. *Conura (Spilochalcis) side* (Walker) (Hymenoptera: Chalcidae) and dipteran parasitoids emerged from some of the cocoons. Their numbers are recorded but not used in the calculation of the percent emergence. Regression analysis was performed ( $y$ =percent emergence;  $x$ =days in storage) using Sigma Plot® (ver. 3.0, Jandel Corp., San Rafael, CA).

### Effect of *D. insulare* Parasitism on Feeding Rate of Diamondback Moth

Diamondback moth eggs were obtained by exposing collard plant leaves in a screened cage containing several hundred moth adults for 24 h. The leaves with eggs were transferred into Dixie™ cups with ventilated lids. Young collard leaves from collard plants uprooted from the field and stored at 4°C were added, and the eggs were held until hatch for 3 d under ambient laboratory conditions of 25±2°C, 70-80% RH and continuous fluorescent light. Two days after hatch, 20 second instar larvae were carefully picked with flexible forceps and put into each of 8 Dixie™ cups with ventilated covers.

One group of 4 cups was used as the treatment (parasitism), and the other half served as the control. Young collard leaves were introduced into each cup. Five 4-d-old mated female *D. insulare* from field-collected cocoons were released by aspirator into the treatment cups through a hole on the side that was then plugged with cotton. The larvae were exposed to attack by the parasitoids for 24 h. Water soaked cotton balls and smears of honey on the inside of the cups served as water and food sources for the parasitoids. After 24 h, the parasitoids were released by opening the cups in screened cages. Old collard leaves were removed and replaced with fresh ones. The larvae clinging to the old leaves were brushed gently onto the fresh leaves. The cups were held in the laboratory under ambient conditions as described previously. Collard leaves were replaced daily to provide an adequate supply of leaf material for the developing larvae. Leaf consumption rate for each replicate was determined by measuring consumed areas of the leaves with a mm<sup>2</sup> plastic grid under a dissecting microscope. No attempt was made to correct for leaf shrinkage or expansion. Observations were made daily until no feeding was observed or until pupation of either diamondback moth or *D. insulae* larvae. Percent parasitism, mean feeding rate per replicate per day, and percent mortality were calculated at the end of the trial. Experimental and control group results were compared by analysis of variance (ANOVA) and the means were separated using the Ryan-Einot-Gabriel-Welsch multiple range test (REGWQ) ( $P=0.05$ ; SAS Institute, 1988).

## RESULTS AND DISCUSSION

Results of the pupal viability test indicated that adult emergence decreased with long term storage at 4°C (Fig. 1). Percent emergence from the cocoons decreased linearly with increasing storage time ( $r^2=0.9603$ ;  $Y=98.3-2.06X$ ;  $P<0.01$ ). No emergence

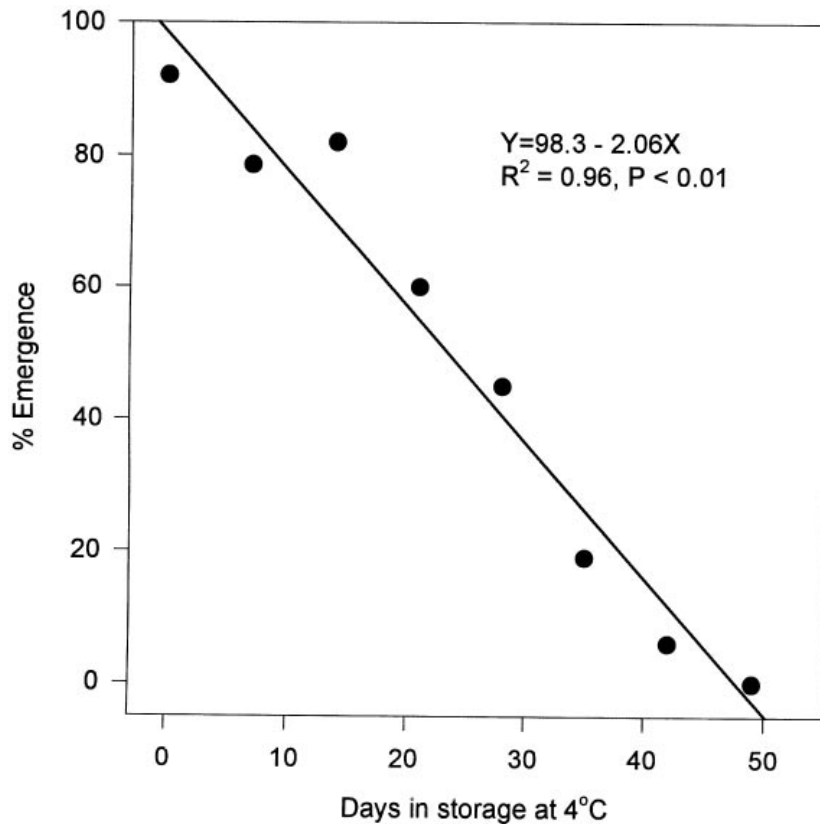


Fig. 1. Percent emergence of *Diadegma insulare* adults from cocoons stored at 4°C for varying lengths of time.

of *D. insulae* was observed after 49 d in storage. The highest emergence of 82% was obtained from cocoons stored for 14 days compared to 92% for cocoons in the control that were not subjected to cold storage. More males than females (127:71) emerged from cocoons stored at 4°C. However, the sex ratio of 1♀:1.79♂ was only slightly higher than that of the control group which was 1♀:1.5♂. The sex ratio for parasitoids emerging from cocoons stored at 4°C possibly was skewed by the low numbers of individuals that emerged after extended periods of cold storage. Data from cocoons collected from the collards in March had a sex ratio of almost 1♀:1♂ (Mitchell et al., unpublished data). The change in sex ratio from March to May suggests that environmental factors and/or increased population pressure favored a shift in favor of male *D. insulare*. The skewed sex ratio favoring males is consistent with those obtained for F1 and subsequent generations when reared in the laboratory (unpublished data), and it is a significant factor in attempts to rear the parasitoid in the laboratory for extended periods.

*Conura (Spilochalcis) side* (Walker) and a dipteran parasitoid emerged from some of the cocoons even when the hosts appeared to be no longer viable, indicating that the

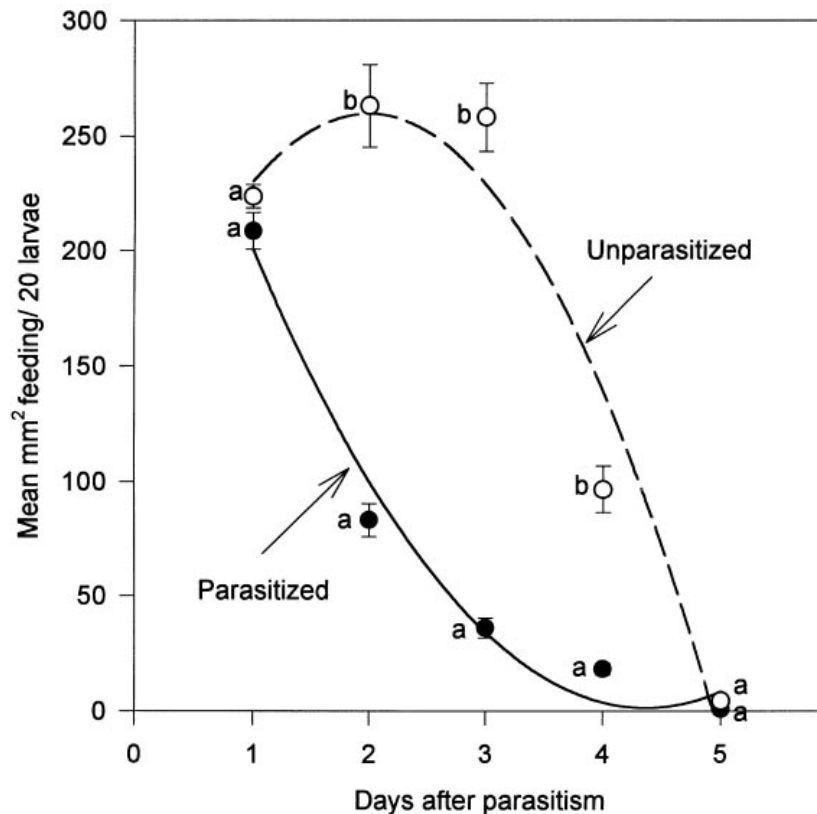


Fig. 2. Daily feeding rate of diamondback moth larvae parasitized by *Diadegma insulare*. Means on the same day with different letters are significantly different (Ryan-Einot-Gabriel-Welsch multiple range test).

parasitoids perhaps were more cold tolerant than *D. insulare*. Dissections of un-emerged cocoons showed dead pharate *D. insulare* adults. The inability of fully formed adults to emerge from their cocoons may be attributed to a lowering of the metabolic rate when exposed to 4°C which resulted in reduced available energy to break through the cocoon. Because *D. insulare* overwinters as a pupa (Harcourt 1960), information obtained could be important in providing insight about the fate of cocoons released during cold spells in the field. The length of time at which viability is maintained also is important with respect to storage of cocoons for use in experiments during periods when *D. insulare* are not readily available in the field.

Figure 2 depicts the effect of *D. insulare* parasitism on feeding rate of diamondback larvae. There was a significant difference in the foliage consumption rate between parasitized and non-parasitized larvae during the period of the experiment ( $F=143.97$ ;  $df=30$ ;  $df=39$ ;  $P<0.05$ ). The multiple range test showed no significant difference in foliage consumption between the parasitized and unparasitized larvae one day after parasitism. The parasitized larvae then became sluggish and fed very little. There were significant differences in feeding rate between the two groups on days 2

through 4. There was no significant difference on day 5 because most of the larvae or parasitoids had pupated. Idris & Grafius (1993b) also reported less feeding by parasitized larvae compared with non-parasitized ones. Parasitism was 85.25%, which was less than the 93% observed by Goodwin (1979) in field settings for other species of *Diadegma* and the 95% reported by Muckenfuss et al. (1990) for *D. insulare*.

In the control group, leaf consumption was high for 3 days after set-up and then dropped dramatically. This was attributed to the insects' preparation for pupation. No mortality was recorded in the control group.

Because development of *D. insulare* is highly synchronized with diamondback moth development and the parasitoid has excellent searching ability (Idris & Grafius 1993a), it is a good candidate for use in IPM programs for control of diamondback moth in cabbage. The information reported here will be useful in developing strategies for managing natural populations of *D. insulare* using various tactics including trap crops, mating disruption (McLaughlin et al. 1994) and pesticides with low environmental impact such as *Bacillus thuringiensis*-based products (Biever et al. 1994).

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