# EFFECT OF AGE OF THE PUPARIUM OF THE APPLE MAGGOT, *RHAGOLETIS POMONELLA* (WALSH) (DIPTERA: TEPHRITIDAE), ON PARASITISM BY *PHYGADEUON WIESMANNI* SACHTL. (HYMENOPTERA: ICHNEUMONIDAE)

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**Abstract**

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The thickness of the wall of the puparium of the apple maggot, *Rhagoletis pomonella* (Walsh), increased as the puparium aged. The force required to pierce the wall of the puparium also increased with age, and this relationship was best described by a cubic polynomial. Increasing thickness and hardness of the wall of the puparium will likely influence the efficacy of the parasite *Phygadeuon wiesmanni* Sachtl.

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**Résumé**

L'épaisseur de la paroi du puparium augmente en fonction de l'âge chez la Mouche de la pomme, *Rhagoletis pomonella* (Walsh). La force requise pour percer la paroi du puparium augmente aussi en fonction de l'âge et c'est une équation polynomiale du troisième degré qui décrit le mieux cette relation. L'épaisseur et la dureté de plus en plus grandes du puparium affectent probablement l'efficacité du parasite *Phygadeuon wiesmanni* Sachtl.

[Traduit par la rédaction]

## Introduction

The apple maggot, *Rhagoletis pomonella* (Walsh), is an important pest of apple in southern Ontario (Hall 1940; Smith 1988). The insect is usually univoltine, although a few second generation adults emerge in the fall in some years. Egg and larval development occur in the fruit; and larvae, when mature, enter the soil where pupation occurs. The insect overwinters as a pupa in the soil. Although natural mortality of the larvae and pupae is high (Hall 1940; unpublished data), parasitism is not an important mortality factor (Monteith 1971, 1978). The results of a survey carried out by the senior author between 1984 and 1987 in several unsprayed (no insecticides) apple orchards in southern Ontario substantiate the findings of Monteith (1971, 1978) as levels of parasitism ranged between 3.0 and 7.4%.

In several areas of Europe, the ichneumonid *Phygadeuon wiesmanni* Sachtl. is a major parasite of the European cherry fruit fly, *Rhagoletis cerasi* L. (Hoffmeister 1988). *Phygadeuon wiesmanni* also successfully attacked pupae of *R. pomonella* in the laboratory and in field cages (unpublished data), and this parasite was first released in southern Ontario against *R. pomonella* in 1985. However, while attempting to mass rear *P. wiesmanni* on pupae of *R. pomonella* that had been stored at 3–5°C for 3–4 months, it was observed that as pupae aged, levels of parasitism decreased. Successful parasitism was reduced from $30.0 \pm 5.2\%$ (SE) ($n = 460$) in pupae <30 days old to $12.9 \pm 2.7\%$ (SE) ($n = 360$) in pupae 30–60 days old and to $5.9 \pm 4.3\%$ (SE) ($n = 332$) in pupae >60 days old. Also, on four occasions, female *P. wiesmanni* were observed walking around in rearing cages with puparia of *R. pomonella* attached to the ends of their extended ovipositors. As the thickness and hardness of the puparial wall increases after formation of the puparium (Snodgrass 1924), studies were undertaken to assess the degree to which these factors might have affected successful parasitism of pupae of *R. pomonella* by *P. wiesmanni*.

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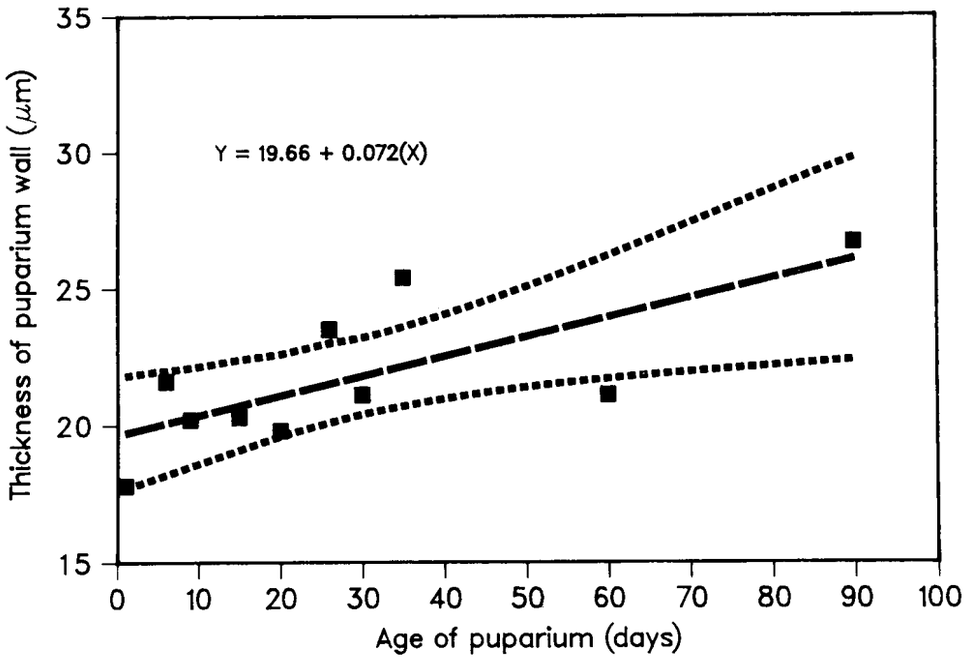


FIG. 1. Relationship between the age and thickness of the puparial wall of *Rhagoletis pomonella*. Dotted lines represent upper and lower 95% confidence limits.

## Materials and Methods

Pupae of *R. pomonella* were collected by placing infested apples in wooden boxes (77.5 by 77.5 by 11.3 cm), the bottoms of which were covered with chicken wire. Each box was then placed over another box of the same size containing a 0.5-cm layer of moist sand or a layer of two to three thicknesses of moist paper towelling. Mature larvae exiting apples fell onto the sand or towelling where pupation took place. Pupae were collected daily and placed in 9-cm-diameter Petri dishes in moist sand and the dishes placed in an open-sided insectary. Six pupae of known age were stuck in the depressions of pieces of corrugated cardboard with Elmer's non-toxic glue and exposed to a male and female pair of adult *P. wiesmanni* that had been held for at least 5 days after emergence in a plexiglass mating cage (11.9 by 6.6 by 10.3 cm) and provided with 50% aqueous honey solution as food. At 2- to 3-day intervals during the life [mean longevity $26.6 \pm 8.3$ days ($\pm$ SD) $n = 28$] of the female, fresh pupae of *P. wiesmanni* were provided. Pupae that had been exposed to the parasitoids were placed in 9-cm-diameter Petri dishes with a piece of moistened sponge, covered and held in an incubator at $22 \pm 2$°C, $\geq$80% RH, and a 16L:8D photoperiod. After 15–20 days the dishes were examined daily for parasite or host eclosion, or both. When eclosion ceased the remaining pupae were dissected to determine if parasitism had occurred and the degree of natural mortality. Pupae that had died (dried up and shrivelled) within the puparium and those that had become infected with fungi were not included in the analyses.

The thickness of the puparial wall was determined by preparing cross-sections of the puparium at different ages. The puparia were frozen at $-20$°C and then fixed in Formalin : acetic acid : 50% ethanol (1:1:18, by volume) for approximately 1 week (Jensen 1962). Ten pupae from each age class (range 1–90 days) were dehydrated in a tertiary butyl alcohol

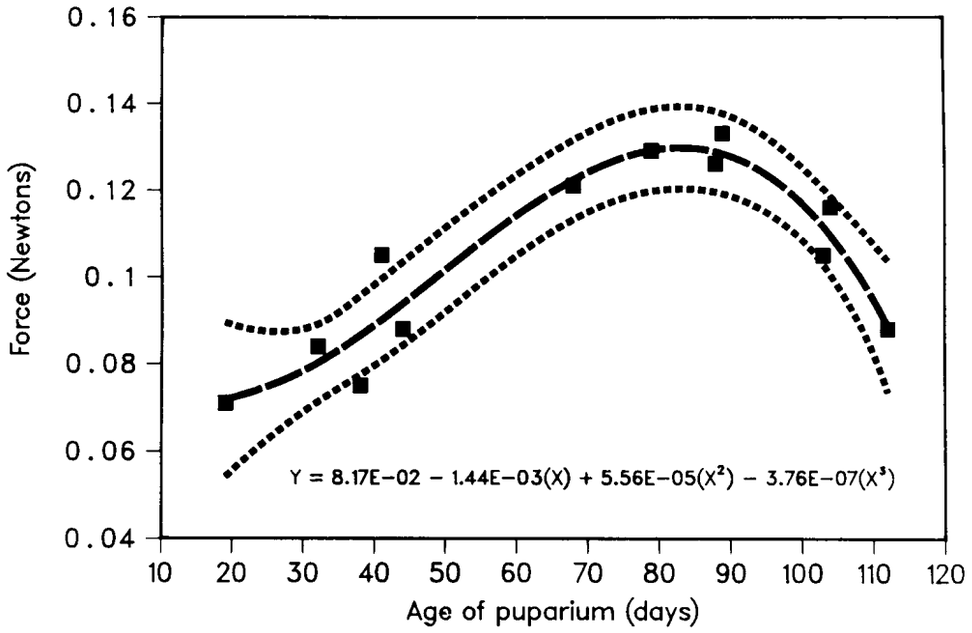


FIG. 2. Relationship between the age of the puparium of *Rhagoletis pomonella* and the force required to pierce its wall. Dotted lines represent upper and lower 95% confidence limits. Measurements made on 26–67 puparia in each age class.

series (Johansen 1940) and embedded in Paraplast Plus (56°C) (Sherwood Medical, St. Louis, MO, USA). Cross-sections were cut at 8 μm on a rotary microtome and mounted on glass slides using a gelatin adhesive. After removing the paraffin wax with two rinses of xylene, sections were stained in 0.5% toluidine blue O for 2 min. Ten glass slides, each containing five cross-sections of the puparial wall, were prepared for each puparial age class. The thickness of the puparial wall was measured at four sites (12, 3, 6, and 9 o'clock) on each section and the values averaged. The peak force (Newtons) required to pierce the walls of the puparium was determined by a computerized procedure (Buckley et al. 1984) using an Apple II computer and an Instron (Model 4201) Universal testing machine operated in the compression mode. The Instron is widely used to measure the mechanical properties of foods (Voisey 1971). A needle probe was mounted in a chuck on the load sensing transducer attached to the moving crosshead of the Instron. The crosshead speed was set at 5 mm per min. Care was taken to ensure that the penetrometer tip was positioned near the mid-line of the pupa, and that penetration was between the bands of the puparium. Force measurements were made on puparia that ranged in age from 19 to 112 days.

The relationship between age and thickness of puparium was established by linear regression analysis (SAS Institute 1985). A non-linear relationship between the thickness of the puparial wall and the force required to pierce the wall was established by examining the distribution of residuals. The best fitting curve was determined using the optimum Adjusted $R^2$ and $F$ statistic for the polynomial mode (SAS Institute 1986).

## Results and Discussion

There was a significant relationship ($R^2 = 0.5239$, $P < 0.05$) between the age and thickness of the puparial wall, i.e. thickness of wall increased as the puparium aged

(Fig. 1). There was also a significant relationship ($F = 24.05$, $P > F = 0.0002$, Adjusted $R^2 = 0.8628$) between the peak force required to pierce the puparial wall and its thickness. This relationship was best described by a cubic polynomial (Fig. 2). These data showed that a greater force was required to pierce the wall of the puparium as the latter aged. The force required to pierce the wall of puparia older than 90 days decreased, probably due to physical or physiological factors, or both, affecting the nature of the cuticle at that time.

Levels of parasitism in pupae ranged between $23.4 \pm 6.3\%$ (SE) ($n = 237$) in pupae <30 days old, $7.4 \pm 2.4\%$ (SE) ($n = 166$) in pupae 30–60 days old, and $4.5 \pm 3.3\%$ (SE) ($n = 128$) in pupae >60 days old. Reduction in levels of parasitism by *P. wiesmanni* might thus have been due to the increased hardness and thickness of the puparial wall as it aged (Zdarek and Fraenkel 1972). However, as the puparia were not examined histologically after probing by the female parasite, it was not possible to determine whether lower parasitism in older pupae was due to inability of the females to pierce the wall of the puparium, or to failure of females to oviposit after successfully piercing the wall. It is apparent, nevertheless, that the proportion of older:younger host pupae in the field at any given time will influence the establishment and efficacy of the parasite.

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