Ethyl acetate: a possible alternative for anaesthetizing insects

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Abstract. In order to anaesthetize insects in a laboratory, chilling and application of diethyl ether and carbon dioxide are commonly used. However none of the above methods is problem free. In our research, we evaluated ethyl acetate as an alternative anaesthetic substance. The effects of ethyl acetate anaesthesia were compared with those produced by carbon dioxide on adult green lacewings (Neuroptera: Chrysopidae). The biological parameters measured were longevity and fecundity. No significant differences appeared between the two treatments and the control. Although further research is necessary, the use of ethyl acetate proves to be very promising and presents a valid alternative to the use of diethyl ether and, in many cases, also to carbon dioxide and chilling.


Keywords: carbon dioxide, ether, chilling, Chrysoperla, green lacewings.

Many laboratory practices require the easy handling of insect specimens. One of the most common is undoubtedly individual marking.

Anaesthesia is almost always the only way to handle the insects without damaging them. Chilling and application of carbon dioxide or ether (diethyl ether) are the most common methods of anaesthesia cited in literature. The following substances are also used: chloroform, nitrogen, nitrous oxide, helium or argon gases, Freon-12 (dichlorodifluoromethane), methylene chloride, carbon tetrachloride and more recently halothane, isoflurane, sevoflurane (Ashburner & Thompson 1978: 48; Southwood & Henderson 2000: 106; Cooper 2001; Lewbart 2006: 216).

However none of the three most common agents is problem free. Many immediate and latent effects of carbon dioxide are known (Nicolás & Sillans 1989). Also chilling can modify the physiology and the behaviour of some species (Wilson et al. 2006; Champion de Crespigny & Wedell 2008; Tanner 2009); as can the use of diethyl ether (Seiger & Kink 1993; Joachim & Curtsinger 1990; Fresia et al. 2001) which is now limited as it is highly inflammable and irritating to mucous membranes.

Ethyl acetate is commonly used in entomology as a killing agent because it is not hygroscopic and it keeps the insect soft enough to allow proper mounting suitable for a collection. It is preferred over others because although it is inflammable, it is not particularly dangerous unless inhaled directly (Gullan & Cranston 2004: 431). The characteristics of this substance are well known by amateurs and professional entomologists.

“Ethyl acetate usually stuns insects quickly but kills them slowly. Specimens, even though they appear dead, may revive if removed from the killing jars too soon.” (Gibb & Oseto 2006: 14).

It is hard to understand why, as far as we know, there is no information about the use of ethyl acetate as an anaesthetic. On the contrary, we have always used this substance in our laboratory for extemporaneous anaesthesia with excellent results. Undoubtedly, as for
ether, insects can be over-anaesthetized by ethyl acetate with the subsequent problems described by Markow & O’Grady (2005: 212) for Drosophila. Specimens which undergo treatment must therefore be kept under observation and the treatment must be stopped a few seconds after they have stopped moving.

We wanted to compare the effects of anaesthesia by ethyl acetate with those produced by carbon dioxide on adult green lacewings (Neuroptera: Chrysopidae). The biological parameters compared were longevity and fecundity.

**Material and methods**

Trials were carried out on a population of Chrysoperla pallida Henry, Brooks, Duelli & Johnson, 2003 from North Sardinia (Italy) which was reared for less than 10 generations in our laboratories. The rearing method used was a simplified version of the one described by Pasqualini (1975). Rearing cages were cylinders open at both ends 10 cm in height and 8 cm in diameter. Food was honeybee pollen loads. Water was constantly supplied. Temperature was 20 ± 1 °C, humidity was 70±10%, and the photoperiod was 16:8 (light:dark).

For anaesthetizing, an individual was placed alone in a glass test vial (12 cm height by 3 cm diameter) closed with a cotton plug. If the insect was treated with ethyl acetate, the plug was simply wet with a few drops of anaesthetic. The specimen was observed until it began to stop moving in order to immediately remove it from the test vial. If it was treated with carbon dioxide, this gas was blown through a plastic cannula inserted between the plug and the tube, for about 30 seconds at a pressure of 3.5 bars at a quantity of 6–8 l/min. All individuals treated with anaesthetic had emerged from 24 to 48 hours and they had been fed and watered for at least 24 hours previously.

For each of the three treatments (ethyl acetate, carbon dioxide and control) 4 groups of 3 male and 3 female peers were used. Three pairs of green lacewings were reared together in the experimental vessels. The control individuals were placed directly in the rearing vessels, the others were placed there about an hour from treatment, when they had completely recovered from the anaesthesia.

The vessels were checked every 24 hours to record any deaths. Once a week fertile eggs were counted using the following procedure: a) all the eggs were removed from the vessel; b) all the eggs laid in the 48 hours, after the vessel was “cleaned”, were collected and saved; c) after another 48 hours it was possible to distinguish the sterile eggs from those with an embryo and these were then counted. The first count recorded the eggs laid on the 9th and 10th day from the beginning of the trial; the last count of

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### Table 1. Analysis of variance (Type III Sums of Squares) for the lifespan of Chrysoperla pallida specimens in relation to their treatments and sex.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>d.f.</th>
<th>Mean square</th>
<th>F-ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>163.444</td>
<td>2</td>
<td>81.722</td>
<td>0.16</td>
<td>0.8561</td>
</tr>
<tr>
<td>Sex</td>
<td>18.000</td>
<td>1</td>
<td>18.000</td>
<td>0.03</td>
<td>0.8537</td>
</tr>
<tr>
<td>Interaction</td>
<td>1508.33</td>
<td>2</td>
<td>754.167</td>
<td>1.44</td>
<td>0.2450</td>
</tr>
<tr>
<td>Residual</td>
<td>34643.5</td>
<td>66</td>
<td>524.902</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (corrected)</td>
<td>36333.3</td>
<td>71</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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![Figure 1](image)

**Figure 1**

A, Average lifespan of the Chrysoperla pallida groups subdivided by sex; B, Kaplan-Meier estimates of the survivor function for the three groups of survival times.
each vessel coincided with the death of all the females.
Number of eggs counted and days of lifespan were compared
using ANOVA (StatGraphics Plus 5.1, 1994–2001). In order
to decompose the variability of lifespan into contributions due
to sex we calculated the interactions by ANOVA table Type III
sums of squares.

To compare the survival data obtained, we plotted the Kaplan-
Meier estimates of survivor function for each of the three
groups of survival times. Differences in survival data between
experimental groups were tested with both the Wilcoxon test
and log-rank test (Collett 2003: 48).

Results

Lifespan of the experimental groups was
86.50 ± 22.19 dd (mean ± SD) in carbon dioxide,
87.00 ± 21.28 dd in the control, 89.92 ± 25.05 dd
in the ethyl acetate group. Lifespan of the sexes was
87.31 ± 27.53 dd for males and 88.31 ± 16.72 dd in
females. The average lifespan of the groups subdivided
in the ethyl acetate group. Lifespan of the sexes was
86.50 ± 22.19 dd (mean ± SD) in carbon dioxide,
89.92 ± 25.05 dd in the control, 87.00 ± 21.28 dd in
females. The average lifespan of the groups subdivided
by sex is shown in fig. 1A. Neither the differences be-
 tween groups, nor the difference between sexes, nor
the interaction between these two factors proved to be
significant (tab. 1).

The Kaplan-Meier estimates of the survivor function for each of the three groups of survival times is plotted in fig. 1B. There are no significant differences between the three survivor functions both according to the Wilcoxon test ($\chi^2 = 0.23; df = 2; P = 0.89$) and the log-rank test ($\chi^2 = 1.12; df = 2; P = 0.57$).

The number of eggs counted per vessel were recorded as 429.75 ± 215.45 eggs (mean ± SD) in carbon dioxide, 556.50 ± 216.51 eggs in the control, 677.00 ± 183.86 eggs in the ethyl acetate group. The differences are not significant according to ANOVA ($F = 1.44; df = 2.9; P = 0.2860$).

Discussion

The main aim of our trial was a preliminary check
of the possible use of ethyl acetate as an anaesthetic
for insects in laboratory practices. From the results
obtained, the use of this substance is very promising.

The longevity and fecundity of the group treated
with ethyl acetate as an anaesthetic were not significantly
different from the other groups, however they were
consistently superior. The only important exception
was the length of the females’ lives which was shorter
in spite of having laid a higher number of eggs.

Ethyl acetate seems to possess all the advantages
of diethyl ether without having all the disadvantages.
In the first place the anaesthetization uses simple
and economical equipment (even just a test vial, a
cotton plug and a few drops of anaesthetic) which
can be used in any laboratory, even small field labs.

Differently from ether, ethyl acetate can be handled
with fewer precautions, it is less irritating and less toxic
(Anonymous 2004, 2006) and therefore easier to use.

Although further research is needed to study the
possible physiological effects of the use of ethyl acetate
as an anaesthetic on different species of insect, this
substance already presents a valid alternative to the use
of diethyl ether and, in many cases, to carbon dioxide
and chilling.

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