Effect of methoprene on the heat tolerance and cold tolerance of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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**Abstract**

Methoprene, a Juvenile Hormone analogue, was evaluated for its ability to alter heat tolerance or cold tolerance of *Tribolium castaneum*, the red flour beetle. Young adults and late instar larvae were exposed to a series of methoprene concentrations. They were held either at 46 °C or 0 °C for different durations, and survival of adults or the adult emergence from larvae was recorded to determine their tolerance to extreme temperatures. At 46 °C, the lethal time to kill 50% of the population (confidence intervals) for untreated adults was 10.8 (9.6–11.8) h compared to 9.3 (8.3–10.0) h for adults exposed to 3.33 ppm of methoprene for 48 h. Higher concentrations of methoprene also caused adults to be less heat tolerant. In contrast, there was no evidence that methoprene reduced the heat tolerance of larvae. At 0 °C, both unacclimated and cold-acclimated insects were tested. Methoprene did not affect the cold tolerance of adults or larvae, regardless of cold acclimation. As seen in other studies, methoprene was not toxic to adults even at 66.6 ppm, but it was highly toxic to larvae (LD50 0.015 to 0.020 ppm). Cold tolerance was slightly greater in both adults and larvae, after being held at 15 °C for two weeks. This is the first study to report that a Juvenile Hormone analogue has an impact on insect heat tolerance.

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1. Introduction

The infestation by insects in stored products dates back to early civilization (Cotton, 1963; Sokoloff, 1972). Red flour beetle, *Tribolium castaneum* (Herbst), has long been reported as a major insect pest of both raw and processed forms of grains (Mickel and Standish, 1947; Daniels, 1956; Champ and Dyte, 1976; Li and Herbst), has long been reported as a major insect pest of both raw and processed forms of grains (Mickel and Standish, 1947; Daniels, 1956; Champ and Dyte, 1976; Li and Herbst), has long been reported as a major insect pest of both raw and processed forms of grains (Mickel and Standish, 1947; Daniels, 1956; Champ and Dyte, 1976; Li and Herbst), has long been reported as a major insect pest of both raw and processed forms of grains (Mickel and Standish, 1947; Daniels, 1956; Champ and Dyte, 1976; Li and Herbst), has long been reported as a major insect pest of both raw and processed forms of grains (Mickel and Standish, 1947; Daniels, 1956; Champ and Dyte, 1976; Li and Herbst), has long been reported as a major insect pest of both raw and processed forms of grains (Mickel and Standish, 1947; Daniels, 1956; Champ and Dyte, 1976; Li and Herbst), has long been reported as a major insect pest of both raw and processed forms of grains (Mickel and Standish, 1947; Daniels, 1956; Champ and Dyte, 1976; Li and Herbst), has long been reported as a major insect pest of both raw and processed forms of grains (Mickel and Standish, 1947; Daniels, 1956; Champ and Dyte, 1976; Li and Herbst), has long been reported as a major insect pest of both raw and processed forms of grains (Mickel and Standish, 1947; Daniels, 1956; Champ and Dyte, 1976; Li and Herbst), has long been reported as a major insect pest of both raw and processed forms of grains (Mickel and Standish, 1947; Daniels, 1956; Champ and Dyte, 1976; Li and Herbst), has long been reported as a major insect pest of both raw and processed forms of grains (Mickel and Standish, 1947; Daniels, 1956; Champ and Dyte, 1976; Li and Herbst), has long been reported as a major insect pest of both raw and processed forms of grains (Mickel and Standish, 1947; Daniels, 1956; Champ and Dyte, 1976; Li and Herbst), has long been reported as a major insect pest of both raw and processed forms of grains (Mickel and Standish, 1947; Daniels, 1956; Champ and Dyte, 1976; Li and Herbst), has long been reported as a major insect pest of both raw and processed forms of grains (Mickel and Standish, 1947; Daniels, 1956; Champ and Dyte, 1976; Li and Herbst), has long been reported as a major insect pest of both raw and processed forms of grains (Mickel and Standish, 1947; Daniels, 1956; Champ and Dyte, 1976; Li and Herbst), has long been reported as a major insect pest of both raw and processed forms of grains (Mickel and Standish, 1947; Daniels, 1956; Champ and Dyte, 1976; Li and Herbst). Current control measures for this beetle, *T. castaneum* or other stored-product insects include the use of contact insecticides such as the pyrethroid cyfluthrin (Arthur and Dowdy, 2003), application of diatomaceous earth (Dowdy, 1999b), fumigation with phosphine (Rajendran, 2000), sulfuryl fluoride (Reichmuth et al., 2003), use of low temperature (Fields, 1992) and high temperature (Arthur, 2006; Beckett et al., 2007). There are concerns with the use of synthetic chemicals to control stored-product insects; presence of residues in food, resistance development by pest species, health risks (Arthur, 1996), increased cost (Hagstrum and Subramanyam, 2006) and toxicity to non-target organisms (Fields, 1992).

Methoprene is an insect growth regulator that is a Juvenile Hormone (JH) analogue (Chanbang et al., 2008), disrupting the development of larvae to adults. JH analogues do not kill adults (Oberlander and Silhacek, 2000), but methoprene can have an indirect impact on adults by reducing their fecundity (Brown and Brown, 1982; Daglish and Pulvirenti, 1998). The effects of methoprene on stored-product insects have been extensively studied (Loschiavo, 1976; Samson et al., 1990; Oberlander et al., 1997; Arthur, 2004). Furthermore, there are several other JH analogues such as hydroproline (McGregor and Kramer, 1975; Mohandass et al., 2006), fenoxycarb (Samson et al., 1990; Oberlander and Silhacek, 2000), pyriproxifen (Aribi et al., 2006), triprene and kinoprene (Loschiavo, 1975). Methoprene, isopropyl 11-methoxy (2E, 4E), 3,7,11 trimethyl 2,4-dodecadienoate (Wilson and Turner, 1992), was registered as an insecticide, Diacon®, in the USA in the 1980s and contained both the R- and S-isomers (Arthur, 2004). It was reintroduced in 2002, with only the biologically active S-isomer of methoprene, by Wellmark International with their product Diacon II® (Chanbang et al., 2008). In the USA, it is registered for direct application to grains, as a contact insecticide or as an aerosol application (Jenson et al., 2009).
Heat was used as early as in 1700 in France to control Sitotroga cerealella (Olivier) in stored wheat (Duhamel du Monceau and Tillet, 1762). It was used to control stored-product insects in flour mills as early as at the beginning of the twentieth century (Dean, 1911). There has been a renewed interest in heat to control insects because of the phase-out of methyl bromide (Fields and White, 2002; Beckett et al., 2007). During structural heat treatments, temperatures of 50–60 °C are maintained for 24–36 h to control all stored-product insects (Fields and White, 2002). However, there are some limitations to using heat as an insect pest management strategy; the increased cost compared to other control methods (Dosland et al., 2006), damage to sensitive equipment (Dowdy and Fields, 2002) and uneven distribution of heat (Dowdy, 1999a). Therefore, it would be useful to determine if heat could be used with other treatments to make it more effective.

There are several examples of combining two treatments to control stored-product insects, which are more effective than either treatment used in isolation (Banks, 1987; Banks and Fields, 1995). Some of these include the combinations of; low temperature and ice-nucleating active bacteria (Fields, 1993), high temperature and diatomaceous earth (Dowdy and Fields, 2002; Beckett et al., 2007). During structural heat treatments, temperatures of 50–60 °C are maintained for 24–36 h to control all stored-product insects (Fields and White, 2002). However, there are some limitations to using heat as an insect pest management strategy; the increased cost compared to other control methods (Dosland et al., 2006), damage to sensitive equipment (Dowdy and Fields, 2002) and uneven distribution of heat (Dowdy, 1999a). Therefore, it would be useful to determine if heat could be used with other treatments to make it more effective.

There are several examples of combining two treatments to control stored-product insects, which are more effective than either treatment used in isolation (Banks, 1987; Banks and Fields, 1995). Some of these include the combinations of; low temperature and ice-nucleating active bacteria (Fields, 1993), high temperature and diatomaceous earth (Dowdy and Fields, 2002), pea protein and parasitoids (Hou et al., 2004), the organophosphate chlorpyrifos-methyl and the pyrethroid cyfluthrin (Arthur, 1994); cyfluthrin and piperonyl butoxide (Possischil and Smith, 1994) and the use of heat, carbon dioxide and phosphine (Mueller, 1994). More importantly, insect growth regulators were considered to be better used in an integrated pest management program, rather than being used alone (Oberlander et al., 1997). Methoprene has been used in combination with other insect growth regulators (Yonggyun and Krafsur, 1995; Daglish and Wallbank, 2005) or with other insecticides (Edward et al., 1993; Daglish, 2008).

Most studies with methoprene and stored-product insects have focussed on the effects on development or mortality. The effect of JH or its analogues on extreme temperature tolerance of insects is poorly studied. A few studies tested the effect of hormones on cryoprotectants and other molecules at freezing temperatures (Tsumuki and Kanehisa, 1980; Horwath and Duman, 1983; Hamilton et al., 1986; Xu et al., 1990). However, none of these studies examined how the combination of treatments affected the survival of insects at freezing temperatures. Furthermore, the effect of acclimation on the survival at low temperatures is well documented. Exposure to an intermediate temperature prior to exposure at the target low temperature increases insect cold tolerance (Fields, 1992). But again, there has been no attempt to explore the potential effect of hormones on cold acclimation. This is the first study to explore the synergistic effect of a JH analogue when used in combination with increased temperatures. The objectives of this study were to determine if the JH analogue methoprene affects the ability of T. castaneum adults or larvae to tolerate high or low temperatures and if methoprene affects cold acclimation.

2. Materials and methods

2.1. Methoprene

Diacon II (Central Life Sciences, Schaumburg, Illinois, USA) was used as the source of methoprene. Tribolium castaneum adults or larvae were exposed to a series of concentrations in heat and cold tolerance experiments. To ensure that any effects were caused by methoprene, the adjuvant mixture of the Diacon II containing all components except methoprene was used at the highest concentration used for methoprene as one of the controls. All trials had two controls; water and adjuvants. All the solutions were prepared immediately before spraying.

Spraying was carried out under a fume hood using an artist’s brush (Paasche Airbrush Company, Chicago, USA). Hard red spring wheat Triticum aestivum L. (14.0–14.4% moisture content) containing 80% whole wheat and 20% cracked wheat was used. For each treatment, 300 g of wheat was laid out into a single kernel layer on a waxed paper. From each concentration, 3 mL was placed in the reservoir of the artist’s airbrush, and was sprayed on to the grain sample. Air pressure of the sprayer was maintained constant throughout the experiment so that the droplet size was the same for all the treatments. Spraying was done in the following order; distilled water, adjuvants and finally the methoprene solutions starting from the lowest concentration to the highest concentration. For a given concentration of methoprene, four separate 300 g grain samples were sprayed; one for each of the four replicates. The same was done with the water and adjuvants. Immediately following spraying, each grain sample was hand tumbled for 30 s (Arthur, 2004) in a plastic bag for uniform dispersion of the compounds in the grain sample. A new waxed sheet was used for the spraying of each 300 g sample of wheat. Wheat was added to glass vials (35 mL); 20 g in each vial and covered with a cap having a wire mesh in the middle for aeration. One 300 g grain sample provided all the grain required for all the durations tested. There were four replicate vials for a particular duration of heat or cold exposure, each originated from independently sprayed grain samples.

2.2. Insects

Tribolium castaneum originating from farm grain bins near Steinbach, Manitoba and cultured in the laboratory since 1989 was used in the experiment. Insects were reared at 30 °C, 70% relative humidity (r.h.), in constant darkness in a medium containing 95% unbleached white wheat flour and 5% brewers’ yeast (ICN Biomedicals, Inc., Aurora, Ohio, USA). The adults and larvae used in both heat tolerance and cold tolerance experiments were produced by allowing two hundred adults to lay eggs on 250 g of the above medium for 3 d. Adults were 9–14 d of age and of mixed sex, and late instar larvae were 10–12 d, when they were placed on wheat treated with different treatments.

Tribolium castaneum cultures were sieved through an 850 μm mesh sieve to separate the 9–14 d adults. The adults were moved and counted using a vacuum line to minimize handling. To separate the 10–12 d old larvae, the rearing media were sifted through a 425 μm mesh sieve. A reference size of larvae was selected and thereby approximately the same-sized larvae were used throughout the experiments. The larvae were scooped on to a piece of paper, and were gently introduced to the vial containing the sprayed wheat. Twenty adults or larvae were introduced into each vial earlier filled with 20 g of treated wheat medium. These vials were held at 30 °C, 70% r.h. and in total darkness for 36–48 h, before being subjected to heat or cold treatments.

2.3. Heat tolerance experiment

In the experiments with adults, wheat was treated with 1.67, 3.33, 16.65, 33.3 or 66.6 ppm of methoprene. For larvae, methoprene concentrations of 0.003, 0.00825, 0.0165, 0.033 or 0.066 ppm were used based on preliminary experiments to have a range of no effect to 100% mortality. Water and 66.6 ppm of adjuvants for adults or water and 0.066 ppm of adjuvants for larvae were used as the controls.

Heat treatments were carried out in an oven at 46 ± 0.5 °C (Model: Salvis Lab, Rotkreuz, Switzerland). For adults, the exposure periods were 0, 6, 9, 11, 13, 15 or 17 h. For larvae they were 0, 3, 6, 9, 12, 15 or 18 h. Temperature was measured by placing...
After the incubation period, the second set of vials was exposed to darkness for two weeks for cold acclimation and was then exposed to a portable cooler containing crushed ice. This container was placed at 2.5 ℃ and insects was exposed directly to 0 ℃. Laboratory acclimation was not included in the total time taken as at 46 ℃ a hormonal impact on cold acclimation. Cold exposure was achieved by removing a group of vials from the oven, there was a drop of 60–90 min with the adults or 10–60 min with larvae for temperature to regain 46 ℃. The warm-up periods and drops in temperature were not included in the total time taken as at 46 ℃. Following heat treatments, the vials containing adults or larvae were placed in an incubator maintained at 30 ℃ and 70% r.h. The survival of adults was determined within 24 h from the termination of exposure at 46 ℃. Effect on larvae was determined as adult emergence and was done after 3 weeks from the heat exposure.

2.4. Cold tolerance experiment

Methoprene concentrations used were the same as for the heat tolerance experiment, but without the 0.066 ppm used with larvae. The controls were as described in 2.1. Following the incubation period of 36–48 h at 30 ℃, one set of vials containing treated grain and insects was exposed directly to 0 ± 0.2 ℃ and 65 ± 10% r.h. After the incubation period, the second set of vials was first transferred to a growth cabinet at 15 ℃ and 60–70% r.h., total darkness for two weeks for cold acclimation and was then exposed to 0 ℃. The purpose of this was to determine whether there is a hormonal impact on cold acclimation. Cold exposure was achieved by putting the vials, covered by a polythene cover, inside a portable cooler containing crushed ice. This container was placed at 2.5 ℃ and thus the vials were exposed to the temperature of melting ice. The temperature inside vials was measured using HOBO data loggers.

_Tribolium castaneum_ adults were exposed for 0, 3, 4, 5, 6 or 7 d if unacclimated with additional treatments for 8 or 9 d if acclimated. The larvae were exposed to 0 ℃ for 0, 2, 3, 4, 5 or 6 d if unacclimated, with an additional treatment for 8 d if acclimated. Upon the completion of these different periods at 0 ℃, the vials were transferred to a 30 ℃ growth cabinet with 60–70% r.h., in total darkness. After three weeks, the adults were counted.

2.5. Data analysis

Experimental design for the heat tolerance experiment was a complete randomized design (CRD) with two-factor factorial. One factor was the different methoprene concentrations and the second factor was the number of hours exposed to the elevated temperature, 46 ℃. The vials were considered replicates. Experimental design for the cold tolerance experiment was a complete randomized design (CRD) with three-factor factorial. One factor was the different methoprene concentrations, second factor was the number of days exposed to 0 ℃ and the third factor was the cold acclimation at 15 ℃. As in heat tolerance experiment, vials were considered replicates.

All means in the text are given with standard errors of the mean. Raw data obtained in the experiments were transformed by taking the square roots of the arcsine and were analysed using the ANOVA procedures of Statistical Analysis System (SAS Institute, 2002–2008). Means were separated by Tukey’s test with the significance level of 0.05, for a particular duration of exposure to 46 ℃ in the heat tolerance experiment or for a particular duration of exposure to 0 ℃, either in unacclimated or cold-acclimated batch of insects, in the cold tolerance experiment. Probit analysis used probit transformation of mortality, loge transformation of concentrations and adjustment for control mortality with natural response parameter (PoloPlus, LeOra Software). Comparison of lethal dose (LD50) or lethal time (LT50) was made using the lethal ratios (Robertson and Preisler, 1992). Lethal time (LT50) for each concentration was compared with the adjuvants solution as the control.

3. Results

3.1. Heat tolerance of adults

For the _T. castaneum_ adults, there was no effect of methoprene alone at 0 h exposure to 46 ℃ (Table 1). In general, there was no difference between the two controls, water and adjuvants, on the adults except at 11 h exposure, where the survival of adults exposed to adjuvants was lower than the water control.

Increased exposure to 46 ℃ caused reduced survival of adults. In general, methoprene at concentrations of 16.65 ppm and higher reduced the survival of _T. castaneum_ adults compared to the controls with water or adjuvants. This is evident with 6, 9, 11 or 13 h exposures at 46 ℃, when the means for survival of adults were separated by Tukey’s test. There are certain outliers (adjuvants at 11 h, 66.6 ppm at 6 h, and 33.3 ppm at 9 h), possibly due to variation of the heat in the oven. The lethal ratios at 3.33 ppm or higher concentrations showed significant differences compared to the adjuvants. The lethal ratio for water was slightly lower than adjuvants (0.92), which may be due to the sudden drop in survival in the adjuvants at 11 h exposure. However, over and above the behaviour of those outliers, the results show that pre-exposure to methoprene made _T. castaneum_ adults more sensitive to heat.

<table>
<thead>
<tr>
<th>Methoprene concentration (ppm)</th>
<th>Survival ± SE (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Duration of exposure (h)</th>
<th>LT50 (95% confidence intervals) (h)</th>
<th>LT50 ratio (95% confidence intervals)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>100 ± 0</td>
<td>98.8 ± 1.3a</td>
<td>0 ± 0</td>
<td>11.68 (11.03–12.30)</td>
</tr>
<tr>
<td>Adjuvants</td>
<td>100 ± 0</td>
<td>98.8 ± 1.3a</td>
<td>0 ± 0</td>
<td>10.75 (9.57–11.78)</td>
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<tr>
<td></td>
<td>100 ± 0</td>
<td>96.3 ± 1.3a</td>
<td>0 ± 0</td>
<td>10.27 (9.55–10.92)</td>
</tr>
<tr>
<td></td>
<td>100 ± 0</td>
<td>95.0 ± 1.3a</td>
<td>0 ± 0</td>
<td>9.25 (8.34–10.04)</td>
</tr>
<tr>
<td></td>
<td>100 ± 0</td>
<td>91.3 ± 1.3a</td>
<td>0 ± 0</td>
<td>9.19 (8.66–9.66)</td>
</tr>
<tr>
<td></td>
<td>100 ± 0</td>
<td>91.3 ± 1.3a</td>
<td>0 ± 0</td>
<td>9.06 (8.33–9.68)</td>
</tr>
<tr>
<td></td>
<td>100 ± 0</td>
<td>86.6 ± 1.3a</td>
<td>0 ± 0</td>
<td>9.38 (8.96–9.75)</td>
</tr>
</tbody>
</table>

<sup>a</sup> For a given exposure, means followed by the same letter in a column are not significantly different at P < 0.05 according to Tukey’s test following ANOVA.

<sup>b</sup> LT50 ratio = LT50 for adjuvants/LT50 for a particular treatment. The LT50 values are not significantly different at P < 0.05, if the 95% confidence intervals for the ratio include 1.0.
3.2. Heat tolerance of larvae

With no exposure to heat (0 h), methoprene was toxic to T. castaneum larvae with a LD50 (confidence intervals) of 0.020 (0.017–0.024) ppm methoprene, and almost complete suppression at 0.066 ppm (Table 2). With increased exposure at 46 °C, there was no difference between the water control and adjuvants, indicating that any effects on larvae exposed to methoprene concentrations were due to either methoprene or the heat. Adult emergence from the larvae treated with 0.003 and 0.00825 ppm methoprene were not different from controls (water or adjuvants), with no heat (0 h). Furthermore, when larvae were exposed to heat, there was no consistent difference between controls and these two lowest concentrations except with 0.003 ppm at 15 h exposure, according to Tukey’s mean separation. For the controls, the emergence ranged from 99 to 100% at 0 h, which reduced only to approximately 50% at the longest exposure time, 18 h (Table 2). Given that the two lowest methoprene concentrations did not show significant differences from the controls both at 0 h exposure and at increased exposure periods and that the LT50 ratios for those two concentrations were not different from that of the adjuvants, we conclude that methoprene did not make larvae more sensitive to heat. Methoprene alone had a strong impact on larvae to prevent their development to adults, as evident at three higher concentrations without any exposure to heat (0 h). This made it impossible to calculate the LT50 due to the high mortality at 0 h (Table 2). Therefore at exposures of more than 0 h at 46 °C, it is difficult to dissect the effects caused by the combination of heat and methoprene from those of methoprene alone. It may be possible that methoprene also reduced the heat tolerance of T. castaneum larvae, as in adults, but this reduction in survival (emergence to adults) was too small to be detected against the huge effects of methoprene alone on larvae.

3.3. Cold tolerance of adults

As in the heat tolerance experiment, methoprene alone (0 d) was not toxic to T. castaneum adults, in both unacclimated and in cold-acclimated groups. Furthermore, there were no significant differences between the survival of adults treated with water or adjuvants among unacclimated adults or among cold-acclimated adults according to Tukey’s mean separation, although slight differences were detected based on the lethal ratios (Table 3).

Survival of adults decreased with the increase in the duration at 0 °C. By acclimating adults at 15 °C for two weeks, the cold tolerance of T. castaneum adults was increased; in the controls LT50 (confidence intervals) increased from 3.59 (3.29–3.77) d for unacclimated adults to 6.02 (5.39–6.46) d for cold-acclimated adults. Furthermore, the lethal ratio (confidence intervals) for cold-acclimated vs. unacclimated adults treated with adjuvants was 1.84 (1.71–1.97) showing a significant increase in cold tolerance due to acclimation. However, neither the unacclimated or cold-acclimated adults showed pronounced and consistent reduction in the LT50 between the controls and the methoprene-treated adults. This is evident from the lethal ratio values, too. Therefore, methoprene has not affected the cold acclimation of T. castaneum adults. Furthermore, the absence of significant differences in the survival of adults in the controls and in the methoprene treatments for a particular duration of exposure, either in the unacclimated or cold-acclimated batch, shows that methoprene does not make T. castaneum adults more sensitive to cold.

3.4. Cold tolerance of larvae

As in the heat tolerance experiment, methoprene was highly toxic to T. castaneum larvae with no exposure to cold (0 d). The LD50 (confidence intervals) for both unacclimated and cold-acclimated larvae with no exposure to cold (0 h) was 0.015S(0.012–0.018) ppm. There were no significant differences between water and adjuvants in terms of adult emergence at any of the exposure periods, either in unacclimated larvae or cold-acclimated larvae, according to Tukey’s mean separation. Lethal ratio at LT50 showed a slight difference between the two controls in the cold-acclimated larvae (Table 4).

Survival of larvae decreased with increased exposure to 0 °C. As in the adults, there was an increase in the cold tolerance by holding larvae at 15 °C for two weeks before the cold exposure at 0 °C. In the controls, unacclimated larvae had an LT50 (confidence intervals) of 2.85 (2.39–3.20) d compared to 4.42 (3.61–5.09) d for cold-acclimated larvae. Lethal ratio (confidence intervals) for cold-acclimated vs unacclimated larvae treated with adjuvants was 1.42 (1.21–1.66) showing a significant increase in cold acclimation in the controls. Furthermore, there were no consistent differences in LT50 values between the controls and the methoprene-treated larvae either in unacclimated or cold-acclimated treatments. This is also evident from the lethal ratio values. This shows that, as in the case of cold tolerance of adults, there is no effect of methoprene on the cold acclimation or cold tolerance of larvae at 0 °C.

4. Discussion

Methoprene reduced the heat tolerance of T. castaneum adults. There are several explanations that would explain the reduced heat tolerance observed in the T. castaneum adults when they were first

### Table 2

Percentage adult emergence (mean ± SE) of T. castaneum larvae treated with methoprene and exposed to 46 °C for different durations (n = 4).

<table>
<thead>
<tr>
<th>Methoprene concentration (ppm)</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>98.8 ± 1.3a</td>
<td>96.3 ± 1.3a</td>
<td>97.5 ± 1.4a</td>
<td>97.7 ± 1.4a</td>
<td>93.8 ± 2.4a</td>
<td>91.3 ± 2.4a</td>
<td>83.8 ± 3.1ab</td>
</tr>
<tr>
<td>Adjuvants</td>
<td>100 ± 0a</td>
<td>97.5 ± 1.4a</td>
<td>95.5 ± 2.5a</td>
<td>97.5 ± 1.4a</td>
<td>85.0 ± 6.1a</td>
<td>87.5 ± 6.3a</td>
<td>LT50 (confidence intervals) (h)</td>
</tr>
<tr>
<td>0.003</td>
<td>100 ± 0a</td>
<td>100 ± 0a</td>
<td>98.8 ± 1.3a</td>
<td>98.8 ± 1.3a</td>
<td>90.0 ± 7.1a</td>
<td>36.3 ± 16.5cd</td>
<td>80.0 ± 4.1a</td>
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<td>0.00825</td>
<td>95.0 ± 2.9a</td>
<td>88.8 ± 4.7a</td>
<td>95.0 ± 2.9a</td>
<td>83.8 ± 2.4ab</td>
<td>68.8 ± 7.2ab</td>
<td>73.8 ± 4.3ab</td>
<td>67.5 ± 4.4a</td>
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<tr>
<td>0.0165</td>
<td>48.8 ± 8.5b</td>
<td>55.0 ± 4.1b</td>
<td>61.3 ± 5.9b</td>
<td>72.5 ± 7.8b</td>
<td>60.0 ± 4.6b</td>
<td>47.5 ± 11.1bc</td>
<td>65.0 ± 7.4a</td>
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<tr>
<td>0.033</td>
<td>16.3 ± 5.5c</td>
<td>11.3 ± 6.6c</td>
<td>7.5 ± 3.2c</td>
<td>16.3 ± 5.5c</td>
<td>11.3 ± 6.3c</td>
<td>0 ± 0d</td>
<td>17.5 ± 7.2c</td>
</tr>
<tr>
<td>0.066</td>
<td>3.8 ± 2.4c</td>
<td>2.5 ± 1.4c</td>
<td>2.5 ± 1.4c</td>
<td>2.5 ± 1.4c</td>
<td>2.5 ± 1.4d</td>
<td>5.0 ± 2.0c</td>
<td></td>
</tr>
</tbody>
</table>

a For a given exposure, means followed by the same letter in a column are not significantly different at P = 0.05 according to Tukey’s test following ANOVA.  
b LT50 ratio = LT50 for adjuvants/LT50 for a particular treatment. The LT50 values are not significantly different at P = 0.05, if the 95% confidence intervals for the ratio include 1.0. LT50 could not be calculated for all methoprene concentrations.
exposed to the JH analogue, methoprene, and then to increased temperature. The death of stored-product insects at increased temperatures occurs due to the changes in the lipids, rate imbalances of biochemical reactions, disturbance in the ionic activities or due to desiccation (Fields, 1992; Denlinger and Yocum, 1998). Insects undergo a wide array of physiological changes in response to heat stress to mitigate these adverse effects. These include synthesis of heat shock proteins (Lurie and Jang, 2007), increased production of insect blood sugar, trehalose (Singer and Lindquist, 1998), increases in glycerol or sorbitol (Denlinger and Yocum, 1998; Wolfe et al., 1998), increases in amino acids (Malmendal et al., 2006) or dopamine (Rauschenbach et al., 1993). One or more of these protective processes may have been adversely affected by methoprene.

Among the many factors responsible for heat tolerance, heat shock proteins are the most well-known (Denlinger and Yocum, 1998). Insects synthesize heat shock proteins when their body temperature rises above the optimal temperature for growth (Lurie and Jang, 2007). Heat shock proteins stabilize denatured proteins (Parsell and Lindquist, 1993), prevent deleterious aggregations of proteins (Parsell and Lindquist, 1993; Becker and Craig, 1994) and degrade misfolded or aggregated proteins (Sonno et al., 2002; Mocow and Tsugane, 2003). Heat shock protein 70 is induced in T. castaneum larvae, pupae and adults when exposed to 40 °C (Mahroof et al., 2005). As only the heat tolerance of adults was reduced by methoprene and not that of larvae of T. castaneum, it is possible that prior exposure to methoprene may perturbate the expression of this particular heat shock protein only in adult T. castaneum, but not in larvae. There is information to support the view that methoprene may trigger such interference. Heat shock protein synthesis is transcriptionally regulated (Lindquist, 1986) and JH acts within the nucleus (Riddiford, 1994), so could regulate the synthesis of heat shock proteins. In Drosophila, JH III and methoprene suppressed the expression of small heat shock protein genes hs 22 and hs 23 mediated byecdysterone (Berger et al., 1992). Therefore, it is possible that exposure to methoprene prior to heat treatment affected the heat shock protein synthesis in T. castaneum adults and reduced their heat tolerance.

One way to test the hypothesis that JH affects the heat tolerance of T. castaneum adults, would be to treat insects with a JH antagonist, precocene, which should decrease endogenous JH and we predict that the decrease of JH titre in the insect body would increase heat tolerance in adults, and have no effect in larvae. Another, but technically more difficult experiment, would be to measure JH levels (Cusson et al., 1994) in insects during exposure to heat. We would expect lower JH levels in response to heat in surviving adults.

Methoprene had no effect on cold tolerance of T. castaneum larvae or adults. Insect cold tolerance is induced by several mechanisms. For freeze-intolerant species, these include elimination of ice nucleators from the body, synthesis of molecules such as low molecular weight cryoprotectants (polyols and sugars) and thermal hysteresis proteins (anti-freeze proteins) (Lee, 1991; Fields, 1992). For freeze-tolerant species, these include synthesis of ice-nucleating proteins, lipoproteins and molecules such as low molecular weight cryoprotectants (Lee, 1991).

There are only a few studies on hormonal effects on cold tolerance in insects (Duman et al., 1991). Application of JH or JH analogues has affected larvae; JH increases the anti-freeze protein levels in the larvae of Dendroides canadensis Latreille (Coleoptera: Pyrochroidae) (Horwath and Duman, 1983; Xu and Duman, 1991), increases glycerol and sorbitol levels in the third instar larvae of gall fly Eurosta solidaginis (Fitch) (Diptera: Tephritidae) (Hamilton et al., 1986), reduces the supercooling point in the larvae of E. solidaginis (Rojas et al., 1987), depresses the supercooling point in beetles.


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