Physiological responses of insects to heat

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Received 19 April 2000; accepted 21 August 2000

Abstract

Postharvest quarantine treatments using high temperatures have been developed for various commodities. There are a wide range of insect pests that are the target of these treatments. In order to make heat treatments effective against these pests, the effects of high temperatures on insect physiology must be understood. Insects, being poikilothermic, are particularly sensitive to heat. Nearly every system studied in insects has demonstrated sensitivity to heat. Studies on the effects of heat in insect metabolism demonstrate some adaptability to thermally challenging environments. Respiration, as to be expected, is also effected by heat, and as the body temperature of the insect increases, there are concomitant increases in both metabolism and respiration up to a critical thermal limit. The effects of heat on the nervous and endocrine systems is another area where elevated temperatures wreck havoc. Changes in behavior and development have been documented as resulting from heat treatments. Among the most studied responses of insects to heat is the elicitation of heat shock proteins. The impact of these proteins on thermotolerance are still being investigated. Models of thermal damage leading to the death of the insect are discussed as well as current studies in describing the events of thermal death. Published by Elsevier Science B.V.

Keywords: Heat; Insects; Respiration; Heat shock; Metabolism

1. Introduction

Insects are known to live in a wide range of thermal climates, but there is very little variability in the maximum temperature (40–50°C) which they can survive (Heinrich, 1981). Postharvest heat treatments to disinfect fresh and stored products have been used for more than 60 years (Jones, 1940). The successful application of these treatments relies on a delicate balance between commodity tolerance and insect intolerance. Understanding how high temperatures affect insect mortality can lead to the formulation of more effective treatments.

Many experiments have been done in which insects have been transferred from one temperature to another and observations made on the effects on metabolism. Clarke (1967) classified these changes in temperature in engineering terms as ‘step function’ and ‘ramp function’. Step function refers to a change from one temperature to another as rapidly as possible. An example would be water bath studies in which insects are immersed directly into heated water (or other aqueous medium) (Sharp and Chew, 1987; Jang,
Step-function transfers reveal how rapidly an insect can respond to a thermal challenge. Ramp-function is when a slower rate of change in temperature occurs (Fig. 1). An example would be in-fruit heat treatments or controlled water bath treatments (Shellie et al., 1997; Neven, 1998a,b). Ramp-function heat treatments can reveal, through examination of the response curve, what mechanisms may be involved in thermal tolerance and indicate whether the tolerance limits of the insect are wider in response to a ramp- than to a step-function (Clarke, 1967). Where applicable, these terms are used below to describe the effects of various heat treatments on insect physiology. In this review, the effects of heat on insect metabolism, respiration, nervous and endocrine systems, and the role of heat shock proteins (hsps) in thermal tolerance are discussed.

2. Metabolism

Due to the exothermic nature of insects, metabolic rate is extremely dependent upon environmental temperature. Optimal growth and development of insects falls within a fairly broad range of temperatures. For example, for codling moth the range is between 10 and 30°C (Rock and Shaffer, 1983). Acute changes in temperature, as experienced in postharvest quarantine treatments, can elicit a range of metabolic responses. Some insects may increase anaerobic metabolism, as in nonfeeding larvae of Cochliomyia macellaria, which results in an increase in polyols and polyphosphates (Meyer, 1978).

One of the most important effects of temperature change is on enzymes. Changes in temperature may affect the binding of a substrate to the enzyme, causing a shift in the Michaelis constant $K_m$, thereby effecting immediate metabolic compensation (Hochachka and Sommario, 1984). Elevated temperatures may also influence the rate of enzymatically catalyzed reactions by determining the proportion of molecules in a given population that possess sufficient energy (energy of activation, $E_a$; enthalpy of activation, $\Delta H$) to react and form an activated complex (Hoffman, 1984). The temperature dependence of the barrier free energy of activation is proposed as a possible molecular basis for spontaneous temperature compensation (Hoffman, 1984). Changes in the fluidity of the phospholipid bilayer of membranes may also affect the activity of membrane-bound enzymes (Hochachka and Sommario, 1984). Temperature may also influence the interaction between enzymes and modulating metabolites (Fig. 2), and affect the conformation of an enzyme, which may in turn alter the kinetic properties of the enzyme (Fig. 3). This effect is probably the most critical in situations where temperatures reach above 40°C.

3. Respiration

The overall metabolic rate of an insect is often measured as oxygen consumption or carbon dioxide evolution. To assess the effects of temperature on insect metabolism a relationship between body weight and respiration over a range of temperatures can be calculated by the van’t Hoff equation.

$$Q_{10} = \left( \frac{R_2}{R_1} \right)^{10(T_2 - T_1)}$$

where $R_2$ is the rate at any temperature $T_2$ (in °C) and $R_1$ is the rate at any lower temperature $T_1$. The formula indicates that the $Q_{10}$ is temperature dependent. An example of the difference in respi-
Fig. 2. Influence of experimental temperature (ET) on the apparent Km of phosphoenolpyruvate (PEP) of pyruvate kinase (PK) from muscle and fat body of three cricket species: *Gryllus campestris* (□), *Gryllus bimaculatus* (▲), and *Acheta domesticus* (○). AT acclimation temperatures (After Hoffman and Marstatt, 1977). Reprinted from Journal of Thermal Biology, Vol. 2, Hoffman and Marstatt, pp. 203–207, 1977, with permission from Elsevier Science.


point, respiration decreases (Fig. 5). It is unclear whether this is an active suppression of respiration, supposedly to conserve stored energy and to buy time in case the temperature lowers and normal metabolism can resume, or if it is an effect of compromised metabolism due to the effects of heat on the respiratory system. Once the insect reaches peak respiration, it is able to recover (Table 2) (Neven, 1998b). However, death occurs soon after respiration rates begin to drop, even if the insect is returned to normal thermal conditions, indicating that systemic cell death is occurring.


<table>
<thead>
<tr>
<th>Temperature</th>
<th>Respiration rate (μlCO₂/mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10°C</td>
<td>0.581</td>
</tr>
<tr>
<td>15°C</td>
<td>0.926</td>
</tr>
<tr>
<td>20°C</td>
<td>1.098</td>
</tr>
<tr>
<td>25°C</td>
<td>1.146</td>
</tr>
<tr>
<td>30°C</td>
<td>1.296</td>
</tr>
</tbody>
</table>
and denatured proteins. Since oxygen is required to produce ATP through oxidative phosphorylation, a lack of ATP would render these proteins relatively inactive. Other effects of an anoxic environment may be observed in the inability to metabolize lipids and carbohydrates to maintain elevated metabolism, inability to synthesize proteins, and inability to alter fatty acids in the phospholipid bilayer to maintain membrane integrity.

4. Nervous system

Changes in temperature affect the central nervous system by differential effects on various nerves. Studies on Periplaneta americana group these nerve chord responses into four classes: (1) those that increase firing frequency with an increase in temperature; (2) those that show transient changes in firing pattern; (3) those that are responsive over a limited range of temperatures; and (4) those unaffected by temperature changes (Kerkut and Taylor, 1957).

The nervous system is important in the regulation of the insect’s response to high temperature by: (1) involvement of the perception of environmental temperature; (2) integration of this information with other sources (i.e. motor neurons, sensillia); (3) adjusting the patterns of insect behavior which will influence activity of the endocrine glands and patterns of muscular activity of the body (Clarke, 1967). The perception of environmental temperatures by the nervous system is accomplished primarily through the sensillae, but may also occur through the antennae, arolia and pulvilli of the legs, and some chemosensors are sensitive to thermal changes (Clarke, 1967).

5. Endocrine system

The role of the endocrine system in response to thermal change is not clear. Obviously, changes in nervous function will ultimately affect the activity of the endocrine system. Many of the changes in insect development and reproduction may result
Table 2
Respiration rates and peak respiration times of fifth instar codling moths during simulated fruit heat treatments (From Neven, 1998b)\textsuperscript{b}

<table>
<thead>
<tr>
<th>Treatment type</th>
<th>Peak time\textsuperscript{a}</th>
<th>Peak rate</th>
<th>Recovery times</th>
<th>Time to 42°C (min)</th>
<th>LT\textsubscript{95} (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min ± se</td>
<td>µlCO\textsubscript{2}/min/mg</td>
<td>3/4 Peak</td>
<td>Full peak</td>
<td></td>
</tr>
<tr>
<td>MFA 44°C</td>
<td>126.3 ± 5.0</td>
<td>3.25 ± 0.23</td>
<td>51.7 ± 4.6</td>
<td>32.7 ± 2.9</td>
<td>97</td>
</tr>
<tr>
<td>MFA 46°C</td>
<td>124.0 ± 3.0</td>
<td>3.66 ± 0.17</td>
<td>29.5 ± 2.6</td>
<td>32.2 ± 2.9</td>
<td>68</td>
</tr>
<tr>
<td>MFA 46°C</td>
<td>106.4 ± 2.5</td>
<td>4.05 ± 0.25</td>
<td>22.2 ± 1.9</td>
<td>25.2 ± 2.2</td>
<td>66</td>
</tr>
<tr>
<td>MFA 48°C</td>
<td>121.2 ± 3.7</td>
<td>4.31 ± 0.23</td>
<td>28.7 ± 10.8</td>
<td>29.6 ± 2.6</td>
<td>73</td>
</tr>
<tr>
<td>MFA 48°C</td>
<td>109.3 ± 2.2</td>
<td>4.11 ± 0.15</td>
<td>31.3 ± 2.7</td>
<td>40.5 ± 3.6</td>
<td>56</td>
</tr>
<tr>
<td>MFA 48°C</td>
<td>116.5 ± 2.8</td>
<td>4.38 ± 0.18</td>
<td>30.7 ± 2.7</td>
<td>42.7 ± 3.8</td>
<td>75</td>
</tr>
<tr>
<td>VFA 44°C</td>
<td>105.7 ± 2.6</td>
<td>4.28 ± 0.17</td>
<td>24.3 ± 2.1</td>
<td>38.2 ± 3.4</td>
<td>58</td>
</tr>
<tr>
<td>VFA 46°C</td>
<td>89.4 ± 3.2</td>
<td>4.88 ± 0.17</td>
<td>33.2 ± 2.9</td>
<td>33.8 ± 3.0</td>
<td>42</td>
</tr>
<tr>
<td>VFA 48°C</td>
<td>90.1 ± 3.5</td>
<td>4.43 ± 0.13</td>
<td>39.0 ± 3.4</td>
<td>39.5 ± 3.5</td>
<td>38</td>
</tr>
<tr>
<td>VFA 48°C</td>
<td>85.5 ± 4.8</td>
<td>4.35 ± 0.20</td>
<td>39.7 ± 3.5</td>
<td>33.5 ± 2.9</td>
<td>32</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Time required to reach maximum respiration.
\textsuperscript{b} MFA: Moist Forced Air treatment, non-condensing environment. VFA: Vapor Forced Air treatment, condensing environment.

From changes in the endocrine system. For example, when codling moth were reared at elevated temperatures, a decline in fertility was observed as the duration of exposure to the elevated temperature was increased (Proverbs and Newton, 1962; White, 1981). Another example comes from research with codling moth and the effects of prestorage heat treatments on larval mortality (Neven and Rehfield, 1995). A ramping function heat treatment at 4°C per hour to a final temperature of 38°C and held there for 4 days did not kill fifth instar codling moth. However, a supernumerary molt to a 6th instar was observed. With the first example, the elevated temperature could have affected the endocrine system and prevented maturation of germ cells and perhaps inhibited the deposition of vitellin in the eggs. In the case of the supernumerary molt, it is most likely that the levels of juvenile hormone were too high to permit the formation of pupae and continuation of development. There are numerous examples in the literature on abnormalities, defects, and delays in development in response to thermal stress (see Denlinger and Yocum, 1998). Many of these may be attributed to the effects on the endocrine system, whereas others may be an effect of high temperatures on DNA. Additional effects of heat on the endocrine systems of insects include: (1) decrease in PTTH levels due to a decrease in the size of the procerebral A1 neurosecretory cells (Jankovi-Hladni et al., 1983); (2) changes in JH titer (Rauschenbach, 1991); (3) effects on JH esterase (Rauschenbach, 1991); (4) changes in ecdysone titer (Rauschenbach, 1991); and (5) changes in AKH and other hormones (Ivanovi, 1991).

Fig. 6. Mortality of 5th-instar codling moth in infested cherries following heat treatments with and without controlled atmospheres. Values represent mean corrected mortality ± SE (From Neven and Mitcham, 1996).
6. Heat shock proteins

The elicitation of specific groups of proteins in response to an acute heat treatment in insects has received considerable attention in recent years (Yocum and Denlinger, 1992; Benedict et al., 1993; Gallie, 1993; Parsell et al., 1994; Marin et al., 1994; Vuister et al., 1994; Coleman et al., 1995; Feder et al., 1997). Most of the research has centered on *Drosophila* (Solomon et al., 1991; Vuister et al., 1994; Feder et al., 1997), but more and more information on heat shock proteins (hsp) in other insects is becoming available. HSPs are characterized in relation to their function and molecular weight (Pardue, 1988). Most of the literature on insect heat shock response centers on the intermediate group of stress proteins in the 60 to 80 kDa range.

The *HSP70* family is the best characterized of the heat-inducible proteins in insects. *HSP70* s are thought to function as molecular chaperones, in which either nascent or denatured proteins are held in situ by the *HSP70* protein, which may ‘chaperone’ the protein to the lysosome for degradation (Chiang et al., 1994) or help the protein re-fold after return to favorable temperatures (Parsell and Lindquist, 1994). The role of *HSP70* s in thermal tolerance in insects is well documented, although, it should be noted that the effects of hsp expression are short lived, and long-term exposure to inducing temperatures exceeds the capacity of these proteins to function and confer thermotolerance. However, in the application of high temperature treatments for disinfestation, in which acute thermal stresses are applied, hsps may play a very important role. Research by Yocum and Denlinger (1992) showed that a mild heat treatment of 40°C for 2 h to the flesh fly, *Sarcophaga crassipalpis*, conferred thermal tolerance to a subsequent normally lethal heat treatment of 90 min at 45°C. This thermal tolerance decayed over time, but lasted 72 h, beyond the time over which the originally induced *HSP70* s were degraded (24 h). This was an excellent example of how pre-conditioning of an insect can confer thermal tolerance to a subsequent higher thermal treatment, but this effect is not necessarily being related to hsps; other factors may participate in thermal tolerance.

![Graphical depiction of the effects of cumulative thermal damage on whole multicellular organisms.](image)

7. Models of thermal damage

What actually causes an organism to ultimately die in response to a heat treatment? Denlinger and Yocum (1998) discuss two current theories, one by Roti Roti (1982) in which it is suggested that the effects of heat on macromolecules are the culprit, and the other by Bowler (1987) which points to damage of the cell membrane as the critical event. However, these views negate the effects of heat on the whole organism. Denlinger and Yocum (1998) point out the hierarchy of thermal resistance: macromolecules > cells > tissues > whole organism (Fig. 7). The progression explains a phenomenon we often see in assessing heat treatments on insects, that of delayed mortality. The insect may seem ‘alive’ following a heat stress, but may fail to complete development under normal growing conditions.

There has been relatively little research done on the effects of heat stress on insect cells. Inferences on the cellular effects can be drawn from other systems. As temperature increases the pH and ion concentrations are altered, and there are also dramatic effects on macromolecules such as proteins, DNA, RNA, lipids, and carbohydrates, and on cellular structures such as cell and nuclear membranes, mitochondria and ribosomes. In general, as temperature increases, pH decreases. The ratio of free hydrogen ions is increased as temperature increases at a ratio of 0.015–0.2 pH units per °C. This change in pH affects the function of proteins, nucleic acids, and membranes.
Heat can also alter the structure of proteins and nucleic acids by destabilizing weak interactions such as van der Waals, ionic and hydrogen bonds. The Tm of many nucleic acids is around 50–52°C. Ribosomal RNA, being shorter in length, is more susceptible to changes in temperature than longer polymers. DNA is particularly vulnerable to heat damage. Lesions in DNA can occur at temperatures above 42°C in Chinese hamster cells (Warter and Brizgys, 1987). These lesions are easily repaired once the cells are returned to normal temperatures. However, if temperatures remain elevated, DNA repair enzymes might not be able or available to repair the damage.

Membranes of the cell, mitochondria, microsomes and nuclei are also vulnerable to thermal damage due to the effects on the phospholipid bilayer and other lipid components. Alteration of the liquid-crystalline fluidity of the membrane can alter the ionic balance of the cell, electric potential, and function of membrane-bound proteins.

The cuticle is also sensitive to temperature changes. The wax of the cuticle is important in protecting the insect from its external environment and maintaining water balance. High temperatures can alter the wax complex to become more fluid and may lead to desiccation (Hepburn, 1985). The effects of high temperature on insect mortality in low humidity environments may be compounded with desiccation stress (Fig. 8) (Beament, 1959). However, a high temperature treatment in a highly saturated environment may lead to drowning, primarily due to the loss of cuticular protection of the spiracles leading to the tracheoles.

So, what is the real cause of insect mortality following a heat treatment? There is no indication in the literature that can point to any one cause. However, recent research may begin to elucidate a cause. Thermal death kinetic studies with codling moth shows an interesting trend (Tang et al., 2000). For larvae of different instars and eggs, the TDT curves have different intercepts but the same slope with an activation energy of 500 kJ/mole. Preliminary work with other immature quarantine pests also show activation energies of 500 kJ/mole (Tang, pers. comm.). Whether this can be related to a single event (i.e. breakdown of the mitochondria, disruption of cellular membranes, denaturation of proteins and/or nucleic acids), or a summation of separate events remains to be seen. Further testing on individual cells, macromolecules, and other isolated systems will be needed to discern this phenomenon. Other work with scanning calorimetry may also help to describe energy balance of insects subjected to anoxic environments and thermal stress (Zhou et al., 2000). There is an increase in the metabolic heat rate (measured in W) of codling moth and omnivorous leaf roller pupae as the temperature is raised from 10 to 30°C. However, at temperatures above 40°C, there is a drop in the heat rate (Mitcham, unpublished data), indicating either a protective mechanism of energy conservation or the inability to produce an adequate supply of ATP to support elevated metabolism.

It is apparent that whatever system is investigated as being affected by heat, differences will be found. The key is to determine the critical or most sensitive point in the system, which can be manipulated to make heat treatments most effective in controlling insects.
References


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