Heat of respiration of Cryptolestes ferrugineus (Stephens) adults and larvae in stored wheat

R. COFIE-AGBLOR 1, W.E. MUIR 1, Q. ZHANG 1 and R.N. SINHA 2

1Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB, Canada R3T 5V6; and 2Agriculture and Agri-Food Canada Research Centre, 195 Dafoe Road, Winnipeg, MB, Canada R3T 2M9. Received 13 February 1995; accepted 25 October 1995.

Cofie-Agblor, R., Muir, W.E., Zhang, Q. and Sinha, R.N. 1996. Heat of respiration of Cryptolestes ferrugineus (Stephens) (Coleoptera: Cucujidae) adults and larvae in stored wheat. Can. Agric. Eng. 38:037-044. The heats of respiration of Cryptolestes ferrugineus (Stephens) (Coleoptera:Cucujidae) adults and larvae were measured both directly and indirectly by CO2 production in an adiabatic respiratory calorimeter. The heat production of 4-week old adults (2500 insects/200 g of wheat) and the second, third, and fourth instar larvae (1250 larvae/200 g of wheat) was determined at five initial grain temperatures (15, 20, 25, 30, and 35°C) and three moisture contents (12, 15, and 18% wet mass basis). Each variable and its interactions significantly affected heat production (p<0.001). Heat production by the adults increased exponentially with increasing temperature from 15 to 35°C. Although heat production increased with increasing moisture content, the rate of increase from 12 to 15% m.c. was greater than from 15 to 18% m.c. Heat production also increased with increasing wheat breakage and the increase from sound to 10% broken wheat was greater than from 10 to 20% broken wheat. Heat production rates ranged from 0.72 μW/insect at 15°C in sound wheat of 12% m.c. to 21.47 μW/insect at 35°C in 20% broken wheat of 18% m.c. Heat production of the larvae increased linearly with increasing level of each variable. Heat production rates of the larvae ranged from 0.37 μW/larva for the second instar at 20°C and 12% m.c., to 17.53 μW/larva for the fourth instar at 35°C and 18% m.c. Interaction between temperature and moisture content on heat production was modelled by exponential and linear regression equations.

Key words: calorimeter, heat of respiration, heat production, temperature, moisture content, grain mechanical condition, insects, larvae, wheat

La chaleur de respiration des adultes et des larves de Cryptolestes ferrugineus (Stephens) (Coleoptera : Cucujidae) a été mesurée directement et indirectement à la production de CO2 par un calorimètre adiatique. La production de chaleur des adultes âgés de 4 semaines (2500 insectes/200 g de blé), des larves de deuxième, troisième, et quatrième stades larvaires (1250 insectes/200 g de blé) a été mesurée à cinq températures initiales de grain (15, 20, 25, 30, et 35°C) et à trois teneurs en eau (12, 15, et 18% d’eau mass). Chaque variable et ses interactions a affecté (p<0.001) la production de chaleur. Chez les adultes, la production de chaleur a augmenté de façon exponentielle par rapport à la température entre 15 et 35°C. Quoique la production de chaleur ait augmenté avec la teneur en eau, le taux de croissance entre 15 et 18% teneur en eau était plus grand qu’entre 15 et 18% teneur en eau. La production de chaleur aussi a augmenté avec le pourcentage de grain cassé et le taux de croissance entre le grain intact et 10% de grains cassés était plus grand qu’entre 10% et 20% de grains cassés. La production de chaleur a varié entre 0.72 μW/insecte à 15°C avec le grain intact et 12% teneur en eau à 21.47 μW/insecte à 35°C avec 20% de grains cassés et 18% teneur en eau. Chez les larves, la production de chaleur a augmenté de façon linéaire pour tous les variables. La production de chaleur a varié entre 0.37 μW/larve au deuxième stade larvaire à 20°C et 12% teneur en eau à 17.53 μW/larve au quatrième stade larvaire à 35°C et 18% teneur en eau. Les interactions entre la température et la teneur en eau sur la production de chaleur sont décrits par les équations exponentielle et linéaire.

Mot clefs: calorimètre, chaleur respiratoire, production de chaleur, température, teneur en eau, condition mécanique du grain, insecte, larve, blé

INTRODUCTION

A bulk of stored grain is a dynamic system often comprised of insects, mites, and microorganisms, which interact with the grain and the environment to affect the nutritional quality and marketability of the grain. Because living organisms obey the laws of thermodynamics, only a part of the energy liberated from the oxidation of metabolic substrates is available for work; the remainder is evolved as heat. In stored grain, the heat of respiration from insects, mites, microorganisms, and the grain itself can lead to the development of hot spots and grain heating.

Insects have long been suspected to be the main cause of heating in dry grain (Back and Cotton 1924; Sinha and Wallace 1966). Oxley (1948) observed that insects are the main cause of grain heating when the grain moisture content is below 15% (wet mass basis, w.b.). Sinha and Wallace (1965, 1966) identified Cryptolestes ferrugineus (Stephens) as a major pest associated with heating and hot spots in grain in Western Canada. Howe (1962) described insect-induced hot spots in grain and noted that hot spots are affected mainly by grain temperature, moisture content, insect developmental age, and insect population density.

Insect metabolism and the accompanying heat production can be measured either directly by calorimetric techniques or indirectly by measuring the consumption of oxygen (O2) and the evolution of carbon dioxide (CO2). The rate of heat production is rarely measured directly and it is usually calculated from the respiratory exchange by utilizing a known energy equivalent (Elliott and Davidson 1975). Lindgren (1935) cautioned about using the respiratory exchange as an index of metabolism and stated that certain factors may lower the respiratory quotient (RQ, the ratio of the amount of carbon dioxide produced and the amount of oxygen con-
There is also the possibility of CO$_2$ sorption by wheat which may lower the amount of measured CO$_2$ (Cofie-Agblor et al. 1993). In view of the problems associated with the indirect measurement of heat production, alternative methods of measuring heat production are desirable. The objectives of the research reported in this paper, therefore, were: 1) to measure both directly and indirectly using an adiabatic respiratory calorimeter, the heat production of Cryptolestes ferrugineus adults and larvae in stored wheat; 2) to compare direct and indirect methods of heat measurement; and 3) to measure the effects of grain temperature, moisture content, and grain mechanical condition on heat production.

**MATERIALS AND METHODS**

**Equipment and materials**

The adiabatic respiratory calorimeter used was similar to the one of Zhang et al. (1992) with minor modifications and described by Cofie-Agblor et al. (1995). The heat capacities of the flasks and the specific heat of wheat at various moisture contents were determined by heating 600 g of distilled water and 300 g of wheat, respectively, with electric resistance heaters, and using the energy balance equation:

\[
Q = E \cdot I \cdot \theta = (m \cdot C_P \cdot \Delta T)_{\text{water}} + (m \cdot C_P \cdot \Delta T)_{\text{heater}} + (C_k \cdot \Delta T)_{\text{flask}}
\]

where:

- $Q$ = total heat supplied (J),
- $E$ = electric potential (V),
- $I$ = current (I),
- $\theta$ = time (s),
- $m$ = mass (kg),
- $C_P$ = specific heat (J kg$^{-1}$ °C$^{-1}$),
- $C_k$ = flask heat capacity (J°C$^{-1}$),
- $\Delta T$ = change in temperature (°C).

The specific heat of wheat was calculated by substituting $(m \cdot C_{Pw} \cdot \Delta T)_{\text{water}}$ for $(m \cdot C_{Pw} \cdot \Delta T)_{\text{heater}}$ in Eq. 1.

The amounts of CO$_2$ produced and O$_2$ consumed were measured with an HP5890A gas chromatograph (Hewlett-Packard, Avondale, PA) which has been described by Cofie-Agblor et al. (1995).

The wheat used for the tests and insect cultures was seed wheat (cv Katepwa, registered seed, United Grain Growers, Winnipeg, MB). Wheat samples were wetted to the desired moisture content by the addition of distilled water to the wheat in moisture-proof polyethylene bags. The samples were stored at 2°C. The particle density of the wheat at each moisture content was determined with an air comparison pycnometer (Beckman Instrumentation Inc., Fullerton, CA) and with a toluene pycnometer method. The specific heat of wheat calculated from Eq. 1 compared favourably with that calculated by the empirical equation proposed by Muir and Viravanichai (1972), as there was no significant difference (p=0.05) between the two methods between 12.0 to 18.0% m.c. The specific heats and standard deviations calculated from Eq. 1 were 1.653 ± 0.105, 1.758 ± 0.041, and 2.003±0.031 kJ kg$^{-1}$ °C$^{-1}$ at 12, 14.5, and 18% m.c, respectively.

The specific heat of wheat at 15% m.c. was calculated from the equation proposed by Muir and Viravanichai (1972). Seeds were broken by passing the wheat through a plate mill and sieving out the fines and smaller fragments with a sieve of 1.17 mm aperture.

The insects were reared in coarsely-ground and broken wheat of approximately 14% m.c. at 30 ± 1°C and 70 ± 5% relative humidity. The larvae were reared on flour made from a mixture of wheat germ and whole wheat (1:4 by mass) at the same conditions of temperature and relative humidity as the adults. Adults of known age were sieved out of the cultures at the appropriate time and each larval instar (stage of insect between successive ecdyses) age was determined by measuring the width of the head capsule (Campbell and Sinha 1978). The age range determined for each instar were: 6-8 d for the first instar, 10-12 d for the second instar, 13-15 d for the third instar, and 17-20 d for the fourth instar.

**Experimental procedure**

The experimental apparatus, wheat, adult insects, and larvae were conditioned at the desired initial temperature for 24 h. The insects and larvae were sieved out of the cultures using sieves with the appropriate apertures and were counted to obtain the desired population density. The wheat samples (200 ± 0.02 g) and insects were poured into the calorimeter flasks and the lids were closed tightly. Air samples (2 mL) were drawn for gas analysis and the experimental set-up was activated to continuously measure the temperature inside the flask. The tests were run for 20 h at the initial temperature of 15-25°C, and for 12 h at 30 and 35°C. At the end of each test, gas samples (two 2-mL samples) were drawn for analysis and the insects were sieved out of the experimental wheat and collected. Adult insects and larvae that remained completely immobile were counted to calculate mortality. The maximum mortality for the adults was 0.5% and for the larvae 3% in the second instar.

**Experimental design and analyses of data**

Completely randomized, factorial experiments were designed to measure the heat production of C. ferrugineus adults and larvae. Each test had four replicates. Adult heat production was measured at five initial grain temperatures (15, 20, 25, 30, and 35°C) and three grain moisture contents (12, 15, and 18% w.b.) with a population density of 2500 insects/200 g of wheat with 20% broken kernels (by mass). Additional tests were conducted at three temperatures (15, 25, and 35°C), three moisture contents (12, 15, and 18% w.b.) and two grain mechanical conditions (sound or 0, and 10% broken wheat by mass) with a population density of 2500 insects/200 g of wheat. Larval heat production was measured at four initial grain temperatures (20, 25, 30, and 35°C) and three moisture contents (12, 15, and 18% w.b.) with a population density of 1250 larvae/200 g of wheat with 20% broken kernels. The larval instars were second (L$_2$), third (L$_3$), and fourth (L$_4$).

Heat production was determined by two methods. In method A, cumulative heat production was calculated from the temperature increase as:

\[
H = \Delta T \left( m \cdot C_{pg} + C_k \right)
\]
where:
\[ H = \text{cumulative heat production (J)}, \]
\[ \Delta T = \text{temperature increase (°C)}, \]
\[ m_g = \text{mass of grain (kg)}, \]
\[ C_{pg} = \text{specific heat of grain (J kg}^{-1} \text{°C}^{-1}), \]
\[ C_k = \text{heat capacity of calorimeter flask (J °C)} \]

HC02 = cumulative heat production associated with CO2 production (J),

QC02 = energy equivalent of CO2 production (J g CO2),

nC02 = amount of CO2 produced (mol), and

\[ M_{CO2} = \text{molar mass of CO2 (44.01 g/mol)}. \]

Heat production (μW/insect and μW/larva) was calculated as the average heat production from the cumulative heat of each test. Analysis of variance was performed on each experiment using the General Linear Models Procedure from PC SAS (SAS Institute Inc., Cary, NC). Testing of the significance of the main effects was done by the Duncan’s new multiple range test.

The maximum error associated with the heat production calculated by Eq. 2 was between 5.1 and 5.8% and that with the CO2 production was 5.0%.

**Assumptions**

The following assumptions were made with regard to the measurement of heat of respiration:

1. The grain, insects, flask, and the box were in equilibrium with the initial grain temperature of the test after conditioning at the desired initial temperature for 24 h.
2. The acclimation of the insects for 24 h at the initial temperatures minimized the effect of thermal shock as the insects were moved from the rearing temperature of 30°C to the desired initial grain temperature.
3. The effect of cumulative CO2 on insect respiration during the tests was negligible.
4. The gases CO2, O2, and N2 behave as ideal gases.

**RESULTS AND DISCUSSIONS**

**Heat production by Methods A and B**

The directly measured heat production (method A) by the adults was not significantly different (p=0.05) from indirectly measured heat production (method B), except at 25°C for all moistures (Table I). Measured rates of heat production by larvae indicated that 57% of the means of the two methods A and B were significantly different from each other (Table II). The differences between the two methods were generally less than 9% at most test combinations, except for three tests where the differences between the methods ranged from 14 to 33%.

**Table I: Heat production (μW/insect) (n = 4) by 4-week old adult C. ferrugineus at a density of 2500 insects/200 g of wheat with 20% broken kernels**

<table>
<thead>
<tr>
<th>Moisture content (%)</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>12</td>
<td>1.74</td>
<td>1.72</td>
<td>3.22</td>
<td>3.19</td>
<td>5.51*</td>
</tr>
<tr>
<td>18</td>
<td>3.71</td>
<td>3.70</td>
<td>4.45</td>
<td>4.52</td>
<td>8.65*</td>
</tr>
</tbody>
</table>

*Method A was calculated from the temperature increase and Method B from the respiratory exchange.

Means determined by the two methods are significantly different from each other at \( P = 0.05 \)
Table II: Heat production (μW/larva) (n = 4) by *C. ferrugineus* larval instars at a density of 1250 larvae/200 g of wheat with 20% broken kernels

<table>
<thead>
<tr>
<th>Moisture content (%)</th>
<th>Larvae (instar stage)</th>
<th>Temperature (°C)</th>
<th>Method*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>12</td>
<td>2 N/A</td>
<td>0.37</td>
<td>1.77*</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.58</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.91</td>
<td>3.52</td>
</tr>
<tr>
<td>15</td>
<td>2 1.28*</td>
<td>0.96*</td>
<td>2.34*</td>
</tr>
<tr>
<td></td>
<td>3 2.23*</td>
<td>2.78*</td>
<td>6.31</td>
</tr>
<tr>
<td></td>
<td>4 4.00*</td>
<td>3.71*</td>
<td>6.93*</td>
</tr>
<tr>
<td>18</td>
<td>2 1.47</td>
<td>1.46</td>
<td>2.75*</td>
</tr>
<tr>
<td></td>
<td>3 2.36</td>
<td>2.39</td>
<td>5.98</td>
</tr>
<tr>
<td></td>
<td>4 5.10</td>
<td>5.06</td>
<td>7.66</td>
</tr>
</tbody>
</table>

*Method A was calculated from cumulative temperature and method B was calculated from the respiratory exchange.
*Means (n=4) determined by the two methods are significantly different from each other at $P = 0.05$.

Although the measurement errors of both methods were low, the observed differences may be due to the inaccuracies and assumptions associated with method B. We assumed that adults and larvae of *C. ferrugineus* metabolize only carbohydrates and lipids even though the insect may oxidize protein for energy. Krogh (1916) cautioned about using RQ calculations because the assumptions on which the deductions from RQ values are made are not strictly valid. It is also possible that, at certain combinations of temperature and moisture content, significant amounts of CO₂ were sorbed by wheat. According to Bradfield and Llewellyn (1982), direct calorimetry is a more accurate method of estimating heat production than an indirect one and if both methods are used simultaneously, each serves as a check on the accuracy of the other.

**Effect of temperature**

Analysis of variance indicated a highly significant ($p<0.001$) temperature effect on heat production by 4-week old adults and by the second to the fourth instar larvae. The means of heat production at all temperature levels were significantly different from each other ($p=0.05$, Duncan's new multiple range test). Heat production by the adults increased exponentially with increasing temperature from 15 to 35°C (Fig. 1a). According to van't Hoff's rule, the factor of increase ($Q_{10}$) in the rate of a chemical reaction for a 10°C temperature rise is between 2 and 3 (Hoffman 1985). Keister and Buck (1974) observed that the respiratory rate of most insects which is low at low temperatures, increases rapidly through mid-range temperatures and levels off sharply as lethal temperatures are approached. Using the mean heat production rates at each temperature in this study as reaction velocity constants, the $Q_{10}$ factor was 2.8 from 15 to 25°C, and was 2.4 from 25 to 35°C. The rate of heat production at 15 and 18% m.c. declined when the grain temperature exceeded 38°C, presumably because the temperature approached the lethal point of 42.5°C (Smith 1965).

Larval heat production increased almost linearly with increasing temperature in the 20 to 35°C temperature range (Fig. 1a). The $Q_{10}$ factor between 25 and 350C was 2.1, which is less than the 2.4 for adults within the same temperature range. The difference between the responses of the adults and the larvae to temperature is due to their different energy demands. Adult metabolism supports maintenance and reproduction, whereas larval metabolism supports growth and development.

**Effect of moisture content**

Heat production by adult *C. ferrugineus* increased with increasing moisture content, but the rate of increase from 12 to 15% w.b. was greater than that from 15 to 18% w.b. (Fig. 1b). Larval heat production, however, increased linearly from 12 through 15 to 18% w.b.

There are two explanations for the trend of heat production in relation to grain moisture content. First, because food forms the most important source of water for many terrestrial insects (Edney 1977), more water becomes available to the insect with increasing moisture content of the food substrate. Consequently, metabolism and other biological processes are accelerated. Second, as moisture content increases, kernel volume increases and this results in a decrease in kernel density which in turn may cause a reduction in kernel hardness. A decrease in kernel hardness enables the adults and larvae to puncture the seed and feed with less difficulty at high moisture contents than at low moisture contents. Sinha and Vosiey (1978) determined that for hard red spring wheat,
Heat production of *C. ferrugineus* adult (4-week old) at a density of 2500 insects/200 g of wheat and larval instars at a density of 1250 larvae/200 g of wheat with 20% broken kernels. (A = effect of temperature, B = effect of moisture content, and C = effect of instar stage, \( n = 4 \).)

**Effect of larval stage**

Heat production was a linear function of increasing instar stage (Fig. 1c); the average heat produced by the fourth instar was 2.5 and 1.4 times that of the second and third instar, respectively. The increase in heat production was due in part to the increase in larva size with age and a demand for energy required for increased rates of growth and development. Campbell and Sinha (1978) reported a similar trend in the heat production of *C. ferrugineus* larvae in wheat of 14% m.c. at 30°C.

**Variable interactions**

The relationships between heat production by adults and larvae and the combined effects of temperature and moisture content were determined by both linear and non-linear regression. The criteria for selecting an equation as the best fit depended on the standard error of the dependent variable, on the standard errors of the coefficients of the independent variables and their respective coefficients of variation (%), and on the coefficients of determination (\( R^2 \)).

The rate of heat production by 4-week old *C. ferrugineus*, in wheat at 12 to 18% m.c. and 20% broken kernels and at 15 to 35°C was modelled as an exponential equation from multiple non-linear regression:

\[
h_p = \exp (a + bM + T^c)
\]

where:

- \( h_p \) = predicted heat production (W/insect),
- \( M \) = moisture content (% w.b.),
- \( T \) = initial grain temperature (°C), and
- \( a, b, \) and \( c \) = empirical constants.

The values of the constants and their respective standard errors (S.E.) are: \( a = -3.969 \pm 0.215 \); \( b = 0.065 \pm 0.008 \); and \( c = 0.499 \pm 0.009 \). The fitted curves are shown in Fig. 2. The \( R^2 \) is 0.99.

For the second and fourth instars, equations were obtained by fitting the heat production by the instar at all three moisture contents and 20-35°C with multiple regression. Because multiple regression gave parameter values with poor statisti-
Fig. 2. The curves fitted to the experimental data (Exp., n=4) of the heat production by 4-week old adult *C. ferrugineus* in wheat with 20% broken kernels.

cal properties, for the third instar separate equations were obtained for each moisture content in the temperature range of 20-35°C (Fig 3).

i) for the second instar larvae at 20-35°C and 15 and 18% m.c.:

\[
\begin{align*}
\hat{h}_2 &= \exp (-4.759 + 0.037M + 0.291T - 0.003T^2) \\
\hat{h}_3 &= \begin{cases} -7.187 + 0.451T, & m.c. = 12\% \\ -8.985 + 0.582T, & m.c. = 15\% \\ -10.968 + 0.667T, & m.c. = 18\% \end{cases}
\end{align*}
\]

where: \( \hat{h} \) = predicted heat production by the second instar (\( \mu W/\text{larva} \)).

The S.E. of \( \hat{h}_2 \) is ± 0.061 and the \( R^2 \) is 0.99.

ii) for the third instar larvae at 20-35°C:

\[
\begin{align*}
\hat{h}_3 &= \exp (-2.832 + 0.039M + 0.243T - 0.003T^2) \\
\hat{h}_4 &= \begin{cases} -7.187 + 0.451T, & m.c. = 12\% \\ -8.985 + 0.582T, & m.c. = 15\% \\ -10.968 + 0.667T, & m.c. = 18\% \end{cases}
\end{align*}
\]

where: \( \hat{h}_3 \) = predicted heat production by the third instar (\( \mu W/\text{larva} \)).

The S.E. and \( R^2 \) of \( \hat{h}_3 \) at 12, 15, 18% m.c. are ± 0.419 and 0.99, ± 0.630 and 0.98, and ± 0.428 and 0.99, respectively.

iii) for the fourth instar larvae at 20-35°C and 12-15% m.c.:

\[
\hat{h}_4 = \exp (-2.832 + 0.039M + 0.243T - 0.003T^2)
\]

where: \( \hat{h}_4 \) = predicted heat production by the fourth instar (\( \mu W/\text{larva} \)).

The S.E. of \( \hat{h}_4 \) is ± 0.063 and the \( R^2 \) is 0.99.

Heat production at all combinations of moisture content and grain mechanical condition increased exponentially with increasing temperature from 15 to 35°C (Fig. 4). At each
SUMMARY AND CONCLUSIONS

Rates of heat production by *C. ferrugineus* adults and larvae were measured in adiabatic calorimeters. The following conclusions were drawn:

1. For the adults, directly measured heat production rate was not significantly different from indirectly measured heat production rate. For the larvae, the two methods were significantly different from each other.

2. Heat production by adult insects increased exponentially with increasing initial grain temperature from 15 to 35°C, whereas heat production by the larvae increased linearly with increasing temperature from 20 to 35°C.

3. Adult heat production increased with increasing moisture content and the rate of increase from 12 to 15% m.c. was greater than from 15 to 18% m.c. Larval heat production, however, increased linearly with increasing moisture content from 12 to 18%.

4. Heat production increased with increasing wheat breakage. The rate of increase from sound to 10% broken wheat was greater than from 10 to 20% broken wheat.

5. The relationships between heat production and the experimental variables were adequately modelled by exponential and linear equations.

ACKNOWLEDGEMENTS

We thank the Natural Sciences and Engineering Research Council of Canada for their financial support.

REFERENCES


