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Microcalorimetric and mass spectrometric methods for determining the effects of controlled atmospheres on insect metabolism

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Abstract

Metabolic heat rates of small insects which fit in 1 cm^3 ampoules can be measured readily using the Calorimetry Sciences Corporation 4100 DSC in the isothermal mode. The rapid thermal equilibration of the calorimeter on changing to a new isothermal temperature also permits the response of the metabolism to change in temperature to be determined quickly. By modifying the ampoules and measuring chamber lid for entry of silica capillaries to allow the headspace in the ampoules to be changed in situ, direct comparison can be made of metabolic heat rates in air and in controlled atmospheres. These results aid in assessing the effectiveness of controlled atmospheres, such as low oxygen and/or high carbon dioxide, as potential replacements of methyl bromide for fumigation of fresh produce. For a range of insects tested, including larval, pupal and adult stages, metabolic heat rates decrease to very close to zero under anoxic conditions. By analysis of the headspace of an ampoule containing an insect using ms, changes in metabolic heat rate can be related directly to compositional changes in the headspace, including those resulting from the insect's metabolism. Important factors in the application of mass spectrometry to the more difficult task of measuring *rates* of oxygen consumption and carbon dioxide production associated with a metabolic heat rate are discussed.

Keywords: Calorimetry; Insect metabolism; Controlled atmospheres; Mass spectrometry

1. Introduction

An environmentally acceptable alternative to methyl bromide fumigation of fresh produce crossing international borders is required urgently. Much effort is being expended in determining whether low oxygen and/or high carbon dioxide atmospheres, commonly called controlled atmospheres, together with changes in temperature, are insecticidal. Such treatments, both in chemical composition of the controlled atmosphere and time of exposure, will obviously need to be compatible with maintaining the quality of the produce.

Observational count of insect mortality is the usual method of determining the effectiveness of a treatment [1]. However, this does not provide information on the state of the metabolism during and after treatment. This can be obtained by calorimetry and should assist in defining the optimum treatment. It was also hoped that calorimetry might assist in deciding whether insects that are moribund after treatment will revive.

The literature on some early calorimetric studies of insect specimens weighing a few tenths of a gram and producing metabolic heat rates in the milliwatt range has been summarised [2]. These results established the

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usefulness of calorimetry in determining variations in metabolic heat rates with time. An adult cockroach (*Periplaneta americana*) produced paroxysms, of the order of an hour or more apart, where the heat rate was up to four times the baseline value and which were associated with the opening of the spiracles of the tracheal system. Power–time curves of differing nature were recorded for the larval, pupal and adult stages of a moth (*Galleria mellanella*). The hatching of the imago was also recorded. The power–time curve for the larva (caterpillar) was irregular, that for the pupa far more uniform while that for the adult had periods of very active thermogenesis interposed between phases of rest.

More recently, Wegener and colleagues [3] have used microcalorimetry to study the effects on insects of graded hypoxia and anoxia. They report that locusts (*Locusta migratoria*) can survive an atmosphere of pure nitrogen for not more than 4 h (at room temperature), whereas hawk moths (*Manduca sexta*) can recover from more than 24 h of anoxia. A review by Wegener [4] and the references cited therein, provide useful background for the non-biologist and an entry point to the relevant biochemical literature. Their interests extend to understanding the biochemical reasons why insects are much more tolerant of longer periods of anoxia than higher vertebrates (5–8 min limit for humans).

Two recent papers [5,6] document the metabolic response of omnivorous leafroller (*Platynota stultana*) pupae to controlled atmospheres using a similar microcalorimeter to that of the present study. Lamprecht and Schmolz [7] have recently produced a compilation of the literature on insect calorimetry and give extended descriptions of the findings, including those of some of the early experiments cited above. Acar et al. [8] used isothermal calorimetry to measure metabolic heat rates and CO_2 production rates (by heat of absorption into aqueous NaOH) of convergent lady beetles as a function of temperature. Production rates of CO_2 were also measured, for comparison, in separate experiments using an infrared analyser.

This paper presents methods developed to determine the response of insects to rapid and large changes in temperature and/or controlled atmosphere. While results obtained using these methods should be relevant to disinfestation goals, we do neglect factors such as acclimation, so they may be less relevant to understanding the behaviour of insects in their natural environment. Over the last decade, microcalorimetric methods have been developed for the rapid acquisition of metabolic heat rates of plant material under a variety of conditions [9]. Our general approach has been to adapt these methods for insect studies.

The simplest method of determining the effects of controlled atmospheres on insect metabolic heat rates, requiring no modifications to the apparatus, is to treat the insects external to the calorimeter and merely measure the heat rates in an air atmosphere. The metabolic heat rate in a controlled atmosphere can be obtained by replacing the lid on the ampoule containing the insect, while it is in a transparent flexible plastic sleeve through which the controlled atmosphere is flowing before placing the ampoule in the calorimeter. However, a much more versatile procedure, described in detail below, is where the atmosphere in the ampoule can be changed while the sample is in the calorimeter. The insect can be taken to the desired temperature, the metabolic heat rate measured in air, then in the controlled atmosphere, and finally in air again to ascertain the magnitude of any irreversible effects.

Changes in metabolic pathway, or substrate, are likely to be reflected by changes in the rate of oxygen consumption, R_{O_2}, or rate of carbon dioxide production, R_{CO_2}, or their ratio to the metabolic heat rate, Φ. Therefore, we have endeavoured to develop mass spectrometry (ms) methods so that R_{O_2} and R_{CO_2}, together with the calorimetrically determined Φ, can be determined simultaneously for a given insect specimen or a collection of smaller insects. A negative sign associated with a Φ value indicates the reaction is exothermic.

Results illustrating the application of these methods are described and aspects for which further development would be desirable are discussed. A preliminary account of part of this work has been given elsewhere [10].

2. Experimental

A Hart DSC model 4207 with 1 cm^3 ampoule size was adapted for these measurements. The current designation for this instrument is CSC 4100 (Calorimetry Sciences Corporation, Spanish Fork, UT). New ampoule lids were machined from Hastelloy C with the

same dimensions as the originals, but with a small hole drilled axially through the upstand. The threads were undercut so that the Viton gaskets fit properly to produce a gas-tight seal which is important if truly anoxic conditions are to be achieved.

To avoid an unduly noisy signal, mobile insects are confined to the bottom of the ampoule by a sliding-fit disk of fine stainless steel mesh with neatly down-turned edges of appropriate height to minimise the chance of injury to the insects.

An alternative, which is especially useful for very agile tiny insects such as thrips, is a circular 6 mm high stainless steel container that is a snug fit in the DSC ampoule. The bottom of the container is internally threaded to accept a thin lid and the top consists of fine stainless steel mesh soldered to the wall. When collecting insects, the lid is replaced by a plastic union that also closely fits a 1 ml Ependorff pipette tip (see Fig. 1(b)). Application of slight suction through the mesh, e.g. from a portable vacuum cleaner of the type used to clean PCs, permits collection of insects via the tip. If desired, a food source such as filter paper impregnated with sugar solution can be included during transport of the insects. If the assembly is slightly chilled the insects can be tapped gently into the container and the union replaced by the lid.

Gas exchange with the ampoule is via silica capillaries, ca. 0.25 mm ID, of the type used for chromatography columns (SGE, Ringwood, Vic., Australia), passed through the small hole in the upstand of the lid and sealed with epoxy. It is advantageous to leave a small collar of metal on the upstand, so that a pair of tweezers with small flats of metal brazed to the tips can be used to remove the ampoule from the snug-fitting holder in the DSC with minimal chance of damage to the capillaries. New thermal covers have an offset upstand and a narrow slit from the centre to the circumference to accept the capillaries.

If measurements are to be made below the ambient temperature of the laboratory, then it is essential that the capillaries exit the measuring compartment through a gas-tight seal. This is to avoid condensation of moisture in the DSC resulting from ingress of laboratory air. A lid (see Fig. 1(a)) was machined from a 35 mm length of 130 mm diameter Acetal rod, hollowed to leave a 15 mm thick roof and sealed to the measuring compartment using an O-ring. The latter was backed on the inside of the lid by a metal band to prevent distortion of the Acetal and consequent failure of the seal. A 45 mm long Acetal plug was drilled axially to accommodate the capillaries and O-ring sealed in a 20 mm diameter hole in the lid directly above the ampoule. The top of the plug was shaped so the neck of a "helium quality" balloon could be stretched over it. The capillaries were sealed with epoxy to an axially drilled $20 \times 3\,mm^2$ brass screw that penetrates the top of the balloon and is sealed to it by tightening down on a washer and nut on the outside. This arrangement allows access to seat the ampoules and covers. The slight back pressure of the purge gas fills the balloon in its unstretched state if it is clamped vertically by the brass screw. Monitoring of a rotameter connected to the purge gas outlet of the measuring chamber provides assurance of the integrity of the seals.

Gases are introduced to the ampoule from a manifold with HiP 15 series valves (High Pressure Equipment, Erie, PA) arranged to permit flushing of all lines, including dead spaces, to avoid cross contamination. Good quality valves are essential if truly anoxic conditions are to be achieved. To ensure there is no ingress of laboratory air, all lines are 1.6 mm OD stainless steel up to where they are sealed to the capillaries with epoxy. Gas flows from outlets are monitored using back-to-back pairs of miniature gas bubblers, about 25 mm high, with a few millimetres depth of water in the second bubbler.

If the composition of the gas phase in the ampoule is to be determined, an additional 2 m length of 50 μm ID silica capillary (part no. 062463, SGE) is included which acts as the inlet to a Dycor Quadrupole Gas Analyser (Ametek, Pittsburgh, PA). To accentuate the changes in the gas phase due to insect metabolism, much of the volume above the mesh can be filled with a hollow glass cylinder containing a central hole for the capillaries. The usual configuration of the ends of the capillaries is for the gas entry to be just inside the lid, and the gas exit to be a few millimetres above, and the ms sample capillary close to, the gauze. Certified air and gas mixtures (BOC Gases, Lower Hutt, NZ) were used to calibrate the ms and to provide the controlled atmospheres.

Disease-free, female green peach aphids (*Myzus persicae* L.) were grown on rape plants in a greenhouse. Codling moths (*Cydia pomonella* L.) were cultured in test tubes in a laboratory at 22 °C.

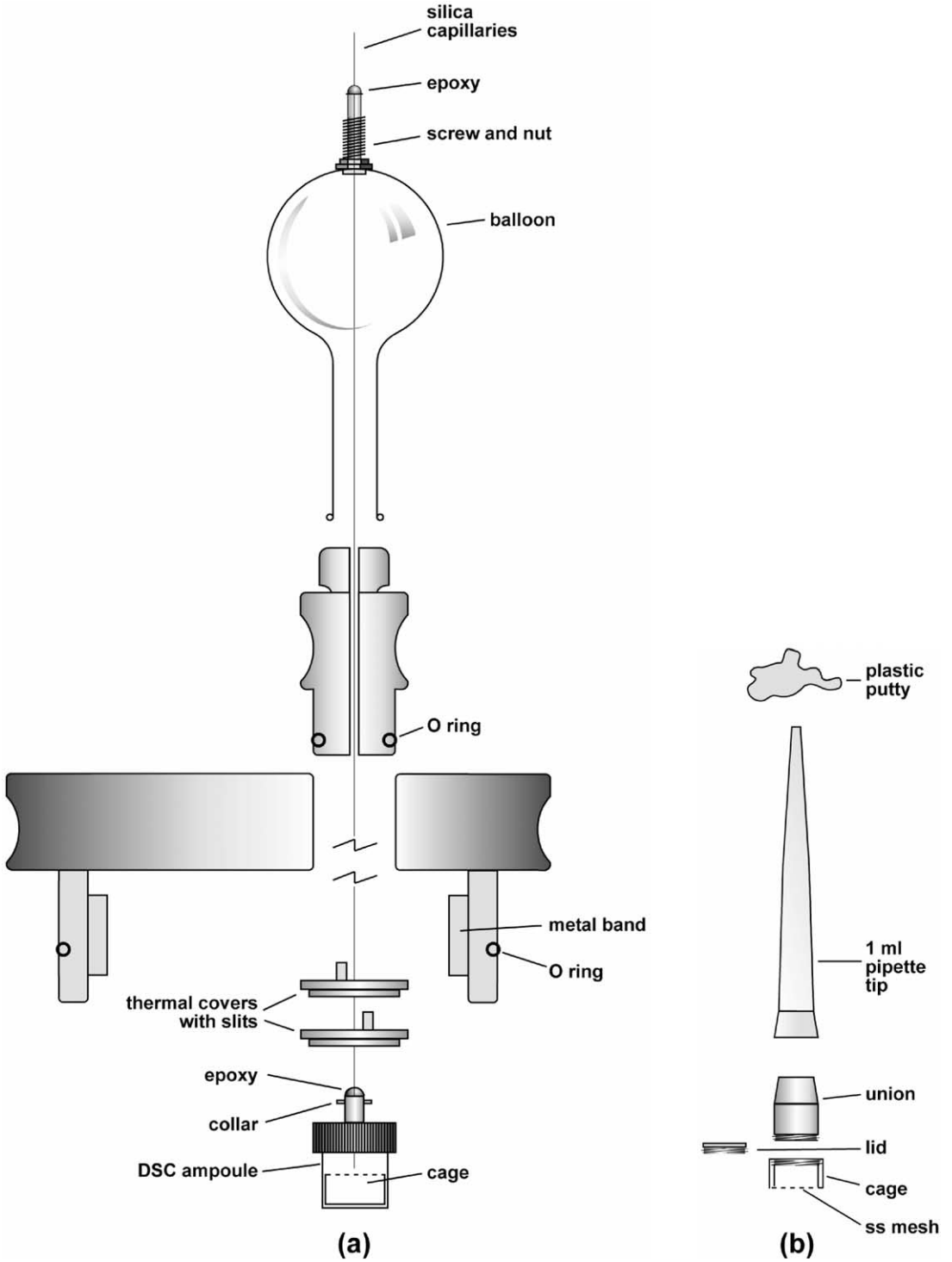

Fig. 1. (a) Modified lid of measuring chamber of calorimeter to permit exchange of ampoule atmosphere in situ. (b) Apparatus for the collection of very small insects and their transfer to the calorimeter ampoule.

3. Results

3.1. Temperature cycling

Two attributes of the present calorimeter, viz. ease of changing the temperature and the rapid thermal equilibration at the new temperature, facilitate the determination of the effects of temperature stress on the metabolic heat rate of insects. No modification of the ampoules is required for these experiments. The calorimeter was programmed so aphids were repeatedly cycled through the sequence at 20 °C, test temperature, 20 °C. They were held at each temperature for 30 min and temperature changes were made at the rate of 1 °C/min. As these experiments extended over several hours, an initial atmosphere of pure oxygen was used to minimise oxygen depletion effects. A guideline for plant material [9] is that maintaining a heat rate of 100 μW for 5 h consumes the oxygen present in about 0.5 cm^3 of air. In separate experiments, not reported here, the metabolic heat rate of aphids in 100% O_2 relative to the value in air was enhanced by about 15% at 40 °C, and 6% at 20 °C, but at 0 °C there was little or no effect. Exposure to 100% O_2, at least for short periods (ca. 1 h), appeared to have no adverse effect on the aphids at 0 and 20 °C, but they did not survive the 40 °C treatment. The temperature coefficient of the metabolic heat rate of aphids was found to be somewhat variable for different batches of insects. Indicative values of the rates, relative to those at 20 °C, obtained by averaging, are 0.18, 0.35, 1.8, 2.7 and ca. 0.1 for 0, 10, 30, 40 and 50 °C, respectively. Starvation, enhanced by the higher metabolic rate, may have contributed to the demise of the aphids at 40 °C.

Metabolic heat rates were measured at the test temperature, as well as at 20 °C, but we have chosen to illustrate the effects of thermal stress by plotting in Fig. 2 the value at 20 °C for each cycle as a percentage of the initial value at 20 °C. It is apparent from the results that the aphids were only slightly affected by cycling to 30 °C and even on cycling to as low as 0 °C the changes were relatively minor. However, cycling to 40 °C, and more particularly to 50 °C, had a much greater effect with the metabolic heat rate declining rapidly as a result of the death of the insects.

Integration of the metabolic heat rates over the time of the experiments indicated the maximum oxygen consumed was less than 10% of that present at the start, so oxygen depletion is not a significant contributor to the decrease in metabolic heat rate (see also later results). Results are not available for the effects of carbon dioxide on metabolism in atmospheres containing mostly air or higher levels of oxygen. But on the basis of a comparison of results for experiments using 5% $O_2 + N_2$ and 5% O_2 + 20% $CO_2 + N_2$ at 20 °C, the effects of the accumulating carbon dioxide resulting from metabolism in the cycling experiments should be minor. Raising the temperature 20 °C above

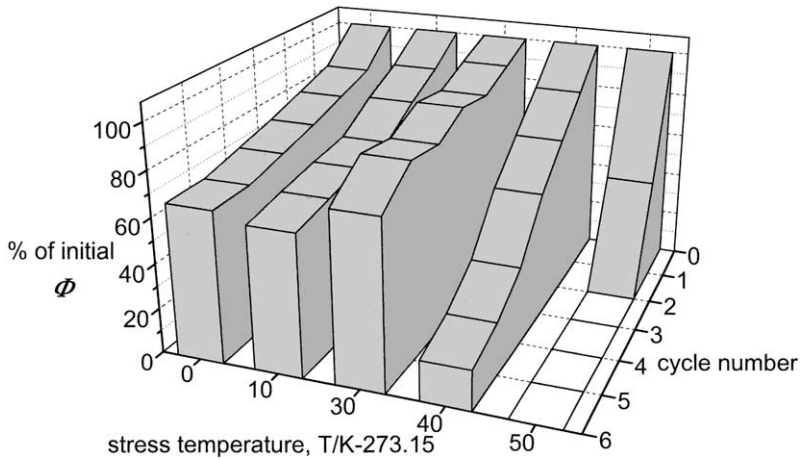

Fig. 2. Effect of temperature cycling on the metabolic heat rate at 20 °C of green peach aphids (*M. persicae*), expressed as a percentage of the original value, as a function of the stress temperature and number of cycles to the stress temperature.

ambient is obviously an effective insecticidal treatment in contrast to lowering the temperature by the same amount. These cycling experiments are of a scoping nature and if such a treatment was being considered for a practical application, the stress response surface would need to be better defined by using more closely spaced stress temperatures. The effects of controlled atmospheres could be investigated in a similar way.

3.2. Metabolism under anoxic atmospheres

The second example, which required the use of a modified ampoule, shows the effect of an anoxic atmosphere on a single codling moth pupa (fresh mass 0.0400 g) at 20 °C. After establishing the metabolic heat rate in air, see Fig. 3, the ampoule was flushed with 60% $CO_2 + N_2$ to remove all the oxygen present. In the time the calorimeter took to re-establish thermal equilibrium, the metabolic heat rate had fallen to $<2\,\mu W$, that is, zero within the repeatability of the instrument at this temperature. After about 2 h under anoxic conditions, air was readmitted, which resulted in the recovery of much of the initial heat rate. The insect was still alive at the end of the experiment and had lost 0.8% in mass. This apparent hiatus in the metabolism, on the introduction of an anoxic atmosphere, as suggested by near zero metabolic heat rates, does not result in damage that is apparent in the short term. Certainly, such behaviour on return to an air atmosphere would result in an insect being classified as "alive" in a border inspection.

In an extension of this type of experiment, aphids were subjected to varying periods of anoxia at 20 °C, primarily in 100% N_2 and 60% $CO_2 + N_2$. A fresh sample of insects was used for each experiment. In all cases, the metabolic heat rate was near zero under the anoxic conditions, as was found with the codling moth pupa. The recovery in metabolic heat rate in air at the end of the experiment is shown in Fig. 4 as a percentage of the initial value in air. After removal from the calorimeter, the aphids were placed in small glass ampoules, with gauze inserts in the lids, for observation as to their life status. With increasing exposure time to anoxic conditions, the aphids took longer to revive. In general, the less than 100% recovery after short exposures was not due to the death of a *proportion* of the aphids. Despite their heat rates being depressed,

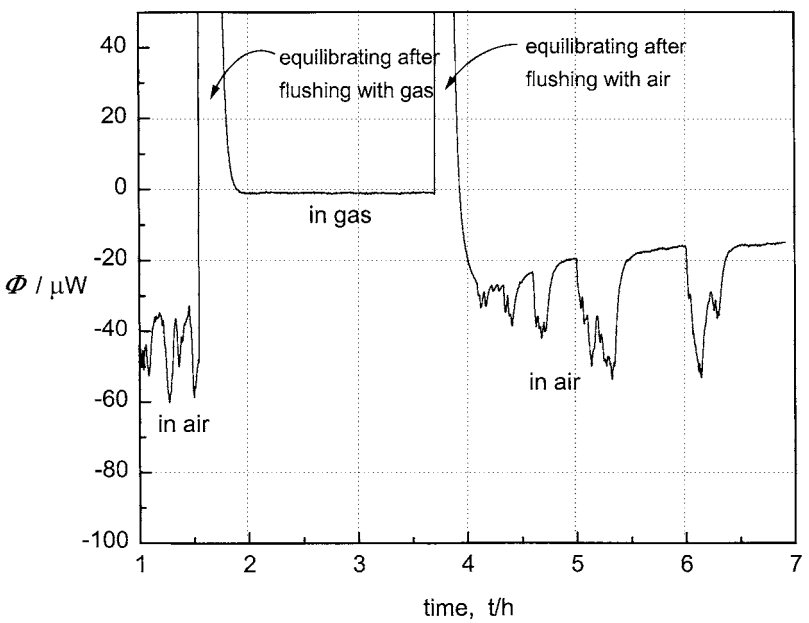

Fig. 3. Metabolic heat rate at 20 °C of a codling moth (*C. pomonella*) pupa, fresh mass 0.0400 g, in air and in a controlled atmosphere of 60% $CO_2 + N_2$.

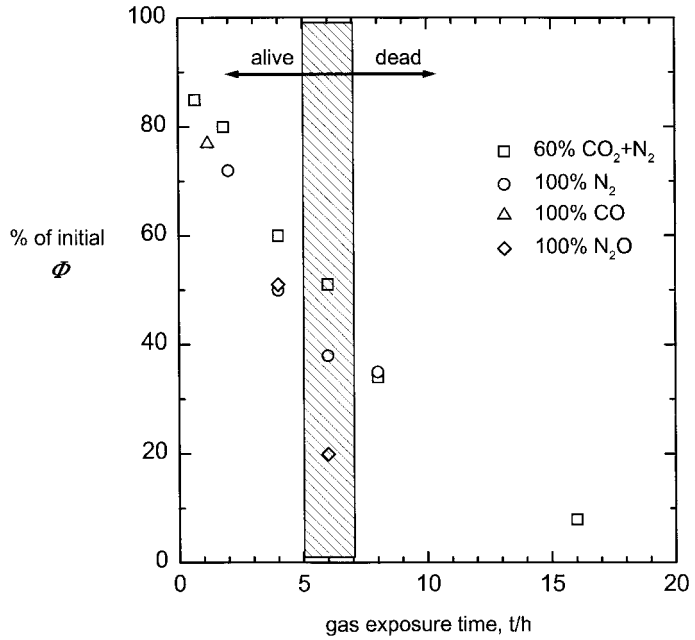

Fig. 4. Response of green peach aphids (*M. persicae*) to anoxic atmospheres at 20 °C, plotted as percent recovery of the initial metabolic heat rate in air, versus the time in the anoxic atmosphere.

all aphids were alive after an exposure time of 4 h. After a 6 h exposure most were dead with only a few smaller (younger?) aphids showing feeble signs of life for a short period a few hours after the test. For longer exposures, although metabolic heat rate recovery was up to 35%, none revived. There was no signal on the trace to indicate death had occurred. These results show the hypercarbic–anoxic atmosphere was no more insecticidal than the anoxic atmosphere. After short exposures to the hypercarbic atmosphere, the aphids, on removal from the calorimeter, were very active physically, but presumably stressed. For these organisms lacking heme pigment, it would appear from a single short term exposure that 100% CO is no more harmful than 100% N_2. Similarly, 100% N_2O did not show a specific effect.

A rather different response, not shown in Fig. 4, was found for aphids when an anoxic atmosphere of 100% dimethyl ether (b.p. −23.65 °C) was introduced. Within 30 min, the metabolic heat rate had decreased to about 24% of the initial value in air and over the next 200 min it fell to 4%. After reintroduction of air at this time the metabolic heat rate recovered to about 9% of the initial value in air. The aphids did not revive and showed some loss of structural integrity. Thus, dimethyl ether had a specific chemical effect in contrast to the other treatments that appear to provide just an inert anoxic atmosphere.

3.3. Calorimetry and mass spectrometry

The additional information that can be obtained by a combination of the two techniques is illustrated by results of experiments designed to determine the effects on aphids of a hypoxic atmosphere, 5% $O_2 + N_2$, and a stressful temperature of 40 °C. After loading the aphids in the calorimeter at room temperature, a slow flow of air was passed through the ampoule while the temperature was raised to 40 °C. When the calorimeter had equilibrated, the airflow was stopped and readings on the ms commenced. This was taken as zero time for the experiment. After 25 min the ampoule was flushed with 5% $O_2 + N_2$ for 8 min. After a further 18 min, the ampoule was flushed with air for 8 min and readings were taken for a further 15 min before ending the experiments.

Metabolic heat rates and oxygen levels in the ampoules were evaluated at 15, 40 and 65 min that

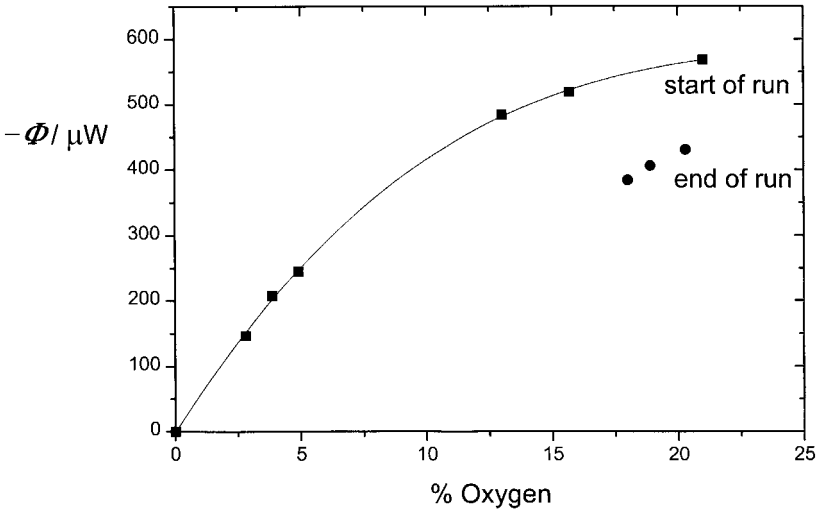

Fig. 5. Metabolic heat rate at 40 °C of green peach aphids (*M. persicae*), fresh mass 0.02203 g, as a function of percentage of O_2 in atmosphere for the sequence with initial compositions of: air; 0.5% $O_2 + N_2$; air. The points on the curve are for initial atmospheres of air and 5% $O_2 + N_2$. The point at 0% O_2 is from another experiment. The points labelled "end of run" are for an initial atmosphere of air at the end of the run.

correspond to near the mid-times for the air, controlled atmosphere, air treatments, together with the values at the beginning and end of each treatment. A short extrapolation was required to evaluate the metabolic heat rates at the start of each period after flushing. These results are shown in Fig. 5; including a value at 0% O_2 that was obtained from other experiments. As noted above, the enhancement in the metabolic heat rate of aphids at 40 °C in 100% O_2 over that in air is about 15%. This indicates that the extension of the curve in Fig. 5 to 100% O_2 would be rather flat in relation to the changes shown for oxygen concentrations less than in normal air.

Criddle and Hansen [9] have noted examples where plant tissues are sensitive to gas phase inhibitors, in particular CO_2, that accumulates in the ampoule as a result of metabolism. However, in this aphid experiment, the initial metabolic heat rate for an atmosphere of 5% $O_2 + N_2$ containing negligible CO_2, falls on the trend line, suggesting the major effect is due to the O_2 level.

The rates of oxygen consumption and carbon dioxide production were evaluated near the mid-times of the treatments. This was done by determining the gradient of a straight line fitted to the data for 5 min on either side of the mid-times. Values of the partial pressures of the gases in the ms are updated every 30 s. The average standard deviation of the gradients for oxygen was 9.0% and that for carbon dioxide was 3.6%. A quadratic gave a marginally better fit but identical gradients at the mid-time. These calculations also require a determination of the volume of the gas phase in the ampoule.

In addition, a significant correction has to be made for the effect of the ms continuously withdrawing gas from the ampoule and its replacement with cylinder air or controlled atmosphere. Because of the importance of this correction, check analyses (BOC Gases) were done with results: air, 380 ± 8 ppm CO_2, 21.0 ± 0.2% O_2; controlled atmosphere, <5 ppm CO_2, 5.0 ± 0.1% O_2. The gas flowrate down the capillary to the ms was calculated using the Poiseuille equation [11]. Average corrections were 27 and 32% of the total rates for O_2 and CO_2, respectively.

The results from the combined calorimetry/ms are given in Table 1. In their development of a plant growth model, Criddle and Hansen [9] noted that plant respiration rate has commonly been measured as R_{O_2} or R_{CO_2}, and less often as metabolic heat rate, Φ. If all three measurements have been made, as in the case of the aphid experiment, the ratios of Φ/R_{CO_2} and Φ/R_{O_2} can be evaluated and these

Table 1
Effect of oxygen concentration on the metabolism of *M. persicae* at 40 °C (fresh weight of aphids 0.02203 g). The times of 15, 40 and 65 min, with their corresponding oxygen values, refer to the approximately mid-times in atmospheres that were initially air, 5% $O_2 + N_2$ and air, respectively

	Units	Minutes from start of the experiment		
		15	40	65
O_2	%	15.7 ± 0.8	3.85 ± 0.2	18.9 ± 0.9
Φ	μW	−519 ± 5	−207 ± 5	−406 ± 5
R_{O_2}	pmol s^{-1}	1210 ± 150	445 ± 34	856 ± 97
R_{CO_2}	pmol s^{-1}	1180 ± 110	586 ± 48	794 ± 54
Φ/R_{O_2}	kJ mol^{-1}	−429 ± 54	−465 ± 37	−474 ± 54
Φ/R_{CO_2}	kJ mol^{-1}	−440 ± 43	−353 ± 30	−511 ± 35
R_{CO_2}/R_{O_2}		0.98 ± 0.15	1.32 ± 0.15	0.93 ± 0.12

are also given in Table 1. They noted that the ratio Φ/R_{O_2} has a value of −455 ± 15 kJ/mol of O_2 for direct combustion of nearly all organic compounds and thus this is the expected value for fully aerobic respiration in the absence of a significant anabolic heat rate. Within the errors given in Table 1 (see also Section 4), all three values of Φ/R_{O_2} agree with this value. Further, the value of R_{CO_2}/R_{O_2} for the initial air atmosphere is approximately 1, suggesting that most of the metabolic heat rate arises from the metabolism of a carbohydrate-type of substrate, probably glycogen. In the controlled atmosphere, the ratio of R_{CO_2}/R_{O_2} is >1. So under hypoxic conditions there is, perhaps, an increased contribution from the degradation of more highly oxidised carbon compounds to form CO_2 and H_2O, or from anaerobic respiration.

4. Discussion

From the outset, it was realised that two simplifications in the adopted procedure would need to be considered when interpreting results. These are: the use of nominally dry gases, and the batch-wise exchange of controlled atmospheres. For determining subtle changes under conditions approaching those of the insects' natural environment, the calorimetric experiments would need to include a continuous supply of thermostatted and humidified air to avoid oxygen depletion, carbon dioxide accumulation, and dehydration effects. The present application is concerned with the consequences of gross changes in conditions, indeed, the life-death boundary is a primary focus.

Nominally dry gases were used because of the perceived risk of condensation in the ms capillary, especially for experiments at above ambient temperature. Equilibration of water between the insects and a static atmosphere in the ampoule has a very minor effect for sample sizes producing ca. 100 μW or more at ambient temperatures. More important is the loss of water resulting from flushing of the ampoule to change the atmosphere, particularly at temperatures above ambient. In the worst cases, losses in mass up to about 5% have been observed for experiments involving three flushing periods. The flushing of the ampoule was possibly excessive due to an overly cautious approach to ensuring the complete removal of the previous atmosphere. A closer examination of this procedure might indicate substantially smaller volumes of gases are adequate. Results for the ms trace for water, assuming the response factor is the same as for oxygen, suggest that the relative humidity is about 45% in the ampoule and it appears to be maintained during flushing. When ms is not being used, humidification of the gases should be considered.

Metabolic heat rates for short exposure times in a particular atmosphere can be obtained by a short extrapolation and, if necessary, by further flushing of the ampoule. Also, by not including the filler in the ampoule and choosing the sample to give a metabolic heat rate of about 100 μW, the rate of change of oxygen and carbon dioxide concentrations would be about one tenth of that for the experiment on aphids at 40 °C (data in Table 1 and Fig. 5). However, the

determination of R_{O_2} and R_{CO_2} by the current ms method would be less precise.

Other than minor perturbations to environmental conditions are expected to lead to short or long term changes in metabolism for most insects. In most experiments, we have used the strategy of taking three sets of measurements: initially under near-normal temperature and/or atmosphere, during the treatment, and then on return to the initial conditions. This allows the effects of the treatment per se to be determined as well as any more permanent changes.

Thus, in Fig. 5 the effect of the controlled atmospheres on the metabolic heat rate is readily apparent as is the indicator of damage in the less than 100% recovery on returning to air. Despite the recovery being 75% initially, albeit rapidly declining, and the aphids being in good physical condition on removal from the calorimeter, they did not revive. It is therefore uncertain as to what life status the ratios listed in Table 1 represent. Aphids did revive after a similar experiment at 20 °C (see below). Comparison of this 75% recovery at 40 °C with the value of ca. 50% that was needed for revival of aphids in the anoxic experiments at 20 °C suggests the critical percentage for recovery is temperature dependent.

In a similar 20 °C experiment with the 5% $O_2 + N_2$ controlled atmosphere (not listed in Section 3), the sample of aphids had an initial metabolic heat rate in air of −400 μW. On the introduction of the controlled atmosphere this decreased to −100 μW and stayed at this value, within <5 μW, for the total exposure time of 66 min. The recovery value in air at the end of the experiment was 96% of the initial value in air suggesting, in contrast to the 40 °C experiment, very little damage had been done to the aphids. At the mid-time of the treatment, the plot of oxygen partial pressure in the ms had near zero slope. Therefore, the major contributor to the calculation of the rate of oxygen consumption is the correction due to the ms removing gas from the ampoule. In view of the uncertainty in this correction (see below) and a problem with the CO_2 trace, these rates are not provided. This near steady-state in the oxygen level in the ampoule resulted in a near-constant metabolic heat rate. Further work is needed to determine if the ratios at 20 °C follow the pattern given in Table 1 for the 40 °C experiment.

Fontana et al. [12] described a method, based on the use of pressure transducers and the same type of calorimeter as used in the present experiments, to determine oxygen and carbon dioxide fluxes, and metabolic heat rates, for redwood meristem tissue in air. Measurements were made in the presence and absence of a trapping solution for carbon dioxide consisting of 40 μl of 0.4 M NaOH. Rates of carbon dioxide production evaluated from the heat of reaction were in good agreement with those deduced from the pressure measurements. Their *listed* errors for the gas fluxes are smaller than those of our measurements (one of the present authors, LDH, advises that for these types of experiment, typical errors in the heat and gas rates are about 3–5%). There are advantages to the ms method: all measurements can be made simultaneously, and thus, in a shorter time, and it should be suitable for use with controlled atmospheres containing up to moderate amounts of carbon dioxide. The simultaneous nature avoids the ambiguities of other methods in relating a particular metabolic heat rate to the gas fluxes. But it would be desirable to refine the ms method.

Errors arise in the ms method principally from two sources: the noise in the variation of the partial pressures in the ms with time, and the uncertainty in the correction due to gas removal from the calorimeter ampoule by the ms. Intermittent connection of the ms in short term experiments would not avoid the problem of gas removal because of the time needed to achieve steady-state conditions in the sampling capillary. The major uncertainty in the calculation of the flowrate in the capillary to the ms arises from the uncertainty of 3.3% in the diameter which is raised to the fourth power in the Poiseuille equation. The manufacturing tolerance on the diameter of 25 μm capillary is twice that of the 50 μm capillary used here, but the calculated flowrate for the same length would be 1/16. If there is not a significant decrease in the response, or an increase in the noise level of the ms, there would be an advantage in using a capillary smaller than the standard 50 μm so far as the total error in the metabolic gas fluxes is concerned. An independent measure of the flowrate down a nominally identical capillary to that used in the metabolic experiments was obtained calorimetrically. A few drops of water were placed in the sample cage in the DSC ampoule at 25 °C which was supplied with dry air and the capillary connected to a vacuum system. This “calorimetric” flowrate was 93±8% of that calculated from the Poiseuille equation. A small endothermic effect (ca. 5 μW at 25 °C) would

also result from evaporative cooling when insects are present and the capillary sampling is in operation, but this correction has not been applied. A capillary of appropriate diameter and length connected to a vacuum system is a convenient means of drawing a small flow of gas through an ampoule without the need, in many cases, to include explicit thermostatting of the gases.

Further biological interpretation of these results will be given elsewhere when larger arrays of data will be presented.

5. Conclusions

The CSC DSC can be readily adapted so the metabolic heat rate of small insects can be monitored as a function of temperature and composition of controlled atmospheres. Connection of a portable ms to the DSC ampoule, via a capillary inlet, permits the simultaneous determination of metabolic heat rates and headspace compositions. Important factors in the more difficult task of relating insect metabolic heat rates to the rates of oxygen and carbon dioxide exchange between insects and the headspace have been identified.

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