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Discontinuous carbon dioxide release in the German cockroach, *Blattella germanica* (Dictyoptera: Blattellidae), and its effect on respiratory transpiration

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Abstract

The discontinuous gas exchange cycle (DGC) was described in the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae) for the first time. Also, the effect of the DGC on water loss was investigated. The CO_2 emission pattern in both insecticide resistant and susceptible *B. germanica* varied with temperature. At 10, 15, and 20 °C the pattern was discontinuous. Cycle frequency increased at 25 and 30 °C, and at 35 °C the pattern became cyclic. In most DGCs, there was no clear distinction between the closed and flutter phases in both strains thus data for these phases were combined and analyzed as the interburst phase. The probability that *B. germanica* would breath discontinuously varied with temperature. Most cockroaches (62.8%) displayed DGCs at 10 °C, therefore measurement of metabolic rate and water loss was carried out at this temperature. Using repeated measures of analysis of variance, the interburst and burst $\dot{V}_{CO_2}$ ($ml\,h^{-1}$) were not significantly different between the two strains. The variability in CO_2 emission during the interburst and burst phases over time was not significantly different from cycle to cycle or between strains. Overall metabolic rate during the entire recording was not significantly different between both strains. There was a significant difference in the duration of the interburst and burst phases between the strains. The susceptible strain had significantly longer interburst and burst phase durations during a complete DGC than the resistant strain. The interburst and burst phase durations were 5.01 ± 0.19 and 6.21 ± 0.13 min, respectively, for the resistant strain, whereas the durations were 7.16 ± 0.37 and 6.73 ± 0.17 min, respectively, for the susceptible strain. This resulted in a DGC of significantly longer duration (13.89 ± 0.44 min) in the susceptible strain compared with the resistant strain (11.23 ± 0.26 min). The duration of the interburst phase was significantly different from the open phase duration in the resistant strain such that during a single DGC lasting ~11.23 min, 43.5% consisted of the interburst phase while the burst phase made up 56.5% of the cycle. The cuticular permeability at 10 °C and 0% RH was $2.26\,\mu g\,cm^{-2}\,h^{-1}\,mmHg^{-1}$ for the resistant strain and $3.42\,\mu g\,cm^{-2}\,h^{-1}\,mmHg^{-1}$ for the susceptible strain. In both strains, cuticular transpiration accounted for ~95% of total water loss. The significantly longer duration of the interburst phase of the susceptible strain was not important in reducing water loss.

Keywords: Discontinuous gas exchange; Transpiration; Resistance; *Blattella germanica*

1. Introduction

It is becoming increasingly clear that gas exchange in many quiescent adult terrestrial insects is discontinuous (see Kestler, 1985; Slama, 1988; Lighton, 1994). A typical discontinuous gas exchange cycle (DGC) begins with a closed-spiracle phase, when little external gas exchange takes place. This is followed by a fluttering-spiracle phase where the spiracles open and close rapidly enabling gases to pass through by convection and diffusion, and finally an open-spiracle phase during which accumulated CO_2 escapes from the tracheal

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system to the surrounding environment (Lighton, 1996). In some insects, DGC is reported to conserve body water (Kestler, 1985; Hadley, 1994; Lighton, 1996), as a result of reduced respiratory water loss during the closed phase, and low water loss during the flutter phase (Lighton, 1996).

In insects that exhibit DGC, it is possible to determine the significance of the discontinuous gas exchange pattern in water conservation since water lost during the open phase (burst) (cuticular and respiratory loss) can be separated from loss during the closed and flutter phases (interburst; cuticular loss) (Hadley, 1994). Even though cuticular transpiration is greatly reduced by lipids associated with the epicuticle, the cuticle is still considered the primary water-efflux path because of the large surface area-to-volume ratio of insects (Hadley, 1986, 1989). Regulation of cutaneous water loss is critical for small insects such as the German cockroach, *Blattella germanica* (L.) because of their small size (<15 mm long) and large surface area-to-volume ratio.

B. germanica is a world wide household pest, which may harbor and transmit human disease-causing pathogens (Ramirez, 1989). Their body parts and feces are also potent allergens to sensitive people (Roberts, 1996). Pyrethroid insecticides are widely used for *B. germanica* control because of their effectiveness and low mammalian toxicity. However, control failures in some field populations have been reported as a result of the development of resistance (Cochran, 1989; Valles et al., 2000). Resistance levels generally decline in the absence of insecticide selection (Tabashnik et al., 1994; Rahardja and Whalon, 1995), and a decrease in resistance may be associated with increased biotic fitness (Tabashnik et al., 1994). Fitness can be observed as changes in survival rate, egg hatch, weight and metabolic rate (Groeters et al., 1994; Idris and Grafius, 1996; Hollingsworth et al., 1997; Dingha et al., 2004).

In cockroaches, the DGC pattern has been reported and described at 20 °C in the American cockroach, *Periplaneta americana* (L.) (Kestler, 1985, 1991; Machin et al., 1991), and *Perisphaeria* sp. (Marais and Chown, 2003). A cyclic O_2 consumption pattern was recorded from the tropical cockroach, *Blaberus giganteus* (L.) at 26.6 °C (Bartholomew and Lighton, 1985). The aim of this study was to describe the respiratory gas exchange patterns at different temperatures, investigate the effects of DGC on water loss, and determine if there are differences in the DGC characteristics between insecticide resistant and susceptible strains of *B. germanica*. To accomplish this, we conducted experiments to test two major hypotheses. First, we hypothesized that *B. germanica* would show a pronounced DGC only at lower temperatures and continuous cycling of CO_2 release at higher temperatures. Since increased temperature generally causes the metabolic rate to rise, the frequency of spiracular opening would increase at higher temperatures (Chappell and Rogowitz, 2000). Therefore, DGCs would be more pronounced in insects at lower temperatures and cyclic at higher temperatures. For example, in some adult insects, such as the eucalyptus-boring beetle, *Phorocantha spp*. (Chappell and Rogowitz, 2000), the California grasshopper, *Melanoplus sanguinipes* (Rourke, 2000), *P. americana* (Kestler, 1985, 1991; Machin et al., 1991) and *Perisphaeria* sp. (Marais and Chown, 2003), DGC was not exhibited at higher temperatures, instead it was observed only at lower temperatures. Second, we hypothesized that genetically resistant *B. germanica* not exposed to insecticide for several generations would have similar metabolic rates and DGC characteristics as a susceptible strain. In addition, we hypothesized there would be no significant difference in water loss between the two strains.

2. Materials and methods

2.1. Cockroach strains

Two *B. germanica* strains were used in this study. ACY (American Cyanamid Co., Clifton, NY), is an insecticide susceptible strain that has been reared in the laboratory without exposure to insecticide for >40 years. The second is Apyr-R (Alabama, pyrethroid resistant); a resistant strain collected from an infested kitchen in Opelika, Lee County, Alabama, USA in 1999 after control failures with permethrin and deltamethrin. This strain was subsequently selected with permethrin and deltamethrin for several generations prior to this study (Wei et al., 2001; Pridgeon et al., 2002). Levels of resistance to permethrin and deltamethrin in Apyr-R were 97- and 480-fold, respectively, compared with the susceptible strain (Wei et al., 2001; Pridgeon et al., 2002). All cockroaches were reared at 25 ± 2 °C and 50 ± 10% RH, with photoperiod of 12L: 12D. Dry dog chow and water were supplied ad libitum.

2.2. Respirometry and metabolic rate measurement

We recorded patterns of CO_2 emission of individual adult male *B. germanica* at 5, 10, 15, 20, 25, 30, and 35 °C using flow through respirometry. Males were selected to avoid complications arising from the metabolic demands of oogenesis in females. To avoid stress (Kestler, 1991; Machin et al., 1991), cockroaches were allowed to crawl into a 3 cm × 1.0 cm transparent Tygon® tubing respirometer chamber which was then sealed at both ends with a rubber stopper and connected to the CO_2 and H_2O analyzer. Cockroaches were acclimated in the respirometry chamber for ~1 h before recordings were initiated. This allowed for acclimation to the initial temperature and predesiccation to remove

any surface moisture. The respirometer was housed in a Sable Systems (Henderson, NV, USA) PT-1 Peltier-effect temperature-controlled cabinet at various temperatures. Outside air was scrubbed of CO_2 and H_2O using a Whatman purge gas Generator (Whatman, Inc., Haverhill, MA, USA), drawn through a computer-controlled base lining system, a Li–Cor CO_2 and H_2O analyzer (LI-6262; LiCor Inc., Lincoln, Nebraska, USA), and a Side-Track mass flow meter (Sierra Instruments Inc., Monterey, CA, USA) with a pump (Gast Mfg. Corp., Benton Harbor, MI, USA) at a flow rate of 100 ml min^{-1} at STP. The gas sample passed through the system and data from the CO_2 and H_2O analyzer were recorded using DATACAN V (Version 5.2; Sable Systems, Herderson, NV, USA) software. The CO_2 analyzer was calibrated with 94.9 ppm span gas (Air Products, Inc.). The occurrence of a DGC in *B. germanica* was most frequent (62.8%) at 10 °C, therefore CO_2 emission and H_2O loss were recorded simultaneously at this temperature for 2–3 h and cockroaches were weighed after each run. However, not every cockroach that was placed in the respirometry system at 10 °C performed a DGC that could be completely analyzed. Recordings in which DGC was interrupted were not included in the analysis. All calculations of rate and duration of CO_2 release and H_2O loss were carried out using DATACAN V software. CO_2 and H_2O recordings were baseline-corrected and converted to $\dot{V}_{CO_2}$ ml h^{-1} and mg h^{-1} for CO_2 emission and water loss, respectively. For each individual cockroach, mean $\dot{V}_{CO_2}$ emission and durations were measured for each of the DGC phases. To minimize handling stress that may cause changes in the $\dot{V}_{CO_2}$, mass loss of animals used within the experiments was calculated by subtracting the body mass after a run from the mean body mass of 160 adult *B. germanica* of both strains that were not used in the experiments; estimates of water loss during the run were consistent with flow-through measurements.

2.3. Data analysis

Analysis of covariance (ANCOVA) was used to estimate the effects of metabolic rate and body mass for each strain of *B. germanica* at 10 °C. Regression analysis was performed if there was a significant effect of mass to estimate the slope of Log $\dot{V}_{CO_2}$ and Log mass relationship. A modified *T*-test was used to test if the slope was significantly different than 1 (Sokal and Rohlf, 1981). In measuring metabolic rate, we examined recordings from 12 susceptible and 19 resistant cockroaches. Eight to 10 DGCs were analyzed for each individual cockroach. For water loss measurements, recordings from 5 cockroaches of both strains were examined and 8–10 DGCs were analyzed for each individual cockroach. Because each DGC represented a replicate that may change over the period of the recording, comparison within and between strains over time was analyzed using repeated measures analysis of variance (ANOVA). The test of fixed effects (PROC Mixed Procedure, ANOVA; Khattree and Naik, 1996, SAS Institute, 1996) was used to determine the effect of strain, DGC by strain interaction and the effect of the sequence of the cycles on $\dot{V}_{CO_2}$ and duration. The DGC characteristics including interburst and burst phase durations, $\dot{V}_{CO_2}$ (ml h^{-1}), H_2O loss (mg h^{-1}), and overall strain effects were analyzed using the general linear model procedure (Proc GLM, ANOVA, SAS Institute, 1996). Mean separation was carried out using the Ryan–Einot Gabriel–Welsch Multiple Range test (REGWQ) that controls both type I and type II error. The significance level was set at $P<0.05$. In view of the lack of a distinguishable closed and flutter phase during the exhibition of most DGCs in these cockroaches, we used a statistical technique that involved regressing water-loss rate against CO_2 release for each cockroach (Gibbs and Johnson, 2004; Johnson and Gibbs, 2004). Water loss rate (mg h^{-1}) at the intercept, where CO_2 release (ml h^{-1}) equals zero, was assumed to represent cuticular transpiration. Respiratory water loss was calculated as the difference between total water loss and cuticular water loss. Cuticular and respiratory water losses (mg) were expressed as percentages of total water loss. Water loss through the cuticle or cuticular permeability (CP) is defined as the micrograms of water lost during the entire recording per unit body surface area (cm^2) per unit time (h) per unit saturation deficit (mmHg) (Edney, 1977). Surface area was estimated for each cockroach strain using Meeh's formula (Meeh, 1897).

3. Results

Carbon dioxide emission patterns of both pyrethroid resistant and susceptible strains of *B. germanica* varied greatly with temperature. At 5 °C there were no recordings of an interburst or burst phase for >1 h and the CO_2 emission pattern remained acyclic throughout this time. At 10 °C (Fig. 1), 15 and 20 °C (Fig. 2), the pattern of CO_2 emission was discontinuous. In most DGCs at 10 °C, CO_2 release never dropped to zero during interburst phase in both strains of *B. germanica* (Figs. 2–4). This suggests that small amounts of this gas diffuse through incompletely closed spiracles during this time. DGC frequency increased at 25 and 30 °C and became cyclic at 35 °C (Figs. 2 and 3). The probability that *B. germanica* would respire discontinuously varied with temperature. At 10 °C, ~62.8% of individuals showed a pronounced DGC ($n=49$ of 78); 28% at 15 °C ($n=7$ of 25); 18% at 20 °C ($n=4$ of 22); 20% at 25 °C ($n=4$ of 20); 10% at 30 and 35 °C ($n=1$ of 10 for

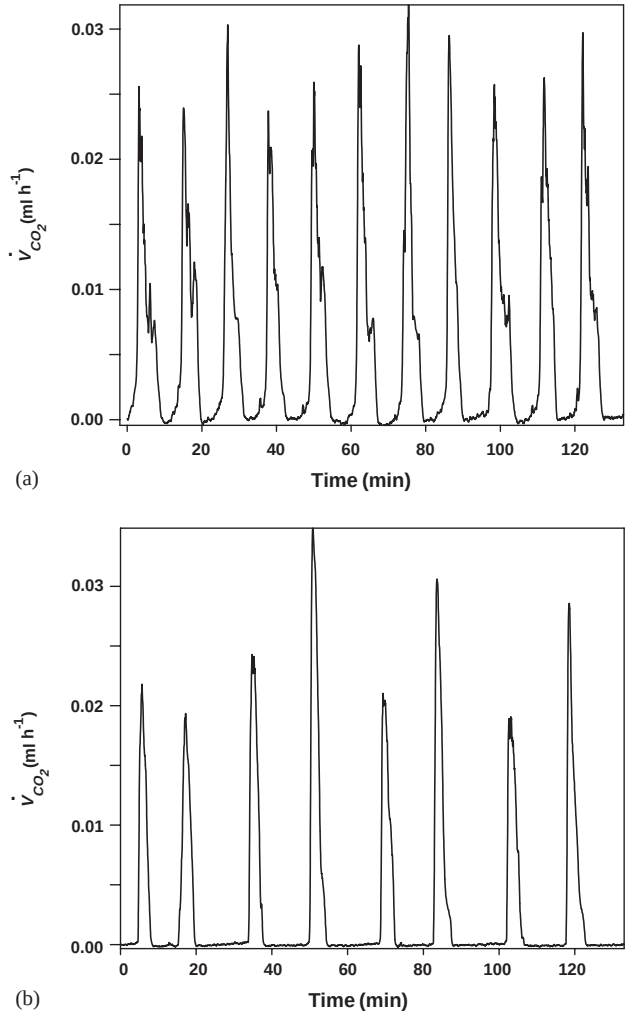


Fig. 1. Typical recording of DGC in (a) resistant (0.045 g) and (b) susceptible (0.049 g) *B. germanica* measured over 1 h at 10 °C.

each). Therefore, measurement of metabolic rate and water loss was carried out at 10 °C. We observed that cockroaches would only exhibit DGC when the temperature in the respirometer with the cockroach was decreased gradually from ambient to the required temperature. If the temperature in the respirometer was already set prior to recording, no DGC was observed and the pattern was always cyclic. Ninety-five percent of the time, it took ~1 h for cockroaches in the respirometer chamber at 10 °C to begin exhibiting DGC (Fig. 4). Out of the 78 DGC recordings obtained at 10 °C, not every recording could be completely analyzed. Recordings in which DGC was interrupted were not included in the analysis. Therefore, overall metabolic rate $\dot{V}_{CO_2}$ (ml h^{-1}) measured as CO_2 release over the, entire recording, was calculated from 12 susceptible and 19 resistant individual cockroaches. From Table 1, the rate of CO_2 emission between both strains was not significantly different ($F_{1,29} = 0.24$, $P > 0.63$). Metabolic rate scaled allometrically with body mass with a slope of 1.22 ± 0.27; this value was not significantly different than 1. The characteristics of the DGC shown in Table 1 were calculated from a total of 96 DGCs from 12 susceptible individuals and 171 DGCs from 19 resistant individuals all at 10 °C. The rate of CO_2 emission ($\dot{V}_{CO_2}$ ml h^{-1}) in each phase is the volume of CO_2 emitted during that phase, obtained by calculating the area under the CO_2 peak and then divided by the phase duration. The phase duration is the time taken for CO_2 to be emitted during that phase and the period is the duration of one DGC (i.e., burst + interburst phase). Interburst and burst $\dot{V}_{CO_2}$ (ml h^{-1}) were not significantly different between the two strains (Tables 1 and 2). The variability over time in CO_2 emission during

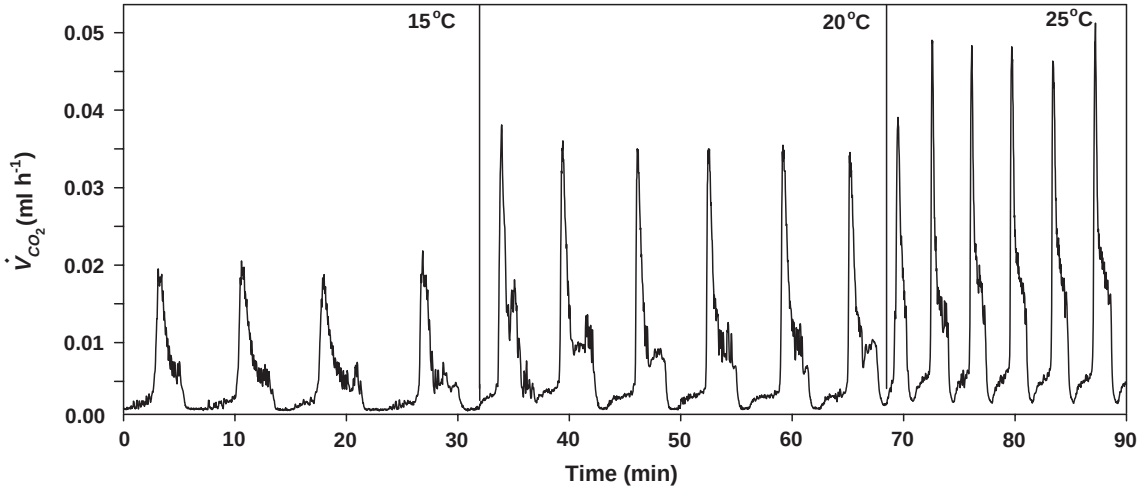


Fig. 2. The effect of temperature (15, 20, and 25 °C) on the DGC frequency illustrated using a susceptible *B. germanica*. Using the same cockroach, temperature was increased after every hour by 5 °C. The CO_2 emission pattern seen here shows DGC at all three temperatures. Note that in some DGCs the CO_2 emission never drops to zero in both strains. Note the increase in DGC frequency as temperature increases.

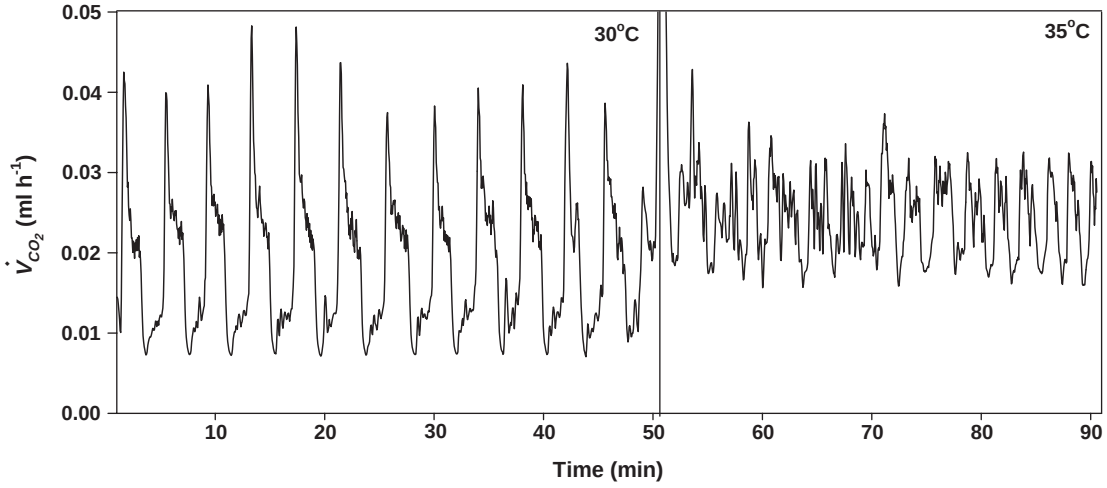


Fig. 3. The effect of temperature (30 and 35 °C) on the DGC frequency illustrated using the same cockroach as in Fig. 2. With cockroach in the respirometer, temperature was increased every hour by 5 °C. Note that in some DGCs the CO_2 emission never drops to zero in both strains. Note the increase in DGC frequency as temperature increases, with the CO_2 emission pattern becoming cyclic at 35 °C.

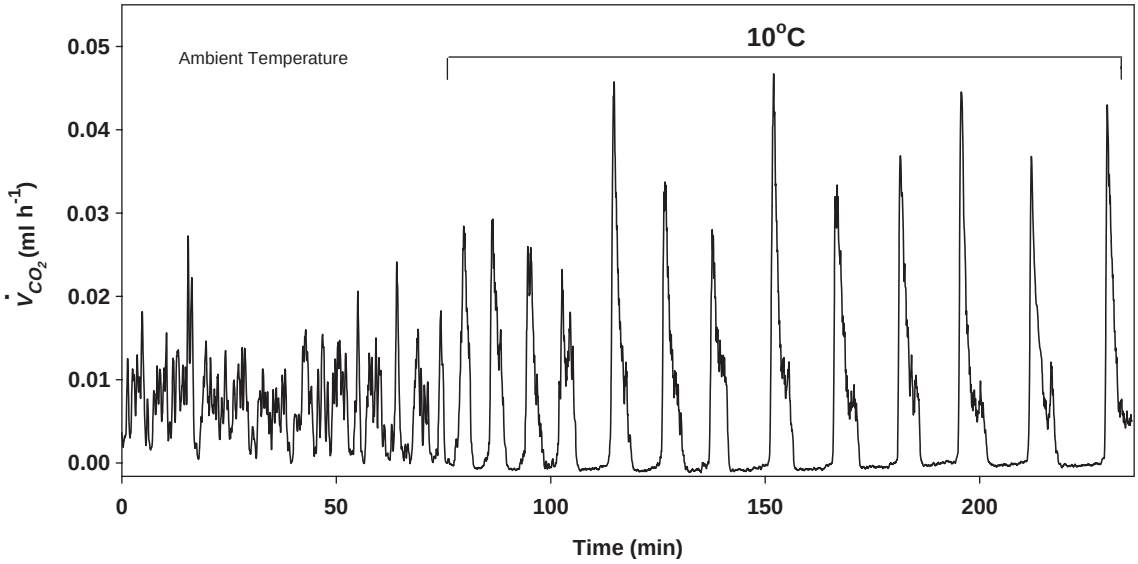


Fig. 4. An illustration of the type of ventilation pattern exhibited by both pyrethroid resistant and susceptible *B. germanica* over time as a result of acclimation to initial set temperature before they begin discontinuous gas exchange. The transition in the CO_2 emission pattern from ambient temperature to 10 °C can be clearly seen. It takes ~1 h before DGC is displayed. Note that in some DGCs the CO_2 emission never drops to zero in both strains.

interburst and burst phases was not significantly different from cycle to cycle or between strains (Table 2). However, there were significant differences in the durations of the interburst and burst phases between the two strains (Table 3). The susceptible strain had significantly longer interburst and burst phase durations during a complete DGC than the resistant strain (Table 1). This results in a DGC of significantly longer duration (13.89 ± 0.44 min) than in the resistant strain (11.23 ± 0.26 min). Also, in a DGC, each phase (interburst or burst) accounted for ~50% of the cycle duration in the susceptible strain (Table 1). In contrast, in the insecticide resistant strain the interburst phase accounted for 43.5% and the burst phase 56.5% of the cycle duration (Table 1). However, in both strains, ~90% of total CO_2 release during a DGC occurred in the burst phase (Table 1). The duration of the interburst phase of both pyrethroid resistant and susceptible strains increased with increasing DGC duration (Fig. 5a and b). However, the duration of the interburst phase in the resistant strain decreased as $\dot{V}_{CO_2}$ decreased, but increased in the susceptible strain (Fig. 6a and b).

Table 1
Characteristics (mean ± SE) of the discontinuous gas exchange cycle (DGC) in resistant and susceptible German cockroaches at 10 °C

	Strain	
	Resistant	Susceptible
Mass (mg)[a]	48.80 ± 0.0004b	54.00 ± 0.0005a
n	19	12
Rate of CO_2 emission		
$\dot{V}_{CO_2}$ (ml h^{-1})	0.024 ± 0.004a	0.021 ± 0.004a
Rate of burst CO_2 emission		
$\dot{V}_{CO_2}$ (ml h^{-1})	0.021 ± 0.004a	0.019 ± 0.004a
Burst duration (min)	6.21 ± 0.134b	6.73 ± 0.173a
Burst duration (% of DGC duration)	56.50 ± 0.008a	50.82 ± 0.012b
Rate of interburst CO_2 emission		
$\dot{V}_{CO_2}$ (ml h^{-1})	0.0029 ± 0.0005a	0.0024 ± 0.0004a
Interburst duration (min)	5.02 ± 0.18b	7.16 ± 0.37a
Interburst (% duration of DGC duration)	43.50 ± 0.008b	49.18 ± 0.12a
Discontinuous gas exchange		
Period (min)	11.23 ± 0.26b	13.89 ± 0.44a
Frequency (mHz)	1.48 ± 0.03	1.20 ± 0.04

The sample size (*n*) is the number of cockroaches measured. Values were calculated from 171 DGCs from 19 resistant and 96 DGCs from 12 susceptible *B. germanica*. Means within rows followed by the same letter are not significantly different at $P \leq 0.05$, REGWQ test.

[a]Initial mass calculated from 160 individuals of each strain that were not used in the respirometry experiments (see Materials and methods).

Table 2
Sources of variation, *F*-statistics, degrees of freedom (numerator, denominator), and probabilities for (A) interburst, (B) burst phases $\dot{V}_{CO_2}$ (ml h^{-1}) of DGC in resistant and susceptible strains of *B. germanica* at 10 °C

Source	(A) Interburst $\dot{V}_{CO_2}$ (ml h^{-1})			(B) Burst $\dot{V}_{CO_2}$ (ml h^{-1})		
	F	df	*P*	*F*	df	*P*
Strain	2.41	1.232	0.1220	0.63	1.232	0.4268
DGC	0.61	7.232	0.7459	0.22	1.232	0.9793
DGC*Strain	0.58	7.232	0.7690	0.21	1.232	0.9841

Values were calculated from 171 DGCs from 19 resistant and 96 DGCs from 12 susceptible *B. germanica*. A DGC is comprised of burst + interburst phases and here it indicates the variability from cycle to cycle.

Mean cockroach body mass differed significantly between strains (Table 1). Mean body mass was calculated from 160 adult male *B. germanica* of both strains. The close synchrony between CO_2 bursts and bursts of water loss provides a means of separating and quantifying cuticular and respiratory water loss (Fig. 7). Respiratory water loss is presumed to be minimal during interburst periods since a reduction in continuous spiraclar opening would reduce water loss. Cuticular water loss was significantly greater in the susceptible strain compared with the resistant (Fig. 8). Also, total water loss (ml h^{-1}) was significantly greater in the susceptible strain (Table 4). In both strains cuticular transpiration always accounted for >95% of the total water loss (Table 4). The overall water loss (mg h^{-1}) was not significantly different ($F_{1,8} = 0.31$, $P > 0.59$) between strains. CP values using Meeh (1897) surface area model estimates were significantly different ($P < 0.05$) between strains. The CP was 2.26 μg cm^{-2} h^{-1} mmHg^{-1} for the resistant strain and 3.42 μg cm^{-2} h^{-1} mmHg^{-1} for the susceptible strain.

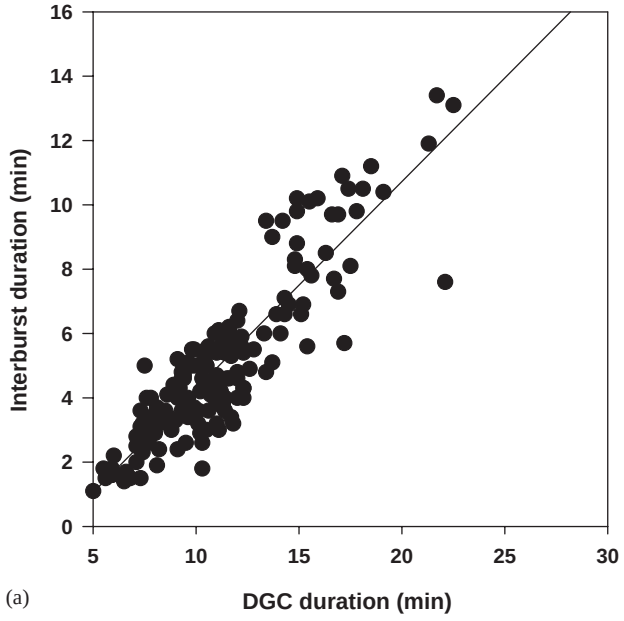
Table 3
Sources of variation, *F*-statistics, degrees of freedom (numerator, denominator), and probabilities for (A) interburst phase duration (min), (B) open phase duration (min), of DGC in resistant and susceptible strains of *B. germanica* at 10 °C

Source	(A) Interburst (min)			(B) Burst (min)		
	F	df	*P*	*F*	df	*P*
Strain	33.27	1.232	0.0001	5.42	1.232	0.0208
DGC	1.76	7.232	0.0968	0.46	1.232	0.8644
DGC*Strain	0.54	7.232	0.8005	0.44	1.232	0.8778

Values were calculated from 171 DGCs from 19 resistant and 96 DGCs from 12 susceptible *B. germanica*. A DGC is comprised of burst + interburst phases and here it indicates the variability from cycle to cycle.

4. Discussion

Variation of metabolic rate with temperature has long been known in insects. The effect of temperature, however, on the gas exchange pattern has not been examined in most adult insects. In the majority (62.8%) of pyrethroid resistant and susceptible *B. germanica* tested in this study, the discontinuous CO_2 emission pattern was observed at 10 °C and in a few individuals at 15 and 20 °C. Increase in temperature (25 and 30 °C) was accompanied by an increase in the frequency of the DGC pattern. At 35 °C, CO_2 emission was cyclic even though relatively few individuals showed discontinuous CO_2 release at this higher temperature. Chappell and Rogowitz (2000) reported similar results in the Eucalyptus-boring beetles, *Phorocantha* sp. which exhibited DGC at 10 and 20 °C, but the pattern changed to cyclic CO_2 emission at temperatures ≥30 °C. However, a few beetles exhibited DGC at these high temperatures. These findings indicate that within a given insect species and when insects are inactive, the majority of individuals exhibit DGC over a certain temperature range above which only a few individuals would exhibit DGC. This inconsistency in displaying a DGC could be due to



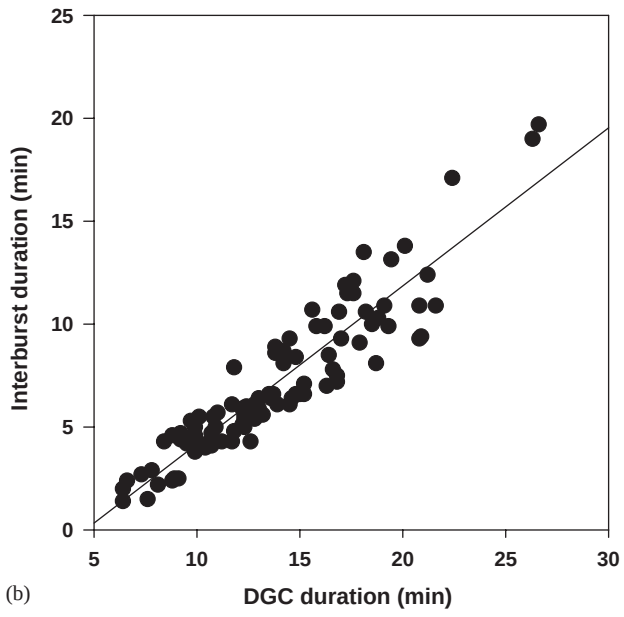
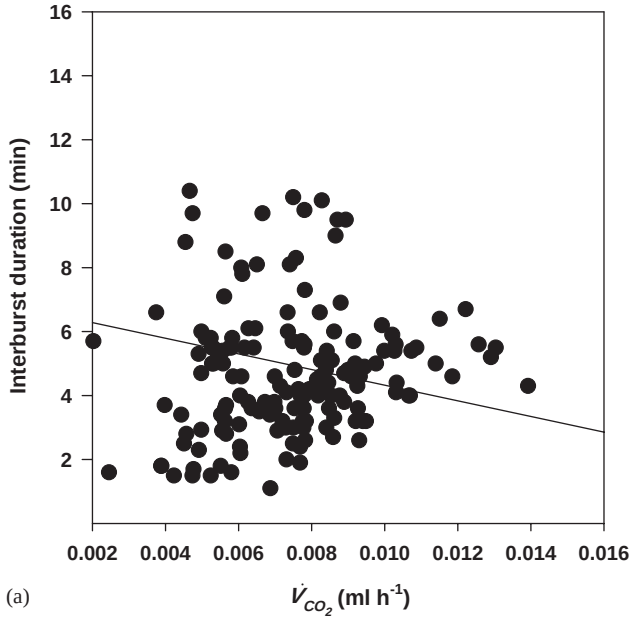


Fig. 5. Relationship between duration of a complete DGC and interburst phase duration in resistant and susceptible *B. germanica*. $N = 12$ susceptible and 19 resistant adult males, with a total of 171 DGCs in the resistant and 96 in the susceptible strains obtained at 10 °C.



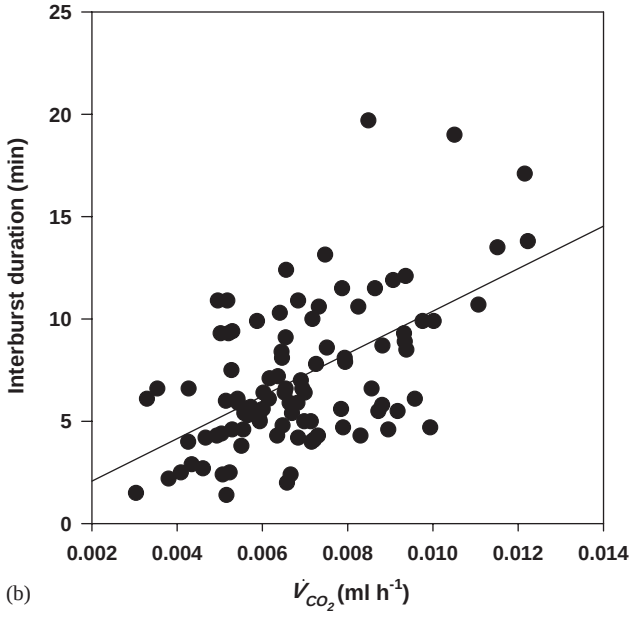


Fig. 6. The relationship between $\dot{V}_{CO_2}$ and interburst phase duration in resistant and susceptible *B. germanica*. $N = 12$ susceptible and 19 resistant adult males, with a total of 171 DGCs in the resistant and 96 in the susceptible strains obtained at 10 °C.

genetic and/or physiological variation in any given population. However, in most published studies, the proportion of individuals that exhibit the DGC is not reported.

The DGCs of both pyrethroid resistant and susceptible strains of *B. germanica* were exhibited only at low to ambient temperatures when cockroaches were quiet and undisturbed. These are similar conditions in which discontinuous CO_2 emission was observed in *R. guttata* (Hadley and Quinlan, 1993), *P. americana* (Kestler, 1991), and *Perisphaeria* sp. (Marais and Chown, 2003). A probable cause could be that at lower temperatures, metabolism is reduced and therefore CO_2 production declines. Thus, the spiracles remain closed longer and are only opened when the concentration of CO_2 within the tracheal system has increased to a certain threshold. Whereas at higher temperatures with increased metabolism, more CO_2 is released from tissues per unit time (Schneiderman, 1960). One implication is that cycle frequency will increase with increased metabolism, and

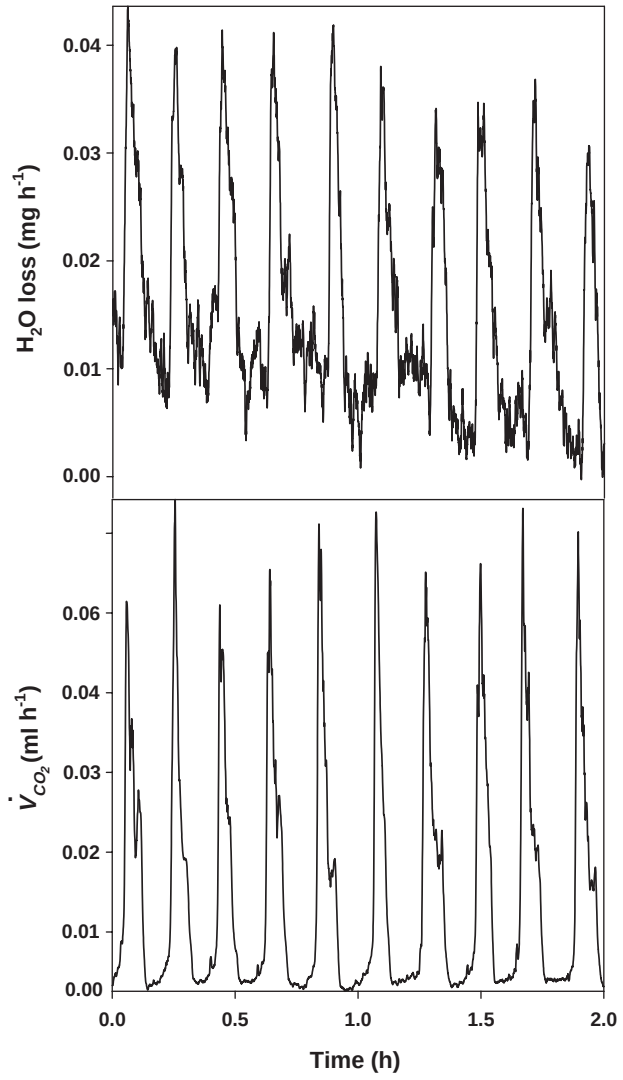


Fig. 7. A simultaneous recording of CO_2 release and H_2O loss in *B. germanica* at 10 °C.

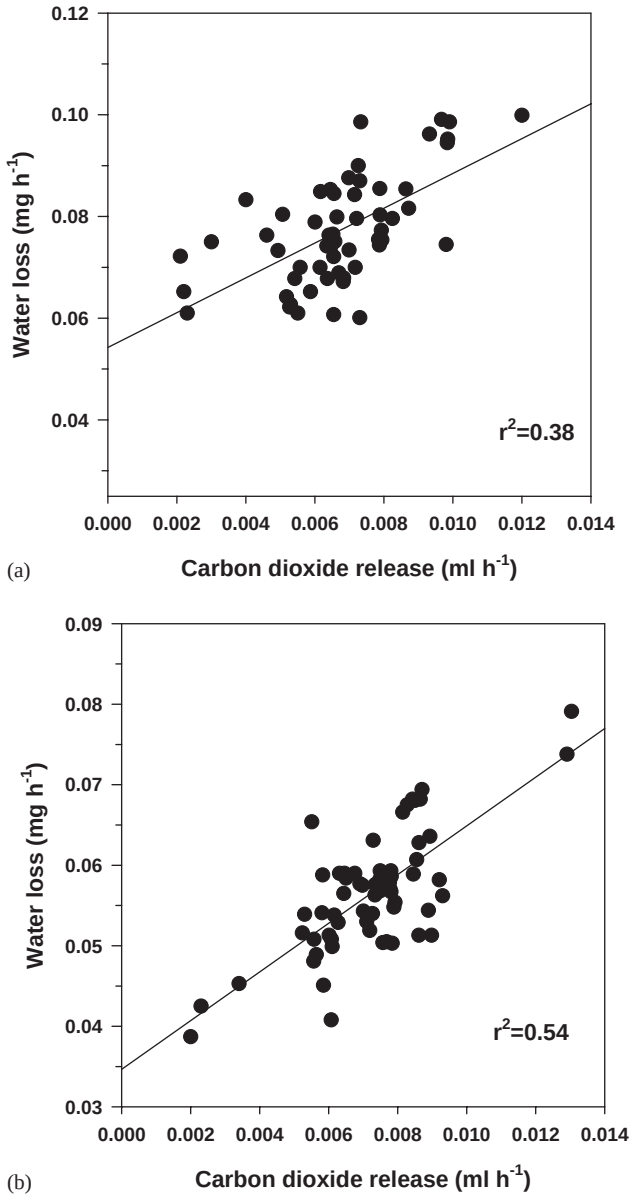


Fig. 8. Plots of water loss (mg h^{-1}) against CO_2 emission at 10 °C for (a) susceptible and (b) resistant *B. germanica*. The arrow in the lower panel denotes the *Y*-intercept of the regression, corresponding to the point at which CO_2 emission is absent and the spiracles are completely closed. The value of the *Y*-intercept thus represents cuticular water loss.

gas exchange will become continuous at higher temperatures as illustrated in Fig. 3.

The discontinuous CO_2 emission pattern in both strains of *B. germanica* differed from the classic "three phase" pattern first described in lepidopteran pupae (Schneiderman, 1960), and that seen in *P. americana* and *Perisphaeria* sp. (Kestler, 1991; Marais and Chown, 2003). The interbust phase could not be clearly differentiated into closed and flutter phases and $\dot{V}_{CO_2}$ never fell to zero in most (~60%) of the DGCs. This is not an unusual pattern; similar observations were also noted in the Eastern lubber grasshopper, *Romalea guttata* (Hadley and Quinlan, 1993), the eucalyptus-boring beetle, *Phorocantha* spp. (Chappell and Rogowitz, 2000), and in the dampwood termite, *Zootermopsis nevadensis* where it was referred to as an acyclic CO_2 emission pattern (Shelton and Appel, 2000). One possibility for this type of pattern is that these insects may be using independent spiracles during respiration, in which case some spiracles are kept open while others are closed or occasionally all spiracles are closed. In addition, it is possible that it is more difficult for insects to precisely coordinate the opening and closing of many spiracles. Adult *B. germanica* have 4 thoracic and 16 abdominal spiracles (Haber, 1926) compared with as few as 4 in many adult ants. Unfortunately, we could not observe spiracular behavior directly during our measurements. Therefore, we do not know whether all the spiracles remained opened at all times.

Table 4
Water loss rates of the discontinuous gas exchange cycle in pyrethroid resistant and susceptible *B. germanica* at 10 °C

Variable	Strain	
	Resistant	Susceptible
Cuticular water loss rate (mg h^{-1})	0.033±0.005b	0.054±0.007a
Respiratory water loss rate (mg h^{-1})	0.002±0.001a	0.002±0.001a
Total water loss (mg h^{-1})	0.035±0.006b	0.056±0.007a
% Respiratory water loss	3.4±1.90a	4.4±2.15b

Note: Water loss values were calculated from ~55–60 DGCs from 5 cockroaches per strain.
Means within rows followed by the same letter are not significantly different at $P<0.05$, REGWQ test.

Change in respiratory pattern from discontinuous to continuous CO_2 emission as a result of a stressor has been documented. For example, in the presence of insecticides (Kestler, 1991), toxic plant extracts (Sibul et al., 2003; Kuusik et al., 2001; Harak et al., 1999) and handling (Harak et al., 1998), the pattern of CO_2 release changed from DGC to continuous emission. In this study, we observed that when the temperature in the respirometer decreased gradually from room temperature to 10 °C, the pattern of CO_2 emission was discontinuous. However, when *B. germanica* was placed directly into a chamber already at 10 °C, the CO_2 emission pattern was continuous. This difference in CO_2 emission pattern could be a response to cold (stressor).

In this study, the metabolic rate between genetically resistant *B. germanica* not exposed to insecticide after several generations was not significantly different from the susceptible strain. Similarly, Hostetler et al. (1994) using closed system respirometry at 26 °C, reported no significant difference in metabolic rate between pyrethroid resistant and susceptible *B. germanica*. Resistant alleles have been shown to exert negative effects on fitness, such as reduced mating (Groeters and Tabashnik, 1993), decreased weight (Brewer and Trumble, 1991; Hostetler and Brenner, 1994; Dingha et al., 2004), reduced egg hatch (Ross, 1991) and increased metabolic rate (Dingha et al., 2004). In the absence of selection, resistance has been reported to decline, for example in *B. thuringiensis*-resistant diamondback moth, *Plutella xylostella* (Tabashnik et al., 1994) and the Colorado potato beetle, *Leptinotarsa decemlineata* (Rahardja and Whalon, 1995). Also, in other pyrethroid resistant *B. germanica* resistance declined from 140 to 1.6 fold in the absence of selection pressure after 15 generations (Cochran, 1993). This reversion is likely associated with increased fitness (Tabashnik et al., 1994). For example, susceptible and resistant *S. exigua* pupae whose larvae were not exposed to Cry1C *B. thuringiensis* had similar metabolic rates, however, this was significantly different from pupae whose larvae were exposed continuously to toxin (Dingha et al., 2004). The mechanisms of resistance to pyrethriod in *B. germanica* include P450 monooxygenase, hydrolase, and altered sodium channels (Wei et al., 2001; Pridgeon et al., 2002). The resistant strain of *B. germanica* may have detoxification mechanisms that would increase metabolic rates but requires the presence of the insecticide to induce accelerated production of the detoxifying enzymes. Furthermore, several studies have documented that production of detoxifying enzymes increase in the presence of pesticides (e.g., Terriere, 1983).

At 25 °C, the metabolic rates for both strains was 0.034 ml h^{-1}, calculated using Q_{10} values of 1.19 and 1.15 for the resistant and susceptible *B. germanica*, respectively (Dingha et al., unpublished). The metabolic rate of other cockroaches such as, *Perisphaeria* sp. (0.315 g) found at high altitudes (950 m above sea level) was 0.0207 ml h^{-1} at 20 °C (Marais and Chown, 2003). The oxygen consumption rate of *B. giganteus* (4.33 g) measured at the end of a 6–10 min run was 0.693 ml h^{-1} at 26.6 °C (Bartholomew and Lighton, 1985). Similar values (0.0316 and 0.034 ml h^{-1}) were reported for pyrethroid resistant (0.048 g) and susceptible (0.043 g) *B. germanica*, respectively, at 26 °C (Hostetler et al., 1994).

Generally, during a DGC the closed phase begins at the end of the last open phase, and lasts until endotracheal O_2 concentration reaches a critical threshold (Levy and Schneiderman, 1966). Once this threshold is reached, the flutter phase is initiated, and during this time, CO_2 accumulates in the hemolymph and continues to accumulate until the open phase is triggered. In both pyrethroid resistant and susceptible *B. germanica* the interburst and burst phase durations were linearly related with the duration of the DGC (Fig. 5a and b). It is therefore possible that both set points influence the duration of the interburst phase. In addition, local concentrations of CO_2 and O_2 in adult insects, including cockroaches, affect spiracular movements (Harrison et al., 1995). Even though we did not measure internal changes in CO_2 and O_2 concentrations, it is possible that different CO_2 and O_2 thresholds trigger spiracular opening in these two physiologically distinct strains (Schneiderman and Williams, 1955; Harrison et al., 1995).

Terrestrial insects generally lose water through several pathways, such as the spiracles, cuticle, oral, and anal openings. The importance of spiracular fluttering in reducing water loss has been emphasized (Miller, 1981; Lighton and Garrigan, 1995). Insects must be able to regulate spiracular opening when challenged with conditions that could result in water loss. Generally, in such situations the burst phase (high respiratory water loss) of the DGC should be short relative to the interburst phase (low respiratory water loss). Only a few studies have actually measured respiratory water

loss during bouts of CO_2 emission and compared these values to rates of total water loss over the same period. Kestler (1978, 1985), measured respiratory transpiration in *P. americana* during DV cycle by recording changes in body mass with an extremely sensitive electronic balance and found the rate of mass (water) loss to be lowest during the interburst phase of the cycle, but increased at the start of ventilation. Machin et al. (1991) also reported that the mean water loss of *P. americana* during the burst phase was higher (87%) than the rate during the interburst phase. The susceptible strain has longer interburst and open phase durations than the resistant strain (Table 1). The duration of the burst phase is less than half and the interburst phase is greater than half the duration of a complete gas exchange cycle. In the resistant strain the duration of the burst phase is greater than half and the interburst phase is less than half the duration of a complete gas exchange cycle. The long interburst phase of the susceptible strain was not important in reducing water loss because more water was lost during this time in comparison to the resistant strain. However, long flutter periods $\geqslant$23 min appear to be important in reducing water loss in arid adapted arthropods, such as the ant *Cataglyphis* sp. (Lighton and Wehner, 1993), and a trogid beetle (Bosch et al., 2000) where the need to conserve water is great.

In the resistant strain, respiratory transpiration was responsible for 3.4% of total water loss while in the susceptible strain respiratory transpiration accounts for 4.4% of total water loss, with cuticular transpiration accounting for the bulk of the water loss in both strains. Despite the high cuticular water loss in both strains ($>$95%) the susceptible strain lost significantly more water in a DGC than the resistant strain. This could be due to the long duration of the interburst phase compared with that of the resistant strain (Table 1). In *R. guttata*, cuticular respiration accounted for 97% of total water loss (Quinlan and Hadley, 1993), in *Taeniopoda eques* 95.2% (Quinlan and Hadley, 1993), 85% in *M. sanguinipes* (Rourke, 2000), and 95% in *Aphodius fossor* (Chown and Holter, 2000). In these insects respiratory transpiration is often less than 20% of the total loss. Our data for *B. germanica* are similar to these values. However, cuticular transpiration and CP were significantly reduced in the resistant strain. Possibly selection for insecticide resistance altered the composition of the cuticle or the epicuticular lipids since decreased cuticular penetration is one of the mechanisms of resistance in the resistant *B. germanica* strain (Wei et al., 2001).

The rate of overall water loss in resistant and susceptible *B. germanica*, when expressed per unit surface area and corrected for saturation deficit, fall within the range of values (0–30 $\mu g\,cm^{-2}\,h^{-1}\,mmHg^{-1}$) exhibited by arthropods that inhabit xeric environments (Edney, 1977). If CP has a Q_{10} value of 2 (Hadley, 1994) then the CP at 30 °C would be 9.04 and 13.7 $\mu g\,cm^{-2}\,h^{-1}\,mmHg^{-1}$ for the resistant and susceptible strains, respectively. However, these CP values were less than half of those obtained from pyrethroid resistant (25.23 $\mu g\,cm^{-2}\,h^{-1}\,mmHg^{-1}$) and susceptible (27.56 $\mu g\,cm^{-2}\,h^{-1}\,mmHg^{-1}$) *B. germanica* in a still-air desiccation study (Appel, 1993). Furthermore, the CP values were not comparable to those of other cockroaches, for example *Diploptera punctata* (20.91 $\mu g\,cm^{-2}\,h^{-1}\,mmHg^{-1}$) and *Pycnoscelus surinamensis* (38.69 $\mu g\,cm^{-2}\,h^{-1}\,mmHg^{-1}$) (Appel, 1991). These differences could probably be due to the fact that Appel (1991, 1993) measured CP from dead cockroaches, which were obviously unable to regulate water loss. It is assumed that spiracular-closing and water-conserving mechanisms are intact in living insects under experimental conditions, whereas water loss in recently killed specimens is mostly by simple diffusion through open spiracles that cannot be controlled (Machin et al., 1991).

In conclusion, during discontinuous release of CO_2 in *B. germanica* $>$95% of water loss occurs during the interburst phase, and respiratory transpiration therefore contributed little to total water loss. Significantly longer duration of the interburst phase of the susceptible strain of *B. germanica* did not contribute to reduction of water loss. DGC tends to be replaced by continuous CO_2 emission as temperature increases and could be due to increased metabolism associated with increased temperature. Moreover, the DGC was only observed at lower temperatures and when the cockroaches were undisturbed. In the insect's natural environment, with temperature $>$26 °C (Appel, 1995), the probability that *B. germanica* would breath discontinuously is about 20% compared with about a 60% chance at 10 °C.

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