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PHYSIOLOGICAL FACTORS RELATED TO BAIT AVERSION IN THE GERMAN COCKROACH (DICTYOPTERA: BLATTELLIDAE)

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Abstract Physiological factors related to bait aversion in the German cockroach, *Blattella germanica* (L.), were investigated in a series of laboratory experiments. We compared the metabolic rates, respiration patterns, and taste receptor perceptions of control and bait averse German cockroaches. Both strains detected glucose and sucrose similarly, but only with the maxillary palp. The metabolic rate of the two strains differed in their response to temperature. Both strains exhibited a discontinuous gas exchange cycle (DGC) at 10°C, but the duration of both the interburst and burst phases was longer in the susceptible strain. The maximum RQ values occurred at 20°C for the resistant and 25°C for the susceptible strains and suggest that lipid and protein served as the metabolic substrates.

Key Words Metabolic rate, DGC, taste receptors, gas exchange

INTRODUCTION

German cockroaches, *Blattella germanica* (L.) are one of the most important pests of domestic environments. They can contaminate foodstuffs and food preparation surfaces as well as animal rearing facilities, and homes with their feces and body parts. German cockroaches can mechanically vector a number of disease organisms and are highly allergenic to sensitive people. In addition, the mere presence of cockroaches can induce discomfort and embarrassment.

A number of insecticidal formulations and active ingredients have been used to control German cockroaches. Although spray, dust, and granular formulations have been used, baits have become the formulation most relied upon by professional pest managers and homeowners. For maximum effectiveness a bait formulation should be attractive, non-repellent, promote consumption of a lethal quantity of bait, and slow acting. Phagostimulants such as various sugars and water have been added to bait formulations to promote attraction and consumption. The historic treatment of German cockroaches with spray formulations containing carbamate, organophosphate, and pyrethroid insecticides has resulted in the development of high levels of insecticide resistance in many populations. Significant levels of resistance remain even in the absence of the selective pressure of continued spraying. Not surprisingly, treatment of German cockroaches with insecticidal baits has also resulted in the development of "resistance". In this case, however, cockroaches developed an aversion to a component of the bait formulation, not the active ingredient. Silverman and Bieman (1993) documented the first case of German cockroaches that had become adverse to glucose-containing baits. This aversion reduced or eliminated consumption of the bait resulting in much delayed or even lack of toxicity and reduced field performance. More recently, Wang et al. (2004) reported both avoidance (behavioral resistance) and physiological resistance to insecticidal baits. They found reduced feeding on a number of sugars used as phagostimulants as well as significant physiological resistance to abamectin and fipronil. Wang et al. (2004) also reported reduced fecundity of resistant cockroaches and suggested a fitness cost for resistance.

Insecticide resistance and stressors such as extreme temperatures, desiccation, and even handling may also affect insect metabolism and respiration. For example, the metabolic rate of third instar *Bacillus thuringiensis* (Bt) resistant beet armyworm, *Spodoptera exigua* (Hübner), larvae is significantly greater when those larvae are exposed to toxin lased diet (Dingha et al., 2004). There is no difference between the metabolic rates of resistant and susceptible larvae in the absence of toxin. Similarly, Hostetler et al. (1994) found that unexposed insecticide resistant German cockroaches did not have different metabolic rates than susceptible cockroaches at a single temperature.

Exposure to stressors such as insecticides and toxins can not only affect metabolic rate, but also alter or disrupt patterns of gas exchange. Dingha et al. (2005) found that the discontinuous gas exchange cycle (DGC) of beet armyworm pupae whose larvae were fed Bt toxin was longer than untreated pupae. The three phases of the DGC were distinct for both treatment groups. However, the duration of each of the DGC phases was significantly greater for pupae previously exposed to toxin. The closed phase was 40 ± 14% longer, the flutter phase 23 ± 19% longer and the open phase was 28 ± 12% longer in pupae exposed to toxin. Insecticide treatment disrupts the DGC of American cockroaches, *Periplaneta americana* (L.), and is therefore an indicator of physiological stress (Kestler, 1991).

The purpose of this study was to compare several physiological parameters of bait adverse and insecticide resistant German cockroaches with those of susceptible cockroaches. Specifically, we examined the metabolic rates, respiration patterns, and perception of sugars in bait averse and susceptible German cockroaches.

MATERIALS AND METHODS

Insects. Two German cockroach strains were used in this study. A control susceptible strain, ACY (American Cyanamid, Clifton, NY), that has been reared in the laboratory for >25 years without exposure to bait formulations or insecticides. The bait averse and pyrethroid resistant strain (Apyr-R) was collected from infested apartments in Opelika, Lee County, AL, US in 1999 after control failures with pyrethroid insecticides and insecticidal baits. This strain was subsequently selected with permethrin for several generations (Wei et al., 2001; Pridgeon et al., 2002), but not during this experiment. All cockroaches were reared at 25 + 2° C and 50 + 10% RH with a photoperiod of 12L:12D. Dry dog chow and water were supplied.

Metabolic rate and gas exchange patterns. One-week old adult males from each strain were selected randomly and weighed to the nearest 0.01 mg with an electronic balance. Cockroaches were transferred individually into respirometers constructed from 3 ml syringes (Becton, Dickinson and Company, Ruthford, NJ, US). Respirometers containing insects were connected to a manifold and flushed with dry, CO_2 free air at the rate of 100ml/min for ~ 10 min (Vogt and Appel, 1999). The plunger was then brought to the 2 ml gradation and the stopcock was adjusted to seal the respirometer. Respirometers were incubated at 5, 10, 15, 20, 25, 30, 35, and 40 + 2° C for 30 min and any activity was recorded with a video camera. Oxygen depletion and carbon dioxide enrichment in each respirometer was determined using a Sable Systems TR-3 respirometry system (Sable Systems, Henderson, NV, US). Outside air was scrubbed of CO_2 and H_2O using a Whatman purge gas Generator (Whatman, Inc., Haverhill MA, US), drawn through a computer-controlled base lining system, a Li-Cor CO_2 and H_2O analyzer (LI-6262; LiCor Inc., Lincoln, Nebraska, USA), a Sable Systems FC-1 Oxygen Analyzer, and a Side-Track mass flow meter (Sierra Instruments Inc., Monterey, CA, US) with a pump (Gast Mfg. Corp., Benton Harbor, MI, US) at 100 ml/min at STP. Exactly 0.5 ml of air from the respirometer was injected into a glass T- injector port upstream of the CO_2-H_2O analyzer. The gas sample passed through the system and data from the O_2 and CO_2 analyzers were recorded using DATACAN V (Version 5.2; Sable Systems, Henderson, NV, US) software. Rate of O_2 consumption was calculated as the volume (ml) of O_2 divided by insect body mass (g), and incubation time (min) resulting in $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}). Respiratory quotient (RQ) was calculated as the ratio of CO_2 produced ($\dot{V}_{O_2}$) to O_2 consumed ($\dot{V}_{O_2}$).

A Sable Systems TR-3 respirometry system was also used to examine the respiration patterns of susceptible and bait averse and insecticide resistant German cockroaches. A single adult male was confined in a glass respirometer chamber and housed in a Sable Systems PT-1 Peltier-effect temperature-controlled cabinet at 10°C. German cockroaches readily exhibit the DGC at this temperature (Dingha et al., 2005). CO_2 free air was drawn across the cockroach at 100 ml/min at STP. CO_2 production by each cockroach was recorded for 3-4 h. The DGC characteristics including interburst and open phase durations (min) and $\dot{V}_{O_2}$ (ml/h) were determined using DATACAN V software.

Electrophysiology. To determine the ability of German cockroaches to detect various sugar solutions we used two electrophysiological techniques. First, the entire terminal segment of the mandibular and maxillary palp was covered with a glass recording electrode containing a test sugar solution (d-glucose or d-sucrose); the indifferent electrode was inserted either into the head or body. The second technique utilized smaller electrodes that were used to cover the distal portion of individual sensory hairs on the terminal segments of the mandibular and maxillary palps. The electrophysiological equipment for this project included a Portable Electrophysiological Recording Unit, Type INR-2, four channel recording system type USB-IDAC (for signal amplification and to facilitate PC processing of data), and an EAG and GC/EAD Signal Recording and Analysis

Program (for data acquisition and analysis). This equipment was manufactured by Syntech (Hilversum, Netherlands).

Data analysis. Metabolic rate data were analyzed using Proc Mixed SAS (SAS Institute, 1996) to determine the effects of cockroach strain on mean body mass, and to determine effects of temperature, strain, and temperature by strain on $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) and RQ. DGC characteristics including interburst, and open phase durations, $\dot{V}_{CO_2}$ (ml/h), and H_2O loss (mg/min) and overall strain effects were analyzed using the general linear model procedure (Proc GLM, ANOVA, SAS Institute, 1996). The significance level was set at $P<0.05$.

RESULTS AND DISCUSSION

The overall effect of strain on $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) was not significant ($P > 0.05$), but the different strains reacted differently to increasing temperature ($F = 3.4$, df = 16, 161, $P < 0.0001$). Generally, metabolic rate increases with increase temperature; however, exceptions from the exponential model have been reported in fish (Jobling, 1994), and insects (Keister and Buck, 1973). Temperature effects were examined by regressing $\log_{10}$-transformed mass-specific $\dot{V}_{O_2}$ over temperature for each strain. For the resistant strain, the equation was: $\log_{10} \dot{V}_{O_2}$ (ml g^{-1} h^{-1}) = 0.044 (+0.016) + 0.0078 (+0.0002) Temperature ($F = 151.2$, $df = 1, 6$, $P<0.0001$, $r^2 = 0.9618$). For the susceptible strain, the relationship was: $\log_{10} \dot{V}_{O_2}$ (ml g^{-1} h^{-1}) = 0.079 (+0.008) + 0.0059 (+0.0003) Temperature ($F = 354.3$, $df =1, 6$, $P<0.0001$, $r^2=0.9833$). The effect of temperature on oxygen consumption of the two strains is presented in Figs. 1 and 2. Q_{10} was calculated by multiplying the slope of the first order log-linear regression of $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) against temperature by 10 then take the antilogarithm. This yields a mean Q_{10} of 1.19 and 1.15 for the resistant and susceptible strain respectively, throughout the experimental temperature range. Although RQ value is not necessarily proof of the identity of a particular substrate used in respiration, it nevertheless allows assumption about the substrate metabolized (Withers, 1992). Generally, RQ values range from 1.00 for carbohydrate, 0.80 for protein, and 0.71 for lipid metabolism (Bartholomew, 1977). RQ values were affected by temperature in both strains (Figs. 3 and 4). The maximum RQ values occurred at 20°C for the resistant and 25°C for the susceptible strains and suggest that lipid and protein served as the metabolic substrates.

DGCs were only observed in motionless cockroaches, and activity, even slight movement altered the DGC and resulted in noticeable changes in the pattern of CO_2 emission. Approximately 62.5% of individuals showed a pronounced DGC (n=25 of 40). During the interburst phase in both strains of *B. germanica*, CO_2 expiration never drops to zero. This suggests that small amounts of this gas diffuse through incompletely closed spiracles during this time (Figs. 5a and b). The duration of the interburst phase of both pyrethroid resistant and susceptible strains increased with increasing DGC duration (Figs. 6a and b). The major difference in DGC characteristics between strains was the interburst and open phase durations. The susceptible strain has a longer interburst and open phase than the resistant strain. In the susceptible strain, the open phase is less than half and the interburst phase is greater than half the duration of a complete DGC. In the resistant strain, however, the open phase is greater than half and the interburst phase less than half the duration of a complete DGC.

Electrophysiological measurements of whole palp and single sensory cells indicated that the mandibular palps did not react to sugar solutions. The maxillary palps, however, did respond to a range of d-glucose and d-sucrose solutions (0.01-1M). There was no difference in the electrophysiological response between bait averse and pyrethroid resistant and susceptible strains of German cockroaches. These results indicate that bait aversion is due to changes in the central rather than sensory nervous system.

In conclusion, bait averse and pyrethroid resistant detect sugar solutions in a similar manner, indicating that aversion is a central nervous system phenomenon. There are also subtle, yet significant, differences in respiration patterns and response to temperature between averse and susceptible strains. Clearly, further study on the physiological differences among German cockroach strains and their importance to cockroach biology and control is indicated.

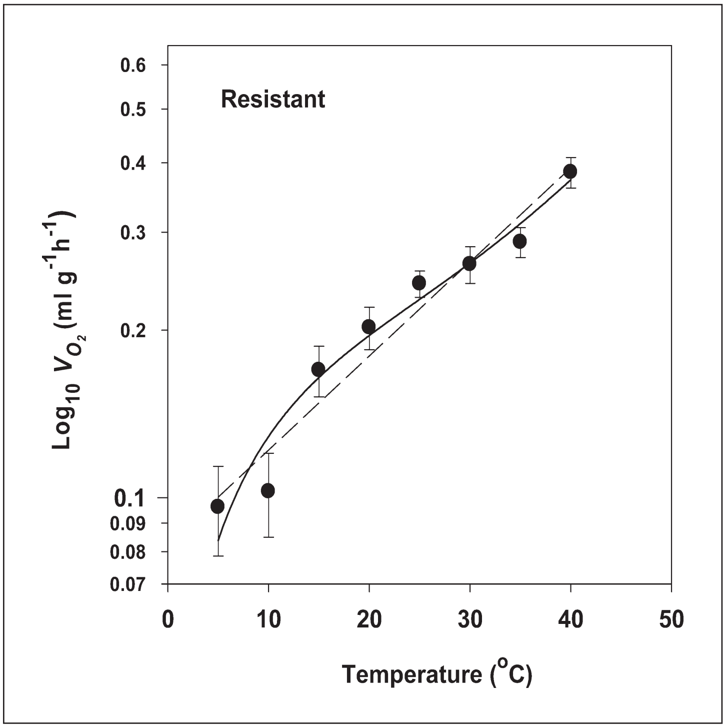

Figure 1. Rate of oxygen consumption in bait averse and pyrethroid resistant German cockroaches at several temperatures. Broken line represents the first-order regression of log transformed oxygen consumption (ml g^{-1} h^{-1}) on temperature, solid lines represents the third-order regression.

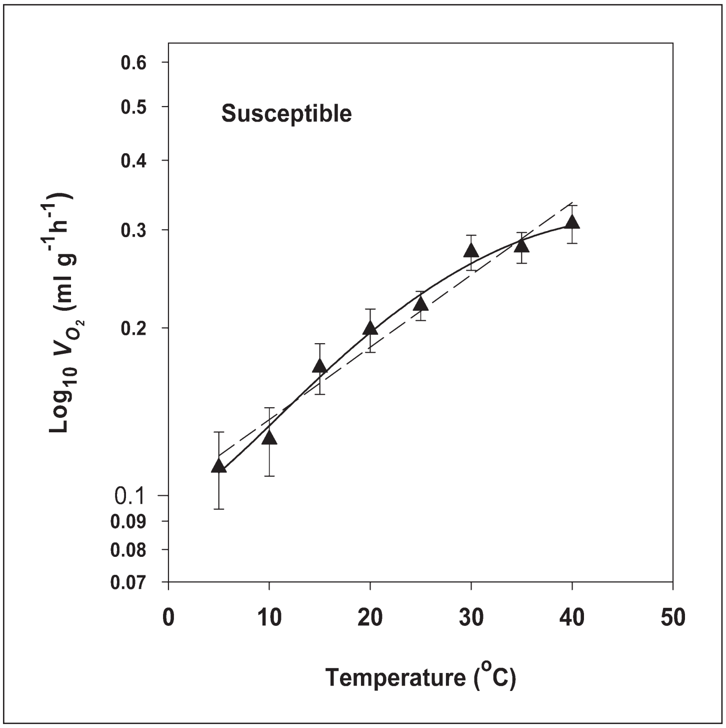

Figure 2. Rate of oxygen consumption in susceptible German cockroaches at several temperatures. Broken line represents the first-order regression of log transformed oxygen consumption (ml g^{-1} h^{-1}) on temperature, solid lines represents the third-order regression.

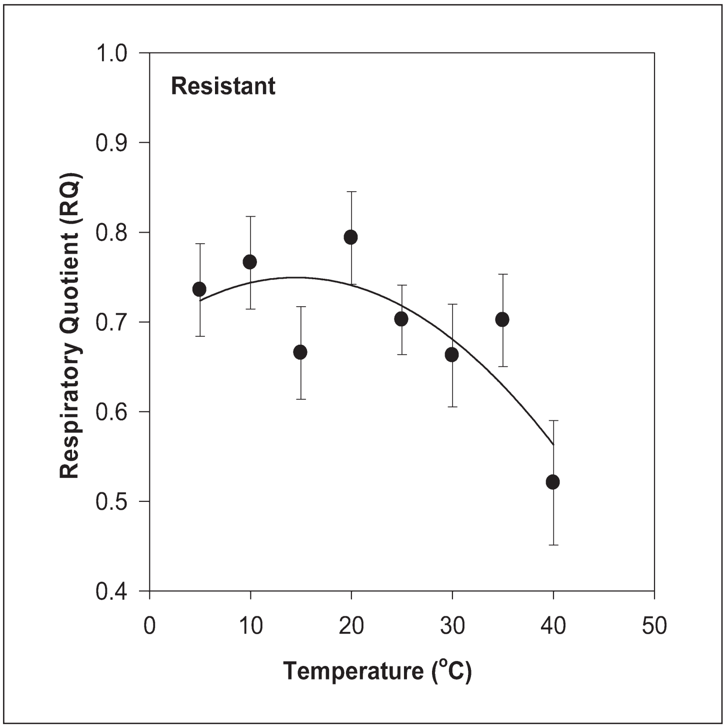

Figure 3. Relationship between temperature and RQ in bait averse and pyrethroid resistant German cockroaches.

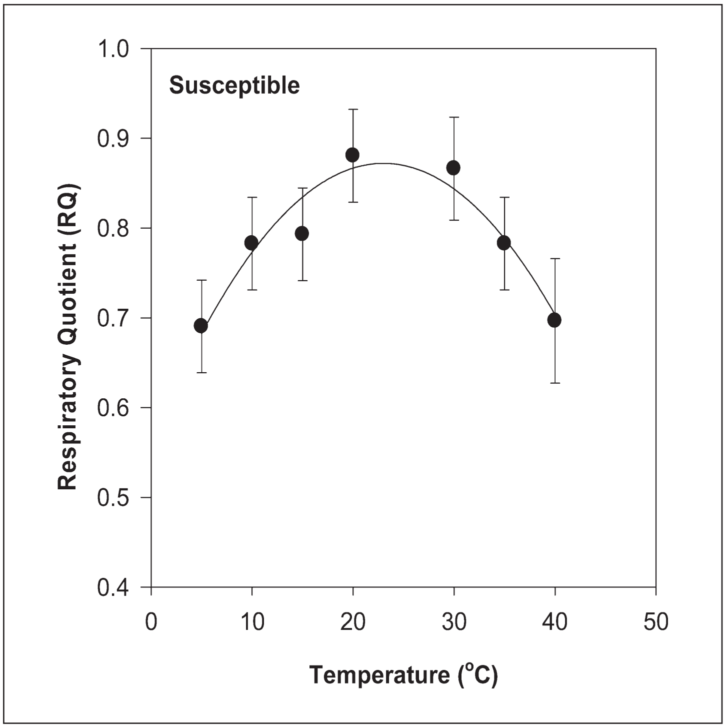

Figure 4. Relationship between temperature and RQ in bait susceptible German cockroaches.

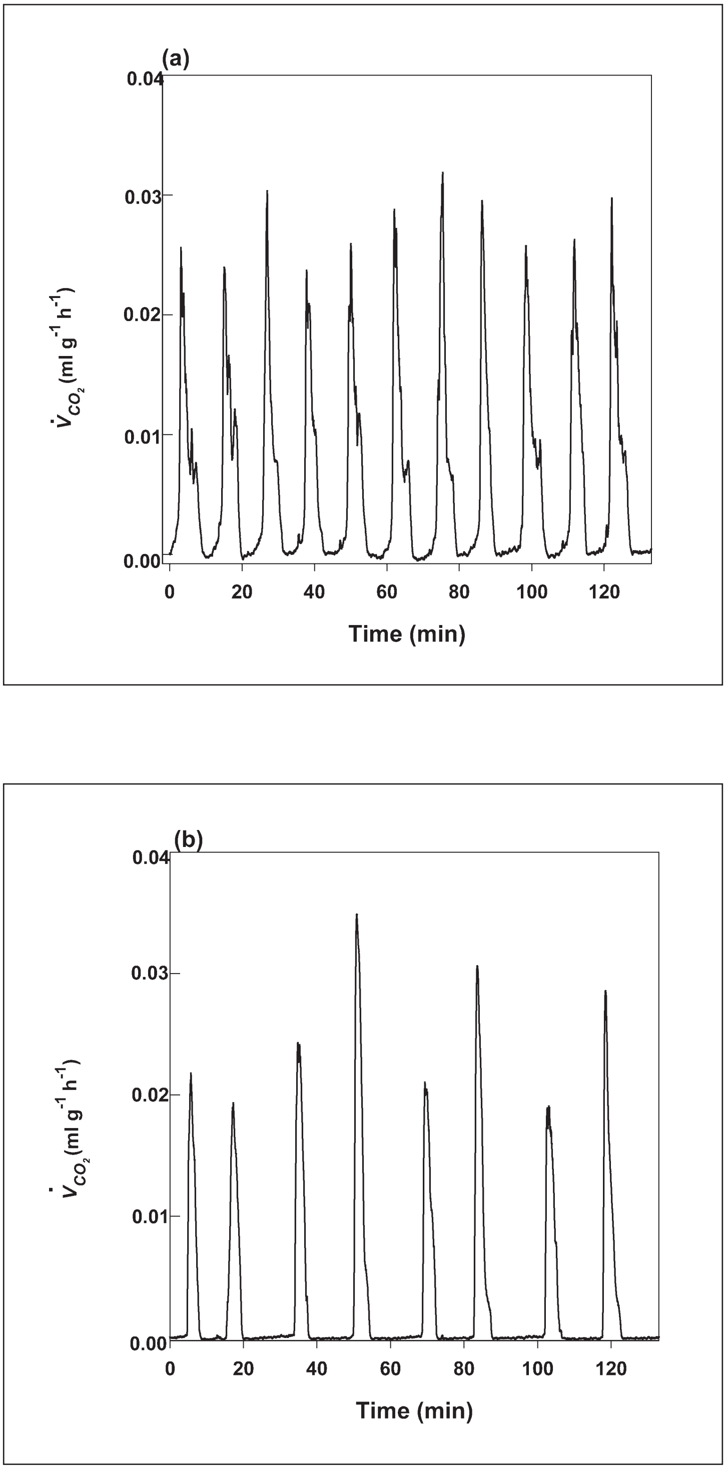

Figure 5. Typical recording of DGC in (a) resistant and (b) susceptible German cockroaches measured over an hour at 10˚C.

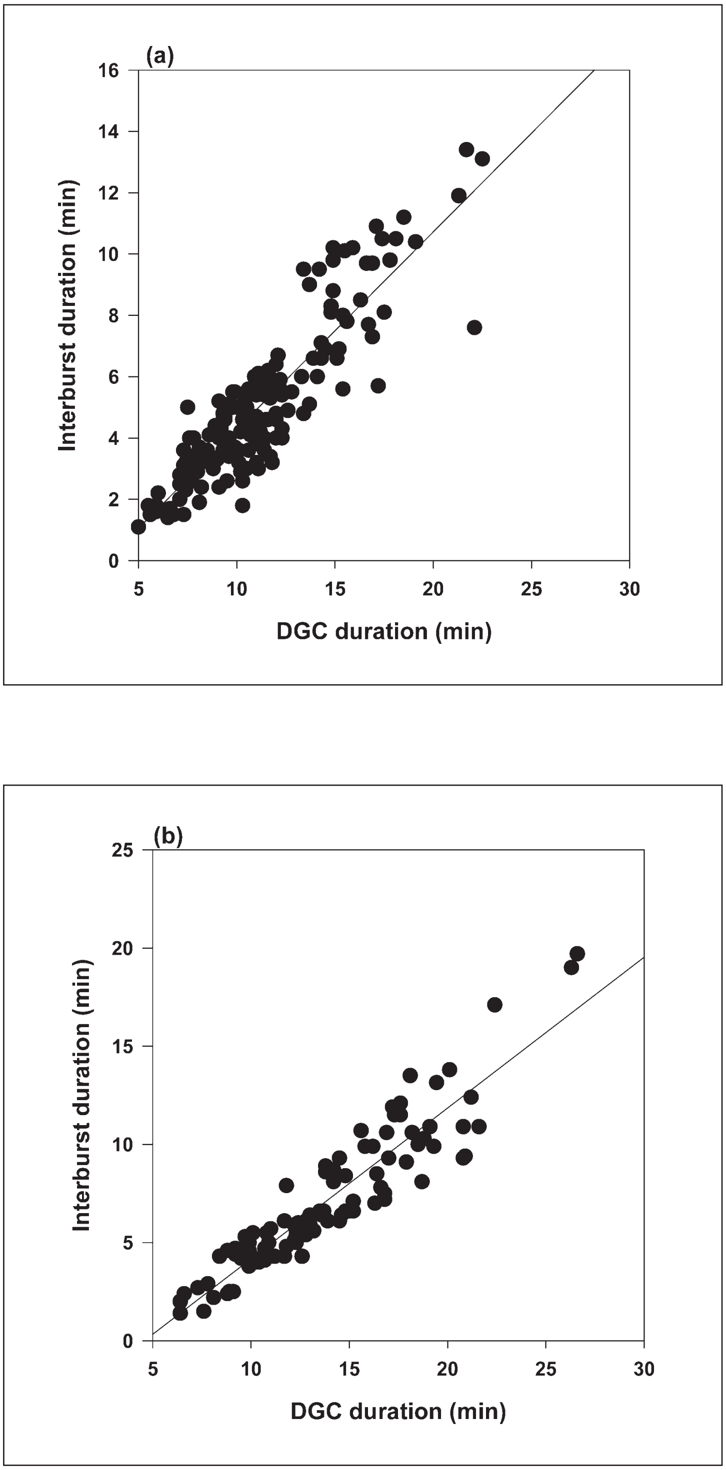

Figure 6. The relationship between overall DGC duration and closed-flutter phase duration in bait averse and pyrethroid resistant (a) and susceptible (b) German cockroaches. N=10 adult males, with 8-10 DGCs per individual.

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