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Efficacy of modified diatomaceous earth on different cockroach species (Orthoptera, Blattellidae) and silverfish (Thysanura, Lepismatidae)

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Abstract Defined populations of American (*Periplaneta americana*), German (*Blattella germanica*), and Oriental (*Blatta orientalis*) cockroaches, and silverfish (*Lepisma saccharina*) were observed after exposure to deposits (25 g/m^2) of a new 1,1,1–trimethyl-N-trimethylsilane-modified, highly hydrophobic diatomaceous earth (DE) formulation by using a computer-aided device measuring motility, circadian rhythm, and mortality under defined environmental and climatic field-simulating and exposure-enforced conditions. In a humid climate (85% relative humidity) with water and food offered *ad libitum*, complete population eradication could be achieved on the sixth day against *B. germanica*, on the eighth day against *P. americana*, and on the ninth day against *L. saccharina*, respectively. No population eradication occurred within 10 days of exposure when testing *B. orientalis*, showing a mean survival rate of 29.4 ± 6.7 % of the populations. When comparing the species-specific mortality rates with the results obtained from corresponding reference control groups, significantly higher mortality rates could be observed in *B. germanica* (F = 66; df = 52; P < 0.00001), *P. americana* (F = 344; df = 66; P < 0.00001), *L. saccharina* (F = 253; df = 24; P < 0.00001), and *B. orientalis* (F = 422; df = 11; P < 0.00001). Overall, the efficacy of the hydrophobised DE examined ranked as follows: *B. germanica* > *P. americana* (F = 51; df = 24; P < 0.00001) > *L. saccharina* (F = 43; df = 24; P < 0.00001) > > *B. orientalis* (F = 9; df = 15; P < 0.000001). DE exposure resulted in complete disruption of the circadian activity in *B. germanica* and *P. americana*, but not when tested against *B. orientalis*, where the species-specific circadian motility peak was still preserved at lower levels after 10 days of exposure. In contrast to the cockroach species examined, no specific circadian rhythm could be measured in the *L. saccharina* control and treatment groups. Results indicate that hydrophobised DE originating from freshwater diatoms modified with 1,1,1-trimethyl-*N*-trimethylsilane can be successfully used for the control of infestations with German and American cockroaches as well as silverfish, but not against Oriental cockroaches. It is concluded that species-specific morphological, physiological and behavioural characteristics of insects influencing DE efficacy as well as the toxicological risk of modified DE to humans deserve further investigation.

Keywords *Blatta orientalis* · *Blattella germanica* · Diatomaceous earth · Efficacy · *Lepisma saccharina* · *Periplaneta americana*

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Introduction

Although used for stored product pest control for more than 5,000 years, the first detailed research work on diatomaceous earth (DE) began in the early 1930s (Polivka 1931; Zacher and Kunike 1931). Primarily marine DE was investigated against the granary weevil, *Sitophilus granarius* (L.), the rice weevil, *S. oryzae* (L.), the bean weevil, *Acanthoscelides obtectus* (Say), and other stored product pests (Zacher and Kunike 1931; Chiu 1939). Nowadays, modified and non-modified DEs are often widely used for integrated cockroach control by professional pest control operators (Quarles 1992; Swadener 1995). Initial attempts to use non-modified DEs for the control of gastrointestinal nematodes and populations of the horn fly *Haematobia irritans* (L.) in cattle were not successful (Lartigue et al. 2004).

The mode of action of DE is generally accepted as a desiccation effect on the insects (Ebeling 1971; Mewis and Reichmuth 1998; Faulde et al. 2006). Insect toxicity primarily depends on its physical properties (Ebeling 1971; Subramanyam and Roesli 2000). Different insecticidal mechanisms have been proposed, including abrasion of the cuticle (Fields 1998), absorption of cuticular waxes from the epicuticle surface (Ebeling 1971; Mewis and Ulrichs 1999; Prasantha 2003), damage to the digestive tract (Smith 1969), blockage of the spiracles and tracheae (Webb 1945), and surface enlargement combined with dehydration (Zacher and Kunike 1931). Recent work revealed that effective absorption of epicuticular lipids and fatty acids is the primary mode of action of DE, leading to desiccation in arthropods (Fields 1998; Mewis and Ulrichs 1999; Prasantha 2003). Additionally, increasing the oil-binding capacity by modification of the surface of the DE particles may increase efficacy (Faulde et al. 2006).

Complete, short-term eradication of populations of medically important cockroach species can be necessary, e.g. for the interruption of transmission chains of communicable, mechanically transmitted disease agents. Under laboratory conditions, deposits of DE have been found to be lethal to the German cockroach, *Blattella germanica* (L.) (Eastin and Burden 1960; Le Patourel and Zhou 1990). Under wet and humid conditions above 81% relative humidity (r.h.), non-modified DEs produce reduced toxic action due to adsorption of water vapour from the surrounding air (Le Patourel and Zhou 1990; Arthur 2000; Prasantha 2003). The toxicity of silica deposits in partially treated choice boxes varied with r.h. and was shown to be effectively eliminated at 95% r.h., or when free water was provided (Le Patourel and Zhou 1990). Successful eradication of an entire *B. germanica* population in an infested area by a single application of DE even under 85% r.h. can only be achieved, when using DE modified by highly hydrophobic silanes. This results in a marked increase in the hydrophobicity of the particle surface and oil-binding capacity while simultaneously reducing water vapour adsorption (Faulde et al. 2006).

Depending on the diatom group (marine or freshwater diatoms), geographical origin, formulation process, oil absorption capacity, and chemical/mechanical modification of DEs, the effectiveness for killing and controlling insect populations can vary significantly depending upon test conditions used (Tarshis 1959; Le Patourel and Zhou 1990; Quarles 1992; Faulde et al. 2006). Control efficacy of DE products may vary greatly depending upon the type of DE and the species being tested (Tarshis 1959; Le Patourel 1996; Prasantha 2003). Therefore, the use of standardised methods under simulated field conditions is necessary to evaluate the effectiveness of DEs as pest control agents.

The purpose of the study described here is to anlayse the toxic and behavioural effects of a single, mechanically modified freshwater DE with a high oil-binding capacity and potentially high insecticidal efficacy under standardised field-simulating conditions against populations of the German cockroach, *Blattella germanica* (L.), the Oriental cockroach, *Blatta orientalis* (L.), the American cockroach, *Periplaneta americana* (L.), and the silverfish, *Lepisma saccharina* (L.). Computer-operated measuring devices were used to monitor motility, circadian rhythm, and control efficacy under defined environmental conditions.

Materials and methods

Test animals

Blattella germanica, *B. orientalis*, *P. americana*, and *L. saccharina* from continuously (≥ 30 years) maintained colonies were reared in glass basins at 28°C and 70% r.h. under a 12-h photoperiod (light-on, 0700–1900 hours). The cockroach species and silverfish were fed on a standard diet consisting of 60% wheat flour, 18% oat flakes, 11% sugar, 8.4% milk powder, and 2.6% egg white. Food and water was offered *ad libitum*. Silverfish glass basins additionally contained two layers of peat plates (10 × 1 cm) which are kept wet continuously in order to increase the humidity in the basin to 80% r.h.

Diatomaceous earth tested

Fossil-Shield 90.0 S White (FS 90.0 SW) was purchased from Bein GmbH (Eiterfeld, Germany) consisting of freshwater diatoms originating from Middle Spain. Hydrophobicity has been highly increased by treatment with 3% Aerosil® treated with 1,1,1-trimethyl-*N*-trimethylsilane. This DE was characterized by a cristobalite content of 0.35% (w/v), a particle size peaking at 5 µm, a pH value of 8.5, an oil absorption capacity of 130 ml/100 g, and a specific surface value of 10 m^2/g.

Automated measurement of motility

The motility and circadian activities were determined with a population of 100 *B. germanica* (25 males, 25 females, 50 nymphs (all stages), 100 *B. orientalis* (25 males, 25 females, 50 nymphs (all stages), 100 *P. americana* (25 males, 25 females, 50 nymphs (all stages), and 100 adult *L. saccharina*. Three locations for measurement were established: one containing the refuge, one in which food was presented *ad libitum*, and one in which water was offered *ad libitum*. These locations were part of a computer-operated monitoring system described by Fuchs and Sann (1981) and Faulde et al. (2006), and were located at the bottom of an air-conditioned chamber. The motility of the test animals were measured by infrared light barriers at a wave length of 900 nm. Twenty five light barriers, having a working distance from each other of 1.2 mm, were combined into a single sensor unit able to detect every object > 1.2 mm in size. The refuge consisted of a black plastic box (45 cm length, 24 cm width, 25 cm height), containing a wooden cube (11×11×11 cm with 16 holes, each 2 cm in diameter) with double sensor units serving as entrances at two sides. Food and water were located in secluded areas of 10×10 cm with four sensor units serving as entrances, one located at each of the four edges as described elsewhere (Faulde et al. 2006). All experiments were carried out at 21°C, 85% r.h., under a preadapted 12-h photoperiod (lights-on 0700–19 00hours). The motility of the test animals was continually recorded as counts of passing at all three monitoring locations. The number of dead test animals within the test chamber was counted daily at 1100 hours.

Effects of DE were tested by homogeneously applying 25 g DE powder per m^2 on the bottom surface of the air-conditioned test chamber. The interior of the sampling sites was not treated. Reference controls were carried out using standard conditions without DE or insecticidal activity. All tests were run continuously for 240 h and were repeated five times.

Statistical evaluation

Statistical analyses were performed using the Statistica for Windows 7.1 program (Statsoft Inc., Germany, http://www.statsoft.com). DE exposition and reference control tests were replicated five times. Values are reported as mean ± standard deviation (SD). The species-specific influence of DE was tested by means of one-way analysis of variance. Two-way analysis of variance was applied to compare the independent variables DE, and test animal species. The Newman–Keuls test was used for post-hoc comparisons. Differences were regarded as significant for $p < 0.01$. The dichotomic response variable "failure rate" was analysed by simple (factor "DE") and multiple binary logistic regression (factor "test animal species").

Results

The lethal effect on the test animal species exposed over a period of 240 h to the modified DE formulation was measured and is depicted in Fig. 1. A 100% kill effect could be achieved on the sixth day against *B. germanica*, the eighth day against *P. americana*, and the ninth day against *L. saccharina*, respectively. Population eradication could not be achieved within 10 days of exposure when testing *B. orientalis* populations, showing a mean survival rate of 29.4 ± 6.7 % within the five test replications. When comparing the species-specific mortality rates with the results obtained from the corresponding reference control groups, significant higher mortality rates could be analysed in *B. germanica* ($F = 66$; $df = 52$; $P < 0.00001$), *P. americana* ($F = 344$; $df = 66$; $P < 0.00001$), *L. saccharina* ($F = 253$; $df = 24$; $P < 0.00001$), and *B. orientalis* ($F = 422$; $df = 11$; $P < 0.00001$). Overall, the efficacy of the hydophobized DE examined ranked as follows: *B. germanica* > *P. americana* ($F = 51$; $df = 24$; $P < 0.00001$) > *L. saccharina* ($F = 43$; $df = 24$; $P < 0.00001$) > > *B. orientalis* ($F = 9$; $df = 15$; $P < 0.000001$). *P. americana* showed a lower mortality rate on the first day of DE exposure, when compared to *B. germanica* and *L. saccharina* results, but increased sharply after the second day of exposure.

The overall daily motility counts of *B. germanica*, *P. americana*, and *B. orientalis* populations exposed to the DE and their corresponding reference control groups are depicted in Fig. 2. The overall daily motility activities in DE-exposed and not-exposed cockroach populations analysed ranked as follows: *B. orientalis* negative control > *P. americana* negative control ($F = 32$; $df = 69$; $P < 0.000001$) *B. germanica* negative control ($F = 11$; $df = 7$; $P < 0.067$) > *B. orientalis* exposed to DE ($F = 24$; $df = 37$; $P < 0.000001$) > *P. americana* exposed to DE ($F = 16$; $df = 51$; $P < 0.000001$) > *B. germanica* exposed to DE ($F = 31$; $df = 44$; $P < 0.000001$). Due to the limited overall motility activity of Thysanura, *L. saccharina* motility counts of DE treated population versus the reference control group are shown in a separate Fig. 3. When compared with the control, a statistically significant reduction in motility beginning on the first day of exposure, was measured in *B. germanica* ($F = 105$; $df = 55$; $P < 0.00001$), *P. americana* ($F = 9$; $df = 74$; $P < 0.000001$), and *B. orientalis* ($F = 42$; $df = 66$; $P < 0.00001$), respectively, when exposed to DE. All DE-exposed cockroach species decreased their overall activity rapidly after the first day of exposure. At the beginning, German, Oriental and American cockroaches were mainly found in the refuge area, although no water and food was provided therein, obviously avoiding body contact to DE. DE-exposed cockroaches aggregated when reaching the monitoring station containing water *ad libutum*.

In *L. saccharina*, DE-exposure increased overall motility activity during the first three days when compared to the reference group (day one: 497 ± 111 vs. 351 ± 92; day two: 409 ± 117 vs. 295 ± 48; day three: 337 ± 121 vs. 297 ± 36). Daily overall motility activity of the DE-exposed population reached statistically significantly ($F = 26$; $df = 80$; $P < 0.0027$) lower motility levels than the reference group on day seven, when the mean mortality rate was 96.8 ± 1.9% (DE-treated) vs. 5 ± 0.7% (control group).

The species-specific circadian rhythms, in conjunction with the preferred areas of motility, of *B. germanica*, *P. americana*, *B. orientalis*, and *L. saccharina* exposed to DE, and their corresponding reference control groups, are shown in Fig. 4. The naturally occurring species-specific circadian rhythms of the cockroach control groups showed peaking activities between 1900 and 0700 hours (lights-off period) for *B. germanica* (Fig. 4a), a

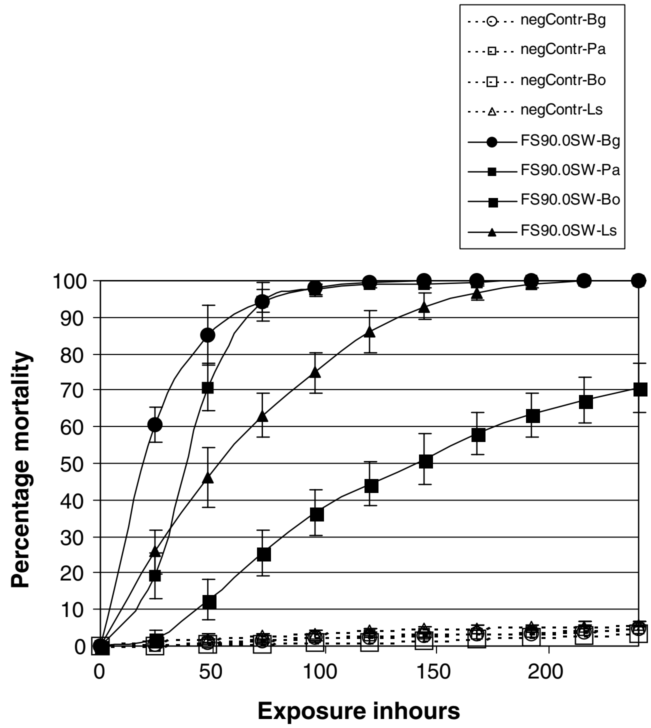

Fig. 1 Percentage mortality rate in populations of 100 *B. germanica* (Bg), 100 *P. americana* (Pa), 100 *B. orientalis* (Bo), and 100 *L. saccharina* (Ls) exposed to modified diatomaceous earth and corresponding not exposed reference controls ($n = 5$, mean ± SD) at 21°C, 85% r.h

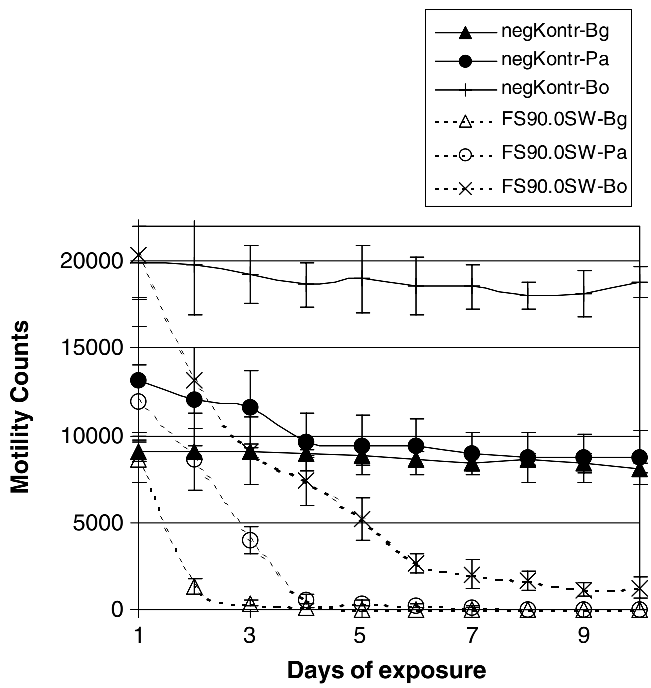

Fig. 2 Overall daily motility activities in populations of 100 *B. germanica*, 100 *P. americana*, and 100 *B. orientalis* exposed to modified diatomaceous earth as well as in corresponding not-exposed reference controls determined by summarizing the motility counts at the refuge and feeding areas at 21°C and 85% r.h. ($n = 5$; mean ± SD)

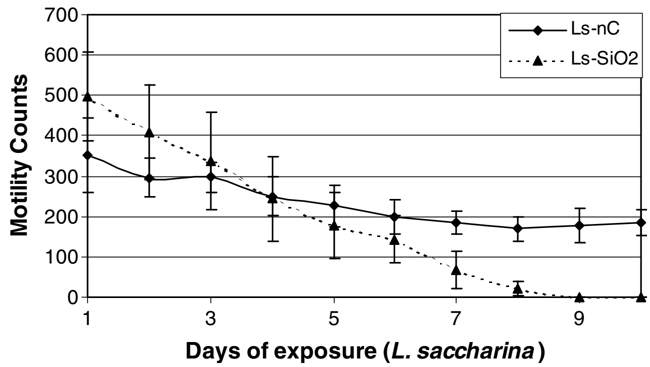

Fig. 3 Overall daily motility activities of 100 *L. saccharina*, exposed to modified diatomaceous earth (Ls-SiO_2) as well as in corresponding not-exposed reference controls (Ls-nC) determined by summarizing the motility counts at the refuge and feeding areas at 21°C and 85% r.h. ($n = 5$; mean ± SD)

predominant peaking activitiy at 2300 hours for *P. americana* (Fig. 4c), and sharp activity peak at 2400 hours for *B. orientalis* (Fig. 4e), respectively. In contrast to the cockroach species examined, no specific circadian rhythm could be found in the *L. saccharina* control (Fig. 4g) and treatment (Fig. 4h) groups. When exposed to DE, motility of *B. germanica* (Fig. 4b), *P. americana* (Fig. 4d), and *B. orientalis* (Fig. 4f) usually increased first at the refuge area at the beginning of the daily activity phase when cockroaches aggregated at the entrance before leaving the refuge area. Thereafter, all three cockroach species began searching for accessible food and water sources with *B. germanica* preferring the monitoring station where food or water has been provided, and *P. americana* and *B. orientalis* primarily choosing the area, where water was accessible. No preference to a monitoring station could be found in *L. saccharina*.

Exposure to modified DE resulted in complete disruption of the circadian activity in *B. germanica* (Fig. 4b) and *P. americana* (4d), but not when tested against *B. orientalis* (4f), where the species-specific activity peak still occurred at low motility levels after 10 days of exposure. The aggregation of *B. germanica* and *P. americana* at the monitoring stations where food and water was offered, resulted in a higher motility actitivity at these monitoring stations until eradication of the population. In Oriental cockroaches, daily motility maxima always occurred at the monitoring station where water has been provided *at libitum*, indicating that *B. orientalis* actively tried to compensate for their water loss resulting from DE exposure. Single, non-circadian rhythm-dependent motility peaks of *L. saccharina* (Fig. 4h) occurred at all three monitoring stations, showing no preferences. Interestingly, *L. saccharina* avoided contact to the DE dusted surfaces and stayed inside the untreated infrared light barriers until forced to leave this shelter, while suffering from starvation or thirst.

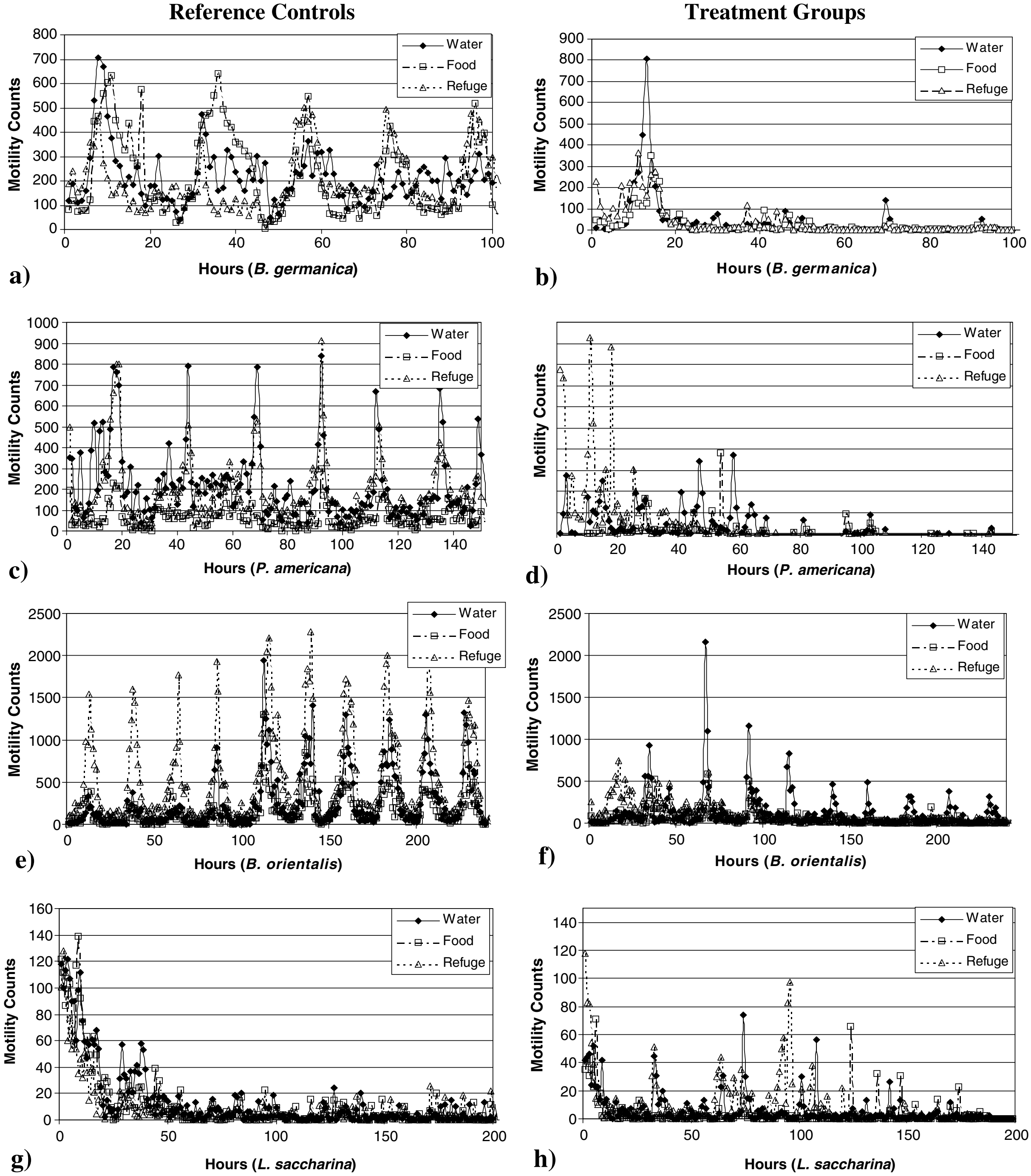

Fig. 4 Circadian activity and hourly measured motility counts at the refuge, the food, and the water location in: **a** *B. germanica* control; **b** *B. germanica* DE-exposed; **c** *P. americana* control; **d** *P. americana* DE-exposed; **e** *B. orientalis* control; **f** *B. orientalis* DE-exposed; **g** *L. saccharina* control; **h** *L. saccharina* DE-exposed at 21°C and 85% r.h., 12-h photoperiod (lights-on 0007–1900 hours)

Discussion

With increasing legislative restrictions being implemented concerning the use of pesticides, safer, but efficient alternatives must be developed to allow least-toxic but most efficient means of integrated pest control (WHO 1974). DE formulations with a cristobalite content of less than 1% are considered as safe (Anonymous 2000; Merget et al. 2002), and increase of the oil-binding capacity by strongly hydrophobic modifications, especially of natural DE originating from freshwater diatoms, are shown to significantly enhance efficacy against German cockroaches even under humid conditions (Faulde et al. 2006). Although widely used against stored product pests, the efficacy of DEs in controlling hygiene pests, especially cockroaches, is still controversial (Le Patourel and Zhou 1990; Swadener 1995; Le Patourel and Zhou 1990; Faulde et al. 2006). Since May 2004, a mass infestation with *B. orientalis* has occurred in the Vechta area in Germany, affecting more than 300 agricultural production plants and stables as well as more than 1,300 homes (Freise 2005). Implemented integrated pest management strategies, which, besides the use of insecticidal formulations, included massive use of non-modified DEs, obviously showed no sufficient control success during 2005. These findings can be partly explained by the results obtained in this study which reveal that, even under enforced exposure to modified and highly hydrophobic DE, no complete control of *B. orientalis* could be achieved under simulated field conditions. Unlike with *B. germanica* and *P. americana*, high oil-binding DEs are not efficient means for the control of *B. orientalis* infestations, even when using a comparable high DE dosage of 25 g/m^2.

Nevertheless, complete population eradication could be achieved after an exposure time between 5 and 8 days in *B. germanica*, *P. americana*, and *L. saccharina* even under humid environmental conditions. This indicates that modified DE is a least-toxic but effective alternative for the control of these insect pests. Results show that cockroach species may differ significantly in their susceptibility and behaviour against DE. It was shown only recently that various DE formulations and modifications may also differ markedly in their blatticidal activity (Faulde et al. 2006). Extrapolation of test results from one DE product to different pest species, as well as from various DE formulations to single species should be avoided, unless specifically tested under standardized and field-simulating conditions.

The increase in insect mortality when using hydrophobised DE, compared to common DE, especially at high humidity levels is due to the higher absorption capacity of cuticle waxes, resulting in death by dessication (Mewis and Ulrichs 1999, Faulde et al. 2006). German and American cockroaches as well as silverfish are obviously unable to compensate for this desiccation effect even at high r.h. and unlimited access to water when exposed to strongly hydrophobised DE. This effect is not necessarily linked to the size and body weight among the different insect orders, since *L. saccharina* was more resistant to DE and desiccation than *P. americana*. Additionally, species-specific physiological resistances to dry environmental conditions should be taken into consideration, as the silverfish is known to contain a relatively high body fat concentration between 20 and 25% (Eidmann and Kuehlhorn 1970), enabling this species to reduce water consumption through metabolism of body fat. The high DE resistance of *B. orientalis* differed markedly when compared with *B. germanica* and *P. americana* because (a) approximately 30% of the *B. orientalis* population survived 10 days of enforced DE exposure, (b) the species-specific circadian rhythm was still intact, and (c) the by far highest daily activities were always found at the monitoring station where an unlimited water source was provided. Whether the reasons for these results are based on morphological (e.g. due to the "oily" surface of *B. orientalis*), physiological (e.g. metabolic water production), or behavioural (e.g. avoiding DE dust, active search for water sources, learning effects) remain unclear and await further investigation. An excessive necrophagic behaviour was observed in all three cockroach species investigated.

Investigations on the circadian system of hemimetabolous insects showed that the compound eye acts as the major photoreceptor and the optic lobe was identified as circadian clock locus (Tomioka and Salaheldin 2004). In cockroaches, where a species-specific circadian rhythm is known to occur, the lobula was previously thought to be the most likely clock locus but the accessory medulla has recently been theorized as the clock centre (Tomioka and Salaheldin 2004). The mechanism, how DE-exposure suppresses the circadian clock in German and American cockroaches, and not in Oriental cockroaches, remains unclear. Lepismatidae are supposed to miss a circadian rhythm since an optic lobe is morphologically absent and the compound eye is usually developed rudimentary within this insect family (Eidmann and Kuehlhorn 1970). No other circadian clock or multioscillatory organisation is known to occur in *L. saccharina*.

From the occupational health point of view, it remains unclear as to what extent newly developed DEs, coated, derived, or mixed with different chemical compounds resulting in modified surface characteristics, could potentially produce adverse health effects in humans. Results presented herein clearly show, that DE originating from freshwater diatoms modified by mechanical coating with 1,1,1-trimethyl-*N*-trimethylsilane-treated Aerosil® can be successfully used for the control of infestations with German and American cockroaches as well as with silverfish, but not against Oriental cockroaches. Finally, it is concluded that morphological, physiological and behavioural characteristics of insect species influencing DE efficacy as well as the toxicological risks of modified DE to humans deserve further investigation.

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