LABORATORY STUDIES ON THE CONTROL

OF

THE GERMAN COCKROACH

Blattella germanica (L.)

By

ZHOU JING-JIANG

B.Sc.(Peking University, China)

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Imperial College of Science, Technology and Medicine

Department of Pure and Applied Biology
Imperial College of Science, Technology and Medicine
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United Kingdom

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Dedicated to my beloved wife, Xiao-Li and to my beloved daughter as well as to our parants without whose support and encouragement this would have been impossible to achieve, and for their enduring patience, love and courage during my absence form home while studying in the United Kingdom.

ABSTRACT

The females *B. germanica* separated from males after first mating produced fewer oothecae than the females caged with males throughout lifetime, but number of eggs, hatch rate and female longevity were not significantly different. Population density did not affect nymphal development rate, density-dependent mortality appeared to be largely due to cannibalism during or immediately after moulting, and there was a linear dependence of final adult density on initial nymphal density.

The effect of a domestic fluorescent light on the resting behaviour of the cockroaches was examined using a 2 chamber arena. Bioassay conditions (absolute light intensity, light intensity difference between chambers, illumination period prior to being given access to shaded chamber, change of photocycle before bioassay, extent of CO_2 anaesthetisation, and division into "dark-preferring" and "light-preferring" groups) had little effect on resting in the shaded arena, and although the cockroaches showed a significant preference for the shaded arena over a number of trials, the effect was not always evident in individual replicates.

Cockroaches allowed to the treated shaded chamber with sub-lethal dose of permenthrin WP following recovery in the untreated subsequently illuminated chamber showed a preference for the illuminated chamber. Cockroaches which pre-exposed to permethrin WP deposits at doses

ranging from 1 to 6.4 mg/m² and recovered in the subsequently illuminated chamber showed a tendency to remain in the illuminated chamber which increased with permethrin deposit concentration. The results obtained with a linear three chmaber arena in which cockroaches were exposed to permethrin WP for 10 min in the centre chamber prior to given access to two escape chambers showed no clear trend towards a greater or a lesser light avoidance reaction with increasing permethrin concentration, there being evidence for a reversal of the normal photophobic response at some concentrations but not at others.

Females poisoned with 1.65% w/w hydramethylnon baits retained their oothecae at death, while a considerable proportion of those killed by boric acid baits deposited their oothecae prematurely at 90% r.h. and either 20°C or 28°C. Oothecae from both 7-14 and 14-21 day old females and poisoned with hydramethylnon showed reduced hatch relative to controls at 20° but not at 28° C, while oothecae from both groups of females poisoned with boric acid showed reduced hatch at both temperatures, and more oothecae retained by the female hatched than those prematurely deposited. The results together with those from experiments examining the % hatch of oothecae either manually removed from females or allowed to remain in the genital pouch until hatch suggested that for neither compound was transfer of toxicant from female to oothecae an important factor in controlling oothecal hatch.

Adult males were exposed to deposits of amorphous silicas in continuous and discontinuous contact bioassays. Homogeneous bioassay results were generally obtained in the continuous bioassays and the LC50's of silicas generally increased with oil adsorption capacity of the dusts. Discontinuous bioassays (1.5-60 min) showed greater variability due to removal of the deposit from body and appendages by cockroaches. Pick-up of 3 dusts by cockroaches in small arenas over a 3 h period was also related to their relative toxicities. The toxicity of silica dusts in half-treated choice boxes under red light varied with relative humidity and was effectively eliminated at 95% r.h. or when free water was provided in the arenas. Cockroaches tended to accumulate in the untreated side of the box within 1 h of being introduced into the arena.

The relative toxicity (24 h) of a number of pyrethroid insecticides to male adults was determined when the compounds were formulated either on one toxic silicas or on talc, using a discontinuous contact bioassay with 3 min exposure. The silica-based formulations were appreciably more toxic than the talc-based ones at a dust concentration of 1 g/m^2. The suitability of silica-based dusts for cockroach control is discussed.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 The German cockroach as a pest species

Cockroaches are an ancient group of insects with origins dating back to the Carboniferous period, at which time they appear from the fossil record to have been extremely abundant. The majority of modern species are tropical or sub-tropical and have no known association with man or his dwellings, but seven species are frequently found in buildings and are regarded as important pests and a number of others have a looser association with domestic environments and acquire pest status on a more irregular basis.

The German cockroach *Blattella germanica* (Linnaeus) is regarded as originating in N.E. Africa between the great African lakes, Ethiopia and Sudan (Cornwell, 1968) and to have been transported to eastern Europe along trade routes which took it into Asia Minor, the Black Sea region and southern Russia, and from thence into western Europe. It is supposed to have arrived in England comparatively recently in bread baskets carried by soldiers returning from the Crimean war (Miall and Denny, cited in Cornwell, 1968). It was recorded as a pest of houses in Australia by 1893, became established in North America during the early part of this century, and is now regarded as a truly

cosmopolitan pest species, capable of flourishing in any building which is maintained at a temperature between approximately 24° and 33° C and where there is free food and water available.

1.2 Cockroaches as passive vectors of disease

Any insect which is synanthropic with man and has omnivorous eating habits has the potential to act as a reservoir and passive vector of bacteria and other microorganisms; however, the major pest species of cockroach have a prediliction for harbouring or feeding in precisely those environments (sewers, drains, rubbish and detritis) which make them most likely to accumulate disease organisms in their gut and on their appendages and thus to contaminate their environment when exploring and foraging for food and water. While the presence of a wide variety of microorganisms (bacterial, viruses, protozoa) has been demonstrated in the gut and on the bodies of cockroaches taken from such environments it has been difficult to demonstrate unequivocally that disease transmission by the insects has occurred in particular instances, although a number of examples are known of the non-specific involvement of cockroaches in outbreaks of human disease, particularly of the excrement group. A large body of circumstantial evidence points to the insect as an efficient carrier and an important contributory factor in diseases transmission (Cornwell, 1968; Burgess, 1984).

In addition to their role as disease carriers, cockroaches also present a potentially serious health risk to allergic individuals living in roach- infested environments. Studies by Richman *et al.* (1984) have shown that whole bodies and cast skins of German cockroaches contain potent allergens, with faeces and egg cases as contributory sources. Cast skins frequently disintegrate and become aerosolized in disturbed surroundings. Stankus and O'Neil (1988) showed that 48% of a group of predominantly inner-city subjects attending an adult allergy clinic in New Orleans had immediate cutaneous reactivity to crude American cockroach extracts, and Morris *et al.* (1986) in a study of 45 inner-city children with perennial rhinitis and/or asthma found that 29% who were skin test negative to house dust were positive to cockroach extract.

1.3 Control measures:

The general public is not as aware of the health dangers of cockroaches as they are of flies (which have a similar capacity to act as passive disease vectors) possibly because of their nocturnal habit and lack of visibility (Wood *et al.* 1981). Nevertheless there is a general dislike of cockroach infestations in domestic and commercial environments even though this may vary considerably from person to person. Zungoli and

Robinson (1984) attempted to define and measure an "aesthetic injury level" (comparable to the economic threshold levels of crop pests) for cockroach infestations in American cities but found it quite variable, the larger the infestation the greater the tolerance to some level of pest infestation. A recent survey by Robinson and Bao (1988) in Hangzhou, China revealed almost identical knowledge of and attitudes to cockroaches as in the American study. It seems likely that as standards of hygiene increase in any community tolerance of pests will decrease, but that pest control programmes need to be designed to meet specific attitudes and requirements which may vary widely from one group to another.

Both incidence of cockroach infestations in domestic environments and successful control have a high correlation with indices of cleanliness, sanitation and domestic clutter (Bennett, 1978; Schal, 1988) and much can be done in most situations where infestations occur to deprive cockroaches of ready access to harbourages, food sources and water. Such methods are long term, and in some situations impracticable, as they would involve major structural changes (buildings rarely being designed with future infestation in mind). Conversely, the use of insecticides provides a rapid and often highly visible means of killing cockroaches. The pest control industry has thus traditionally relied on fast-acting neurotoxic insecticides applied at regular intervals as residual deposits to surfaces and into harbourages as the principal

component of cockroach control measures, and it is only recently, with increased concern about the safety of such measures in domestic or hospital environments together with the increase in resistance to particular products, that the use of alternative strategies of control and novel types of chemical action have been seriously investigated. Developments in this area have been greatly stimulated by the present and projected increases in the market for cockroach control products, particularly in the USA. Thus Robinson and Zungoli (1985) discussed the development of integrated control programmes for German cockroach control in multiple-unit dwellings which incorporated surveys of attitudes among tennants, resistance levels to insecticides in the pest population, an education programme for the tennants, a training programme for management and pest control personnel, specific control programmes involving more than one type of pesticide and application method, and a post-treatment evaluation of the programme.

1.3.1 Residual deposits of neuroactive insecticides

Since the initial development of the synthetic organic insecticides acting on the insect nervous system during the 1940's cockroach control has been largely a process of applying residual deposits of organochlorine, carbamate, organophosphorus, or pyrethroid insecticides to foci of infestation in buildings, supplemented on occasions by the

use of fogging aerosols of non-persistent products such as allethrin or dichlorvos. Table 1.1 shows the principal insecticides used for cockroach control, although not all these products are approved for use in all countries. When a toxic dose of such insecticides is picked up, cockroaches show characteristic symptoms of hyperactivity (Carle, 1982; Hutzel, 1942) followed by knockdown in which the insect is incapable of coordinated movement and normally on its back, and death usually within 12-24 h (Cornwell, 1976; Chadwick, 1979).

There have been three major drawbacks to this approach to the control of *B. germanica*: firstly the rapid generation time of the species has led to the development of resistance to particular products by mechanisms which make the selected populations cross-resistant to other compounds in the same, and sometimes other, chemical groups; secondly, a characteristic action of acutely neurotoxic insecticides on female *B. germanica* carrying an ootheca in their genital pouch is to make them drop the ootheca prematurely, possibly in an inaccessible harbourage. Provided they are beyond a critical period of development, a large proportion of such oothecae subsequently hatch (Barson and Renn, 1983), providing a new focus of infestation and a reason to call the pest control operator back for a subsequent treatment cycle.

1.3.2 Resistance development

Resistance to chemical insecticides in pest populations is an aspect of the evolutionary process by which organisms adapt to changing environments through a selection process. The acquisition of resistance occurs through the survival of particular insects in an insecticide-treated environment as a result of the possession of a particular gene (through normal random mutation processes) which confers greater fitness in that environment. There is no evidence to suggest that insecticides cause mutations which result in such fitness. Following survival, the normal rules of Mendelian inheritance govern the transmission of the fitness gene within the population. The rapid generation time and frequent occurrence of isolated populations of *B. germanica* both aid the rapid increase in resistance levels in populations subjected to intense selection pressure from insecticide application.

The extent of resistance to conventional insecticides in *B. germanica* is difficult to assess since, although it has been extensively documented in the USA (Grayson, 1966), and to a lesser extent in the UK (Green *et al.*, 1961; Barson and McCheyne, 1978) and Denmark (Keiding, 1964), the lack of reports from other localities may reflect inadequate monitoring rather than continued success with particular products. *B. germanica* is a rapid breeder, and when heavy selection pressure is

applied with insecticides resistance appears to appear quite rapidly, both in laboratory experiments and in the field.

Resistance to chlordane was recognised at a site in Texas in 1951, only a year or two after the introduction of the compound for cockroach control (Heal *et al.*, 1953) and as each successive group of acute neurotoxicants has been introduced resistance has appeared at a local level, reflecting the intensity and the duration of its use. Mechanisms of resistance have been investigated in some strains, and two which are regarded as particularly important are *kdr* (a decrease in sensitivity of the nervous system particularly to pyrethroids) (Scott, 1987), and enhanced microsomal oxidase levels giving broad cross-resistance over a number of different chemical types (Cochran, 1987). Resistance to the carbamate carbaryl in one strain of *B. germanica* was ascribed to a reduced rate of penetration through the cuticle (Ku and Bishop, 1967).

1.3.3 Baiting

The development of concern about the toxic hazard of residual deposits of insecticides in domestic and certain other environments (zoos, animal houses, food preparation and processing areas, aircraft) has led to a renewed interest in the use of baits for cockroach control since

they can be manufactured in the form of sealed containers which are unopenable by children or pets, with small openings accessible only to cockroaches.

There have been a number of investigations into the potential for making bait formulations attractive to cockroaches at a distance by incorporating food components with the insecticide. Tsuji (1985) classified such compounds as volatile olfactory stimulants (such as hexane extracts of bran) or gustatory stimulants (such as sugars). As all the pest species of cockroaches are omnivorous it is not suprising that compounds have yet to be found which are either species-specific (although Adler (1985) claimed that a product called "Dried Distillers Grain" was specifically attractive to the Brown Banded cockroach) or uniquely attractive. Rust and Reierson (1981) reported that successful baiting was more influenced by positioning of bait stations relative to harbourages and cockroach runs than the composition of the formulation, and demonstrated that cockroaches which had food and harbourages available to them were not attracted even short distances to materials used in commercial baits. An alternative approach used in iodofenphos baits was to prepare a dilute gel containing the insecticide and rely on the requirement of *B. germanica* for frequent access to water to serve as a stimulus to sample the material (Cornwell, 1976).

Baiting can be a successful component of a cockroach control strategy,

and current interest in it has stimulated the development of new products based on uncouplers of oxidative phosphorylation which act as delayed toxicants suitable for use in bait stations and which do not limit ingestion because of immediate neurotoxic effects on the gustatory system. Two compounds of this type, hydramethylnon and sulfluramid (Reid *et al.*, 1990), have been marketed in the USA, and one was investigated in the present work to explore the limitations of the baiting approach due to lack of effect on oothecae carried by poisoned adult females.

1.3.4 Integrated strategies

The major methods of using insecticides to control cockroaches each has its own advantages and disadvantages, and there is an obvious case for designing strategies which employ optimised combinations of them for particular infestation situations. Such strategies can be combined with other techniques, such as harbourage limitation, sanitation, education, survey work and infestation monitoring for maximum effectiveness. Integrated control programmes of this type have been described by Robinson and Zungoli (1985) for infested apartment blocks in Norfolk, Virginia (USA) and by Beasley *et al.* (1988) for apartment blocks in Manchester, UK. In both cases, the same approach was adopted: there was an initial monitoring program, which in the

American study included a determination of the insecticide resistance profile, and this was followed by a programme of resident education involving discussion groups and informational literature. The personnel involved in the control programme were given training in the effective application of pesticides, and this was followed by a sequential programme of pesticide application with formulations designed to match the different environments and harbourages within the infested blocks. Infestation monitoring and resident participation was continued for some time after the application programme had finished.

Integrated approaches have obvious advantages over the piece-meal contracts given to many pest control companies, and the opportunities for successful control or eradication have been significantly increased by the recent availability of effective residual juvenoid insecticides which sterilise adult female cockroaches when applied to the last nymphal stage (Staal *et al.*, 1985) and by the delayed-action respiratory inhibitors applied in bait form referred to above.

Use of insecticides of various types and applied in various ways will continue to be the principal method of cockroach control in the forseeable future, and it is therefore important to acquire as much knowledge as possible about their action in relation to the biology and behaviour of pest species in their natural habitat - which is the domestic environment of man. It is hoped that the work described in the present

thesis will be regarded as a small contribution towards this goal.

1.4 Aims of the thesis

Integrated approaches to cockroach control require the limitations and advantages of each component of the strategy to be researched. This thesis describes preliminary work to characterise the strain of *B. germanica* used and to investigate aspects of its development, and then discusses investigations into aspects of the use of three such components: residual pyrethroid insecticides; delayed-toxicity bait formulations; and silica dusts. The detailed aims within each of these areas were as follows:

1. to characterise the productivity and development times of nymphal stages of the colony of *B. germanica* using a culture method in which synchronous populations were reared;

2. to investigate the effect of nymphal crowding on development, with the aim of characterising density-dependent mortality in synchronous populations;

3. to develop a bioassay system with which to identify any changes in the light-avoidance reaction of adult *B. germanica* following treatment

with a residual pyrethroid insecticide;

4. to study the timing and extent of oothecal hatching following treatment of adult females carrying oothecae at different stages of development with two delayed-toxicity bait formulations: hydramethylnon and boric acid;

5. to investigate the response of *B. germanica* to deposits of amorphous silicas, and to design bioassays with which to study the intrinsic toxicity of different types of amorphous silica;

6. to compare the toxicity of residual pyrethroids adsorbed on toxic desiccant silicas and on talc, and to investigate the comparative pick-up of such formulations by *B. germanica* in small arenas.

Table 1.1 Insecticides used for cockroach control

chemical type	insecticide	application
organophosphorus ester	acephate	residual
	chlorpyrifos	"
	diazinon	"
	dichlorvos	vapour phase
	dioxacarb	residual
	fenitrothion	"
	iodofenphos	gel bait
	malathion	residual
	pirimiphos methyl	"
	propetamphos	"
carbamates	bendiocarb	residual
	dioxacarb	"
	propoxur	"
pyrethroids	bioresmethrin	aerosol
	alphacypermethrin	residual
	cypermethrin	"
	deltamethrin	"
	permethrin	"
	pyrethrins	flushing agent
	tetramethrin	"
	lambdacyhalothrin	residual
	cyfluthrin	"
juvenoids	hydroprene	residual
	fenoxycarb	"
respiratory inhibitors	hydramethylnon	bait
	sulfluramid	"
other	silica dust	residual
	boric acid	bait/residual

CHAPTER 2

ASPECTS OF THE BIONOMICS OF *BLATTELLA GERMANICA* (L.) IN LABORATORY CULTURE

2.1 Introduction

Blattella germanica achieves its pest status not least because of the ability of residual populations to survive changing and potentially adverse conditions. This adaptability makes it difficult to predict or analyse the growth of field populations, habitats being highly variable and development correspondingly adjusted to the local environment. Only in the controlled conditions of laboratory culture is it possible to produce definitive data on rates of growth through the nymphal stages and on the fecundity of adults. Existing information on the bionomics of the species is widely scattered in the literature, and has been reviewed by Cornwell (1968).

The data on the development and fecundity of the colony of *B. germanica* used in the current studies was produced for two reasons. Firstly it was considered that the colony was highly productive under the culture conditions used compared with a number which have been described in the literature, and definition of the culture method, which could produce nymphs and adults at defined stages of growth, could be useful to other workers in this area; secondly it was hoped to accumulate sufficient

data to define a density-dependent model of population growth for the species, even though such a model would be limited to defining growth under optimal conditions. Such a model is seen as an increasingly important research tool in the investigation of integrated control strategies using different types of insecticide (acutely toxic residual compounds, slow-acting baits and juvenoids acting as chemosterilants).

2.2 Materials and Methods

2.2.1 Culture of *B. germanica*

The colony of *B. germanica* (L.) was obtained from Rentokil Laboratories, East Grinstead in November 1986. It is regarded as a susceptible strain, having been maintained at Rentokil for a number of years and used as such for standard insecticide testing procedures. The cockroaches were reared at 28-30° C in a LD 12:12 light cycle. Humidity in the rearing room was low (< 40%) to minimise the possibility of mite infestations developing and spreading, and to reduce survival of escapees.

Culture tins were 5 litre paint tins with the central part of the lid replaced with fine copper mesh. Each tin stood in a shallow container of liquid paraffin to prevent escapes.

The calf pellets used for food were obtained from Grain Harvesters Ltd. and analysed as: oil (2.5%), protein (15.5%), fibre (5.5%), ash (6.0%), vitamin A (10000 iu/kg), vitamin D3 (300 iu/kg), vitamin E (60.0 iu/kg) and selenium (0.1 mg/kg) (manufacturer's data).

Culturing was carried out on a weekly basis as follows:

Newly emerged males and females adults were removed from tins containing 7 week old nymphs following CO_2 anaesthetisation. Paper harborages, water and food pots, and filter paper base were removed. any cockroaches still attached being shaken off. Adults were then separated from VI instar nymphs, removed using soft forceps and placed in adult tins (females alone, males alone and mixed females and males). Females already carrying oothecae were separated and destroyed at this point. Approximately 200 adults were put into each tin. The culture normally produced 400 mixed adults each week to continue the culture, together with 400 males plus 400 females in separate tins for experiments. Cockroaches produced in excess of these numbers were killed and disposed of without being sorted. Tins containing last instar nymphs which had not moulted to adults were amalgamated into a single new nymph tin and returned to the culture for sorting the following week. New rearing tins contained a close fitting filter paper base, an 8 oz jar containing water and fitted with an absorbent

cotton wool plug, a small tray containing approximately 25 g of sterilised calf pellets, and a folded paper harbourage (approximately 10 x 20 cm).

"Hatching tins" were set up by placing about 30 oothecae-carrying females into a small breeding cage (Figure 2.1) together with a small water dispenser and a few pellets of calf food. The females chosen carried oothecae which showed a green line of meconium under the brown casing, such oothecea being expected to hatch within a week. The water dispenser was a 20 ml specimen tube provided with an absorbent cotton wool plug inserted in the top. This plug was pushed in tight and was flush with the neck of the bottle so that small nymphs would not become entangled. The breeding cage was placed in a prepared tin on supports to allow emerging nymphs to escape.

The breeding cages from corresponding tins set up the previous week were shaken and removed, young nymphs being gently brushed from the cages into the tins. Females still carrying oothecea in the breeding cage were transferred to a new cage or destroyed. A fresh water bottle, a container of calf pellets, and a folded harbourage were added to the tin, which was then labelled.

Tins containing developing nymphs were checked, and water and food replenished as required. Carbon dioxide was used as little as possible

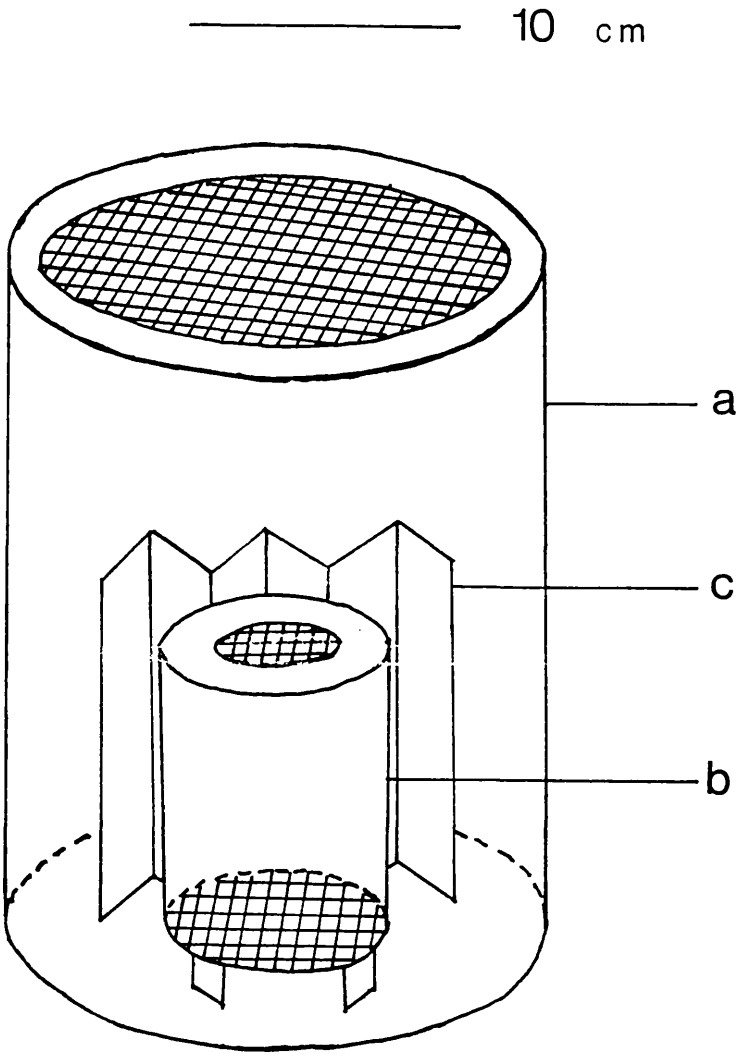


Figure 2.1 Culture tin (a), hatching tin (b) and habourage (c).

and only for later stages (IV - VI).

Each tin was labelled with a reference number, the date the first instar nymphs were separated from the adult females, and where relevant the number of cockroaches in the tin and their sex. Tins containing adults were dated from the point at which newly emerged adults were separated from the last instar nymphs. All handling and sorting of cockroaches was carried out with tins kept in their liquid paraffin moats.

2.2.2 Ootheca development and hatching in females kept with or without males

Glass jars (1 lb) were fitted with a screw-on metal lid with a 5.5 cm hole in the centre to take a filter paper insert. The surface area of the jar was estimated to be 360.5 sq. cm. Food trays (2.2 cm dia. x 1 cm deep) containing 4 calf pellets (total 4.1 g) and water dispensers (20 ml specimen tubes, as in Section 2.2.1) were placed in the jars. The water dispensers were replaced every 10 days.

(a) Newly emerged female adults (2) were placed in the glass jars with male adults (2) together with food and a water dispenser, and the jars maintained at 28° C. The female and male adults were kept together

throughout the experiment, and daily observations recorded the number of oothecae produced by each female adult and the time required by female adults to develop each oothecae. The number of egg cells/ootheca, the number of nymphs emerging successfully from each ootheca, and the size of the ootheca were also recorded (3 replications of 2 pairs).

(b) The experiment was repeated as in (a) with males removed from the jars when the first oothecae became visible on the females.

(c) The experiment was repeated with 6 virgin females isolated at the time of adult moult in three separate jars.

Carbon dioxide anaesthetisation was not used in any of these experiments. Humidity in the rearing jars was measured with a hair hygrometer calibrated against saturated salt solutions (giving an estimated accuracy of ±10%) and averaged 60-70%. The temperature was 28° C ± 2°.

2.2.3 Development times for nymphal stages in synchronous populations of differing densities

Newly hatched first instar nymphs were placed in jars as in Section 2.2.2 with food and water. They were monitored every day until adult emergence without using carbon dioxide anaesthetisation. The numbers of each nymphal stage at each examination was recorded. Five replicates were set up for initial population densities of 10, 20, 30, and 50 nymphs/jar.

2.2.4 Density-dependent mortality in synchronous nymphal populations

Cannibalism was considered to be the major density-dependent mortality factor in the nymphal populations of this colony of *B. germanica* and preliminary observations on the colony suggested that it almost always occurred during or just after a nymphal moult, when the insect was least able to defend itself against attack either by escape or aggression. A developed nymph attacking a newly emerged nymph might alternatively be diverted to eat its exuvia. Since these phenomena affect the life expectancy and rate of adult recruitment of the cockroaches an experiment was set up to examine the effects of crowding within the culture on the extent of cannibalism in nymphal populations.

Populations of 10, 20, 35, and 50 first instar nymphs emerging from

oothecae on the same day were set up in jars (360.5 cm^2 internal surface area) with a water dispenser (20 ml specimen tube) and 2-3 calf pellets. Each population was replicated 5 times. The numbers of live nymphs were then counted every 10 days (using CO_2 anaesthetisation to examine the populations) for 60 days during the nymphal development period, and the number of nymphs successfully moulting to adults recorded. In a second experiment, 2 replicate populations were set up for each of 40, 170 and 330 nymphs/jar and the same data collected.

2.2.5 Cannibalism in mixed nymph/adult populations

To examine the effects of cannibalism by adults on nymphal survival and adult recruitment the newly hatched nymphs from a single ootheca were caged together with adult populations of either 10 male adults, 10 female adults, or 5 male plus 5 female adults with food and water *ad lib.* The survival of the nymphs was examined at 10 day intervals using CO_2 anaesthetisation to examine the populations. Jars containing the same populations of nymphs but no adults were set up at same time for comparison. Four replicates of each test were set up.

2.2.6 Effects of CO_2 anaesthetisation on nymphal development

Nymphs (approximately 40) newly hatched from the same oothecae were placed in a jar with food and water *ad lib.* and a harborage. Ten such jars were prepared and divided equally into two treatments to investigate the effects of CO_2 on the nymphal development. One group of 5 jars was treated with CO_2 daily throughout the development period, and the other group of 5 jars maintained without CO_2 treatment. The cockroaches were not handled during the development period. The number of days from the date of oothecal hatch to the date at which the first and the last adult in each jar emerged was recorded.

2.3 Results

2.3.1 Ootheca development

The results of the experiment in which females were caged with males throughout their reproductive life are shown in Table 2.1. All these females continued to produce oothecae throughout their adult life, and in 5 out of 6 cases observed this corresponded to 5 oothecae, all of which hatched. The interval between adult emergence and hatch of first ootheca, and between hatchings of subsequent oothecae was fairly constant (24.5 - 29.8 days). There was a clear correlation between the

ordering of the oothecae and the number of egg cells contained in them (r_s = 0.74, $p < 0.05$ Spearman Rank correlation), egg cells declining from a mean of 46.7 to 33.2 (first to fifth ootheca). The same trend was evident in the number of nymphs hatching from the oothecae, the mean hatch rate ranging from 99 - 95%. The length of the oothecae produced also showed a decreasing trend with the number of the ootheca (r_s = 0.96, $p < 0.05$). The mean longevity of the adult females was 211.8 days with an s.e. of 25.2 days.

The results from the second experiment in which females were caged with males only up to the emergence of the first oothecae were closely similar (Table 2.1). All females from the 6 replicates produced 4 oothecae but only 3 of the 6 females went on to produce a 5th ootheca. The mean number of egg cells and % hatch was slightly smaller for each oothecae than for the previous experiment but the difference was not significant at $p < 0.05$ in any case. Also the size of the oothecae was uniformly smaller than those of females caged with males throughout their reproductive life. The mean longevity of the females was 163.2 $\pm$ 34.6 days, not significantly different than that of the females kept with males at $p < 0.05$.

In the experiment in which newly-emerged unmated female adults were isolated in jars without males the females produced three or more oothecae but these were all non-viable (no hatch) and no counts were

TABLE 2.1

Ootheca development and hatching in females kept with or without males

	mean (s.e.)			
ootheca	days[1]	egg cells	nymphs hatched	size (mm)
oothecae produced by females caged with males throughout life time				
1st	28.3(1.2)	46.7(4.3)	45.5(5.0)	7.8(0.8) x 2.3(0.3)
2nd	24.5(2.4)	43.3(3.7)	42.2(3.8)	7.3(0.4) x 2.2(0.3)
3rd	25.2(1.2)	41.3(4.0)	40.7(4.1)	7.3(0.7) x 2.6(0.2)
4th	28.0(0.9)	36.8(4.0)	36.0(4.1)	6.7(0.9) x 2.2(0.3)
5th	29.8(2.5)	33.2(4.8)	31.4(3.7)	5.9(0.6) x 2.1(0.2)
female's life duration 211.8(25.2) days				
oothecae produced by females caged with males up to first ootheca production				
1st	27.3(1.5)	46.0(3.6)	43.5(4.1)	7.7(0.4) x 2.1(0.2)
2nd	25.5(1.8)	42.3(4.7)	42.3(4.5)	6.9(0.4) x 2.2(0.3)
3rd	24.2(1.2)	39.3(5.5)	36.5(3.5)	6.9(0.7) x 2.3(0.4)
4th	28.8(2.1)	32.3(5.3)	27.2(7.5)	6.2(1.0) x 2.2(0.3)
5th[2]	35.3(5.5)	28.0(0.0)	27.0(1.0)	5.3(0.6) x 2.0(0.0)
female's life duration 163.2(34.6) days				

female's life duration without mating with male in life time 173.3(38.7) days

[1] mean number of days from adult emergence for first ootheca or from hatch of privous ootheca to day on which the oothecea hatched.

[2] only 50% of the females produced 5th oothecae.

made of the number of egg cells in them. The mean longevity of these females was not significantly different from those recorded for the previous two experiments (Table 2.1)

2.3.2 Development times for nymphal stages in synchronous populations of variable density

Mean times for development of each instar are given in Table 2.2 for populations of initial densities ranging from 10 to 50 insects. The observations were made without anaesthetisation by using small jars (Section 2.2.2) and making daily observations. The various nymphal stages of *B. germanica* can be distinguished with practice on the basis of body size, coloration and shape.

The initial density of the nymphal population in a jar did not appear to affect the development time of each instar provided food and water were provided *ad lib.*. There was no significant difference between the time of moulting of the nymphs at the four densities investigated. A feature of the experiments was the small variance in the timing of the nymphal moults, which was atypical and differs from reports using other colonies and culture methods.

TABLE 2.2

Development times for nymphal stages in synchronous populations of different densities

moult	days (mean, s.e., n=5) from ootheca hatch on which nymphs moulted with an initial population of			
	10	20	30	50
1st	7.2(0.8)	7.0(0.0)	6.8(0.5)	6.9(0.5)
2nd	13.0(0.7)	12.4(0.6)	12.4(0.6)	11.8(0.8)
3rd	17.0(0.7)	17.0(0.0)	18.0(0.7)	17.6(0.9)
4th	26.4(0.9)	23.8(0.8)	24.4(0.9)	22.8(1.1)
5th	33.6(1.7)	30.8(0.5)	31.4(0.6)	29.6(1.1)
6th	39.0(2.9)	41.2(2.5)	40.2(2.3)	40.4(1.1)

TABLE 2.3

Linear regressions ($y = a_o + a_1 x$)[1] of nymphal populations at each stage on initial densities[2]

	nymphal stage					
	1st	2nd	3rd	4th	5th	6th
r^2	0.814	0.807	0.800	0.894	0.905	0.968
a_o	97.95	96.27	94.72	91.85	88.65	88.34
a_1	-0.039	-0.056	-0.059	-0.063	-0.079	-0.147

[1] (% survival) = $a_o + a_1$ (initial density).

[2] cockroaches kept in a glass jar (360 cm^2) with food, water and harbourage at 28°C ± 2°C.

2.3.3 Density dependent mortality in synchronous nymphal populations

The results of the experiment are shown in Figure 2.2. and the raw data in Appendix 2.5. Dead cockroaches were observed in the jars during the experiments but many nymphs were cannibalised and the remainder were difficult to separate from the exuviae and frass so reasons for mortality could not be established with certainty. Increased crowding consistently reduced the percentage of nymphs surviving to adulthood, in spite of free availability of food and water. The two highest initial populations of new nymphs (means 170.5 and 331.5) were reduced to approximately equal populations of adults (means 101.5 and 108.0 respectively), possibly indicating that this was the maximum carrying capacity for the surface area (360 cm^2) available to them.

The moult to adult took place over the range 36 - 55 days from hatching. Figure 2.2 indicates that the greatest % reduction in population took place during the early nymphal stages (possibly corresponding to 1st instar nymphs being cannibalised before or at moulting by 2nd instar nymphs) with a secondary period of reduction at 20 - 30 days. There appeared to be little further reduction in population level following adult emergence except in the two highest density population groups.

There was a linear relationship between initial mean density of instar

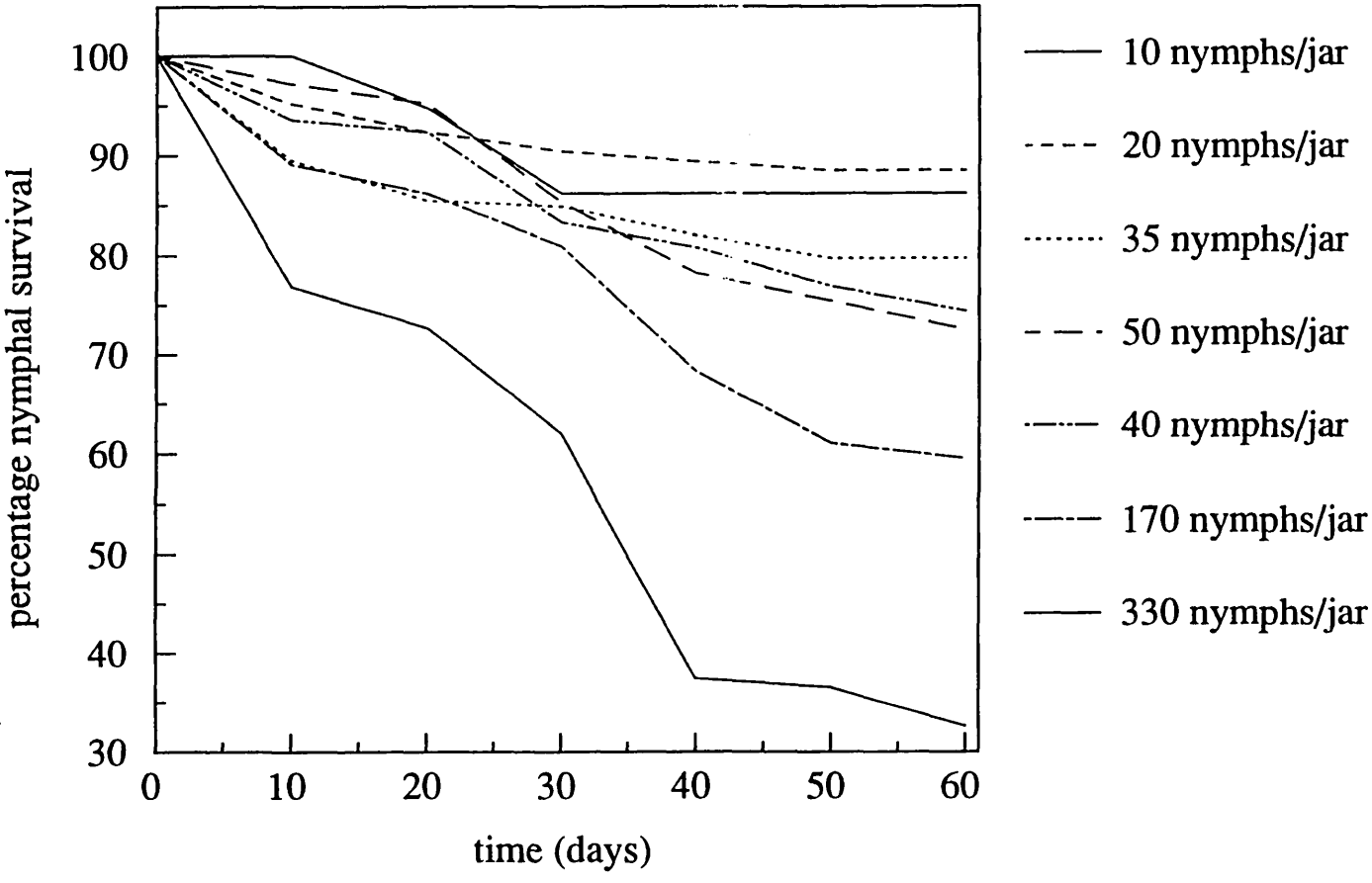


Fig. 2.2 Survival of B. germanica nymphs as % of initial population of first instar nymphs over time at various initial population densities.

f nymphs and final mean density of adults ($y = 85.6 - 0.16x$, $r^2 = 0.96$) over the range 11 - 331 nymphs/jar. Using mean times for moulting obtained in Section 2.3.2 and interpolating to obtain approximate nymphal densities at these times from Figure 2.2, linear regressions of nymphal populations at each stage on initial densities were also obtained. These are shown in Table 2.3.

2.3.4 Cannibalism in mixed nymph/adult populations

The effect of adding adults to a nymphal population on survival and adult recruitment is shown in Figure 2.3 with raw data in Appendix 2.7. The nymphal population size was close to that of population 5 (Figure 2.2) and the total population size close to that of population 4 (Figure 2.2) of the previous experiment. The presence or absence of adults in the population had little effect on adult recruitment, and the mean % reduction of the nymphal populations at the time they moulted to the adult stage were not significantly different regardless of whether adults were present throughout the development period or not.

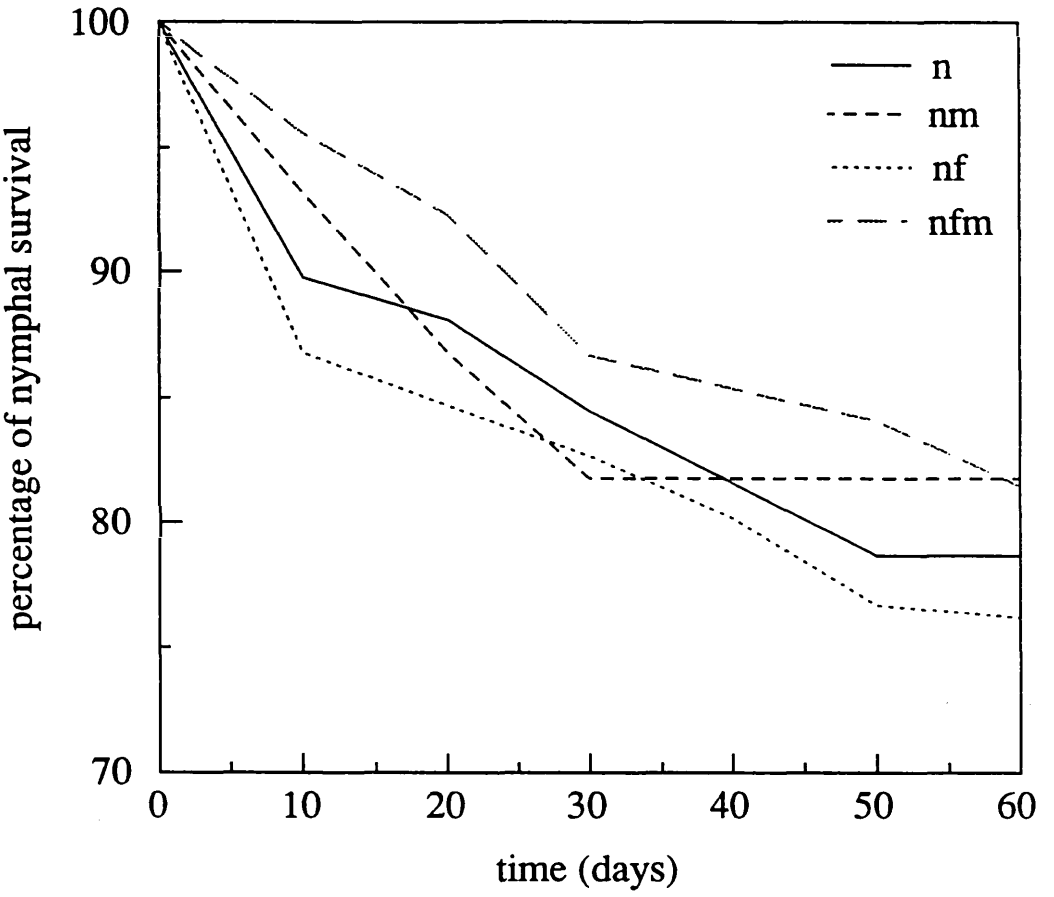


Fig. 2.3 Survival of B. germanica nymphs (40) as % of initial population of first instar nymphs in the presence of adults (10) over time.
n. nymphs (40) alone,
nm. nymphs (40) + male adults (10),
nf. nymphs (40) + female adults (10),
nfm. nymphs (40) + female adults (5) + male aduls (5).

2.3.5 First to second moult and final moult: observations

It was evident from the previous two experiments and from general observations on the cockroach cultures that the period at which nymphs were most vulnerable to cannibalisation was during and immediately after the first moult. Conversely, it was observed that cannibalisation rarely took place at the final moult. The cuticle of the newly emerged second instar nymph is soft and the insect is sluggish, making it susceptible to attack by developed second or later stage nymphs. The relevance of nymphal moulting to cannibalism in *B.germanica* does not appear to have been previously recorded and the following notes taken during observations of a number of first to second nymphal moults and final moults are intended to provide a more complete description of the process than is available in the literature.

Approximately five days from emergence from the ootheca the first instar nymph takes up a position on the vertical side of the culture jar with its head pointing downwards. It remains in this position for a period of 1-2 hours without moving, the abdomen held clear of the surface and the antennae pointing forwards from the body. This positioning is not invariably followed, and does not appear crucial for successful ecdysis. Initiation of splitting of the old cuticle is marked by a series of movements of the antennae. At this stage,

according to Cornwell (1968), air is taken into the crop to distend the old cuticle, but this was not readily visible in the first instar moult. The old cuticle splits at the thorax along the dorsal midline and the second instar nymph starts to emerge by pushing the back of its thorax through the opening in the old cuticle. The legs follow as soon as the head and thorax has been pushed clear of the old cuticle but are not used to provide any traction for subsequent emergence, remaining clear of both the surface and the old cuticle. The antennae are then brought to a position in which they are pointing backwards from the body (vertically upwards) and are then drawn out. The new nymph slides forward out of the cuticle covering its abdomen, until the whole body is positioned in front of the old cuticle. The legs are not used to assist this process.

The time from the first split in the old cuticle to complete emergence is about five minutes. At this stage the newly emerged nymph is translucent and thinner than its final shape. The change to an egg-shaped structure occurs over a period of about 3 minutes with the nymph either stationary or moving within a radius of about 2 cm. During this time it turns round to face the old cuticle, sometimes crawling onto the top of it, and eats it starting from the head end. The process of eating the old cuticle takes about 30 minutes and it is at this stage that the new nymph appears most vulnerable to cannibalisation by developed second or subsequent nymphs. Normal

approach of two developed nymphs to within contact distance may result in no particular reaction or to antennal sparring with the nymphs turning to face each other. A newly emerged nymph appears less able to respond to a potential aggressor in the latter manner. If the nymph itself is not attacked, or if it diverts an attack, its old cuticle may be eaten by the other nymph.

Positioning of the final instar prior to the moult is the same as that of the first instar, but again does not appear crucial for successful ecdysis. The nymph puts its antennae back and close to the abdomen prior to splitting the old cuticle at the dorsal thorax mid-line. It takes about 2 min for the head, thorax and wings to emerge from the old cuticle and 10 minutes for the entire body to emerge. However, it was noticable that during the process other fifth instar nymphs and adults did not disturb or attack moulting nymphs to the same extent as at earlier moults, and if they touched it with their antennae they turned away. The time from initiation of ecdysis to visible melanisation of the new cuticle is about 2 h.

2.3.6 Effects of CO_2 anaesthetisation on nymphal development

The results of this experiment are shown in Table 2.4. The mean period of nymphal development was 36.2 - 46.6 days without treatment with

carbon dioxide and 55.0 - 66.2 days with daily carbon dioxide treatment. The differences between the times to first and to last adult emergence were significant at $p < 0.05$ (Table 2.4).

TABLE 2.4

Effect of carbon dioxide treatment on number of days required by nymphs to become adults in nymphal population (40 nymphs per jar)

observation	days required	p[1]
1st adult emergence		
with CO_2 treatment	55.0(3.6)	
without CO_2 treatment	36.2(0.8)	**
all adult emergence		
with CO_2 treatment	66.2(1.9)	
without CO_2 treatment	46.6(1.1)	**

[1] student's t test for significance of difference between means ** $p < 0.01$

2.4 Discussion

2.4.1 Ootheca development and hatching in females kept with or without males

The fecundity of *B. germanica* under various culture conditions has been investigated by a number of workers; their results are summarised in Table 2.5. From these data it would appear that *B. germanica* is highly flexible in this aspect of its reproductive biology, oothecal production being dependent on culture conditions (food quality and crowding) and temperature. In some of the cases given in Table 2.5 the authors indicate that adverse culture conditions (inadequate food, high temperatures) limited fecundity in their cultures. It would appear that the culturing procedure (which was adapted from that used at Wellcome Research Ltd., Berkhamsted: J Lucas, pers. comm.) and the strain used in the present work resulted in a highly productive colony, possibly approaching the upper limit for female fecundity in this species.

The difference between fecundity of females caged with and without males indicates that the first mating of the female largely determines its subsequent oothecal production although there is some indication of reduced fecundity in females caged in the absence of males after the first mating. Cornwell (1968) states that a single mating is adequate

TABLE 2.5

Fecundity of female *B. germanica* under various culture conditions

temp. oC	no. of oothecae	mated	development time (days)	no. of egg cells	no. of nymphs	reference
24	4.4	x	16.6	-	(29.9)	Gould & Deay (1940)
29	-	x	12.4[1]	-		
29-37	4.5(0.3)	o	-	-		
29-37	-	x	-	33.2˜0.18	29.9˜0.7	Roth & Willis (1956)
30	6.6(0.5)	x	7.8 (0.3) 1 22.0 (0.3) S	(36.7˜0.9)	(27.8˜2.2)	Willis *et al.* (1958)
30	-	x	18.0 (0.1) 1	-	-	Perkins & Grayson (1961)
24-25	-	x	12.8 (0.3) 1	-	-	Roth & Stay (1962)
		o	14.4 (0.1) 1	-	-	
25	3.9	x	-	-	27	Brooks (1965)

[1] oothecal number not recorded: presumed mean of all ootheca produced
o unmated females
x mated females
1 first ootheca
S subsquent oothecae

for production of all the oothecae formed by a female in her lifetime, the spermatothecae providing a highly favourable environment for storage of spermatozoa transferred in the initial spermatophore. However, Kunkel (1966) considered that while mating is not necessary during every reproductive cycle, the maximum number of eggs per oothecae is obtained when males are continuously present. The present work suggests that the proportion of females producing fifth oothecae after a single mating may be reduced relative to the proportion producing them when allowed to mate freely (although this experiment needs to be repeated with larger numbers of cockroaches) but that the fertility of fifth oothecae is not affected.

Control operations against cockroaches with residual insecticides are usually effective at killing a high proportion of the existing nymphal and adult populations (particularly when harbourages are relatively exposed) due to the initial flushing and subsequent toxic action of the spray deposit. Carter and Chadwick (1978) have reported that the residual life of deposits of many formulations is comparatively short (of the order of a few days) on many of the surfaces found in normal domestic and industrial environments, so that survival of a mated female is not an unlikely occurrence. The data presented here indicates that the fecundity of such a female would be minimally affected by the absence of males and could be predicted on the basis of its fecundity in normal mixed populations. Under the highly

favourable conditions of the culture described here the female would produce a maximum of approximately 200 new nymphs over a 4-5 month period.

2.4.2 Density-dependent mortality in synchronous nymphal populations

The effects of population density on the development of cockroaches under laboratory conditions have been examined by Landowski (1938) for small colonies of *Blatta orientalis* (up to 16 individuals/container) and some preliminary results have been reported for *B. germanica* (Chauvin, 1946). The results obtained were similar to those found in this work: mortality increased with density but the number of moults was unaffected provided food and water was supplied *ad lib.* The current study also established that time of moulting was unaffected by nymphal density, at least within the experimental density range. Density-dependent mortality in these laboratory populations of *B. germanica* is attributable primarily to cannibalism at moults prior to the final one, and particularly during the period immediately after moulting. Cannibalism at the final moult to adult and between adults appeared to be a minor effect relative to the cannibalism of earlier instars, particularly at the first instar to second instar moult.

It has been reported from field investigations in the U.S. that

population numbers in apartments with established cockroach infestations are rarely limited by access to food and water, and that in natural mixed age populations control of population density is at the level of oothecal production. Neither relative humidity nor temperature (range 21 - 30°) were correlated with density or age structure (Sherron et al., 1982), *B. germanica* being reported to have little preference for the humidity of its immediate surroundings provided it has easy access to free water (Gunn, 1934). In natural populations the proportion of adults is generally higher than laboratory populations, although the reason for this has not been clearly established (Huber, 1932).

The experiment was designed to simulate the effect of a control operation which had eliminated nymphs and adults from a harbourage and left the oothecae deposited by poisoned females (see Chapter 4). In such a situation the population developing from such oothecae would be limited either by available alternative harbourages, food or cannibalism. That cannibalism is a major determinant of population structure in field populations is indicated by the consistent tendency for a decrease in number with age and instar in infested appartments (Ross, 1976; Ross and Wright, 1977). On the other hand, it has been reported that intermediate instars disperse more than first and final instars in field populations (Ross and Bret, 1984) so that the density-dependent effect of cannibalism is moderated by migration if

alternative harbourages are available.

Density-dependent nymphal mortality was included as a parameter by Larter and Chadwick (1983) in their population model for *B. germanica* as a means by which population numbers stabilised following control operations. Daily survival rates were empirically established to achieve a stable population, and density dependence then included such that the daily survival rate varied inversely with the total number of insects in each stadium (an arbitary measure of insect age based on 7 day intervals). Surprisingly, in view of the potential of population models to suggest effective integrated control strategies, Larter and Chadwick's model remains the only attempt to describe the population dynamics of *B. germanica*; the highly variable nature of the development pattern of the species in response to environmental parameters is the principal disincentive to such an approach. The present data suggest that overall linear dependence of probability of survival to adulthood on initial population density is a good first approximation at least for synchronous nymphal populations, the correlation coefficient being 0.95 over the population densities examined. It is possible to (tentatively) extend the analysis of density dependent mortality by interpolating nymphal densities from Figure 2.2 at the mean times of moulting obtained in Section 2.3.2. This is not a strictly valid procedure since the former results were obtained for populations subject to CO_2 anaesthetisation every 10 days. However, with this

proviso, good linear regressions were obtained for mean proportions surviving at the beginning of each instar on mean initial nymphal populations for each of the six instars (Table 2.3).

Addition of adults to nymphal populations appeared to have little effect on the nymphal mortality, confirming initial observations that competition between nymphs is the principal determinant of survival to maturity.

B. germanica is a highly active insect and a good climber, making it difficult to handle in the laboratory. Accordingly, it is common practice to use carbon dioxide to anaesthetise cultures of the insects during handling, transfer and counting of individuals. Brooks (1957, 1965) and Tanaka (1982) reported on the effects of repeated anaesthetisations on the number of instars, nymphal duration and adult body size of *B. germanica*; quantitative data on these aspects of laboratory cultures need to be obtained without using such anaesthesia if they are to be related to the biology of the pest in the field.

Tanaka and Brooks used weekly anaesthetisations of their experimental populations up to maturity, a less intense regime than was used in the present work, and the growth retardation reported here was thus probably closer to the maximum value obtainable without unacceptable mortality. Tanaka (1982) reported a retardation of 19.8% for males (6

instar type) and 23.3% for females (6 instar type) treated twice during each instar up to the third and then twice a week until adult emergence, compared with the 52% retardation (first adult emergence) or 43% (all adult emergence) found in the present work (in which no distinction was made between the sexes). The results of Tanaka (1982) suggest that most of the growth retarding effects of carbon dioxide are established in the first 3 instars, with comparatively little effect in instars IV-VI.

Work on *B. germanica* in subsequent chapters of this thesis were carried out on adult stages of the insect, and carbon dioxide was used particularly during handling of later stage nymphs (which are more difficult to control than early nymphs) during culturing and when preparing adults for experimentation. The behavioural effects of carbon dioxide on adults are discussed in Chapter 3 (Section 3.4) and Chapter 5 (Section 5.4) in the context of light avoidance reactions and pick-up of dust deposits respectively. While it is virtually impossible to prepare for experiments on *B. germanica* without immobilising them by some means, the stress and other physiological effects so induced must be considered when interpreting the results. The author has observed that cockroaches which are immobilised by cooling to 0° C exhibit the same type of hyperactivity on recovery as do adults which are anaesthetised by carbon dioxide, and it would be interesting to establish whether or not the same growth retarding

effects are obtained using this alternative means of controlling cockroaches during handling.

CHAPTER 3

THE TOXICITY OF PERMETHRIN DEPOSITS AND THEIR EFFECTS ON THE LIGHT RESPONSE OF *BLATTELLA GERMANICA* (L.)

3.1 Introduction

Blattella germanica is normally a nocturnal animal, aggregating in dark resting sites during the day. Its diurnal rhythm of activity was investigated by Wille (1920), and was considered by Gunn (1940) to be entrained by the change from light to dark conditions within the 24 h cycle. Cockroaches are rarely seen during daylight hours in buildings except in heavily infested premises but most cockroach control operations are carried out during the day, and rely for their effect partly on the flushing action of neuroactive insecticides (which causes cockroaches to emerge from harbourages and contact the fresh deposits) and partly on contact with deposits during foraging activity in the following and subsequent dark phases.

The pyrethroid insecticides which are increasingly used as residual control agents for cockroach control are known to produce responses in cockroaches during the initial phases of acute intoxication or at sub-lethal doses which can be interpreted as due to the progressive loss of neural control, disruption of normal behavioural responses and

other physiological functions. These responses are worthy of study because in some cases they may enhance the overall effect of the chemical application and in others reduce its effect. Pyrethroids and some neuroactive carbamate insecticides have an excitatory or activating effect on cockroaches during the initial phase of intoxication, leading to an increase in locomotory activity. The activating effect of natural pyrethrins on the German cockroach was studied by Hutzel (1942) who showed that topical application at a rate of 1 µg/insect to adult males produced a short period of continuous running at nearly 4 times the normal speed. Similar results were reported by Carle (1982) for deltamethrin. The initial excitation produced when aerosol droplets or vapour of pyrethroids penetrate into harbourages during spray application gives rise to the characteristic "flushing" action of these compounds (Fuchs, 1989). In the case of natural pyrethrins, which are not highly toxic to cockroaches, the effect is used to locate infestations and to estimate their size. Permethrin has a weak flushing action relative to pyrethrum and other pyrethroids (Fuchs, 1989).

The flushing effect of synthetic pyrethroids has not been examined in any detail, particularly with respect to the orientation of intoxicated cockroaches moving from harbourage positions. The observed action implies that those cockroaches which emerge are sufficiently disorientated to overcome their normal aversion to brightly illuminated

surroundings during the resting phase of their diurnal cycle. However, if activation of cockroaches harbouring in large void areas (pipe ducting, wall voids, false ceilings) was not also accompanied by disruption of the light aversion reaction it could result in them being driven further into the harbourage. Alterations to the light response have practical importance since it is well established that adult females carrying oothecae deposit them prematurely when agitated, or following exposure to neuroactive insecticides. If most cockroaches emerge into the light following sub-lethal exposure such oothecae will be dropped on treated surfaces and any nymphs hatching from them killed. Conversely, if they retreat further into the harbourages oothecae will be deposited well away from insecticidal deposits and re-infestation will occur as nymphs hatch from them. Differences between insecticides in this respect could be of importance in determining their relative efficacy for long-term control.

The tendency of cockroaches to settle and harbour in a dark rather than an illuminated area is the biological principle underlying the design of a choice box used by Ebeling *et al.* (1966) to measure the repellency of insecticides. Their box was two chambered, one chamber being darkened and treated with insecticide, while other was illuminated and provided with food and water. Cockroaches were released into the illuminated chamber and their subsequent mortality noted at intervals of 24 h. It was assumed that the tendency to settle in the darkened

chamber was counteracted to a greater or lesser extent by the "repellency" of the insecticide, so that an intrinsically toxic product producing little or no mortality under these conditions would be assessed as highly repellent. The Ebeling choice box has been used by a number of workers to measure the repellency of insecticidal formulations although Busvine (1971) commented that the number of experimental variables involved made it difficult to interpret results unambiguously.

The following experiments were designed to explore the tendency of *B. germanica* to move towards a dark area (harbourage) from an illuminated area when suddenly exposed to light during the dark-harbouring part of their diurnal cycle, and to investigate the effect of exposure to lethal and sublethal deposits of permethrin wettable powder (WP) on it. Cockroaches were allowed to move freely in a small arena containing an illuminated and a shaded area, and the extent to which they chose to settle in the shaded area determined under various experimental conditions. Miall and le Patourel (1986) reported a reversal of the normal dark resting response of *B. germanica* during the initial phases of poisoning with a number of pyrethroid insecticides, and the present experiments were designed to confirm and extend this result.

3.2 Materials and Methods

3.2.1 Insects

Adults males (2 weeks from final moult) of the German cockroaches were used for all experiments. They were taken from cultures following anaesthetisation with carbon dioxide and used immediately. Unless otherwise stated, cockroaches were taken for experiments 2 h from the beginning of the photophase.

3.2.2 Chemical and formulation methods

Permethrin (25% W/W WP) was obtained from Wellcome Research Laboratories. The formulation has the trade name "Coopex".

Whatman's No 1 filter paper (10 cm sq) was treated with the wettable powder formulation as an aqueous suspension using a Potter Tower. Treatment concentrations were calculated from weights of spray formulation in petri dishes weighed at the same time as the filter papers, using a conveyer belt arrangement under the funnel of the tower. Filter papers were dried in the laboratory for 1 hour prior to use.

3.2.3 Bioassay arenas

A bioassay arena was contructed on a glass base using two inverted plastic petri dishes (10 cm x 10 cm x 2 cm) butted together with a wide inter- connecting hole (8 cm x 2 cm) [Figure 3.1]. The base of the arena was covered with a piece of filter paper which was changed after each experiment. The cockroaches were constrained to move on this surface by painting the top and sides of each petri dish with a PTFE emulsion ("Fluon"). Three sides of the arena were covered with black tape, and the top of one of the two chambers (the shaded chamber) were covered with black paper, and the other chamber (the illuminated chamber) had a hole (3 cm dia.) in its top through which cockroaches could be introduced. The arenas were orientated with their long side either parallel or perpendicular to, and at varying distances from a fluorescent strip light with a maximum output at 550 nm (Thorn EMI 36 W Pluslux 3000, Warmwhite) on a bench [Figure 3.2 a,b].

Light intensity (mW/m^2) in the wavebands 350-390 nm and 420-680 nm was determined at various distances from the source and at different positions in the arena using a photocell calibrated against a standard light source (Young, 1986). The orientation of the photocell and arenas used for measurement are shown in Figure 3.2.

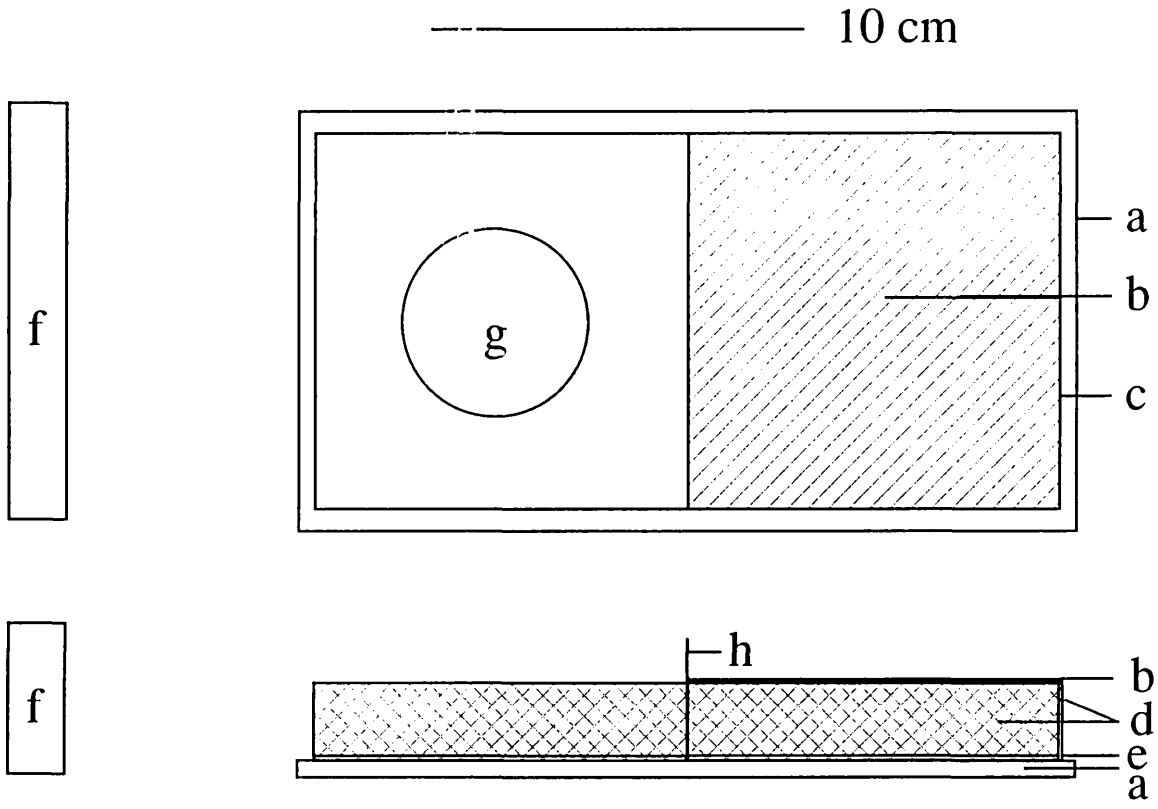


Figure 3.1 Arena (two chamber) for bioassay of resting position of B. germanica relative to a fluorescent light source.
a. glass base, b. black paper, c. petri dish, d. black tape, e. filter paper, f. light source, g. port for introducing cockroaches to arena. h. removable card separating chambers.

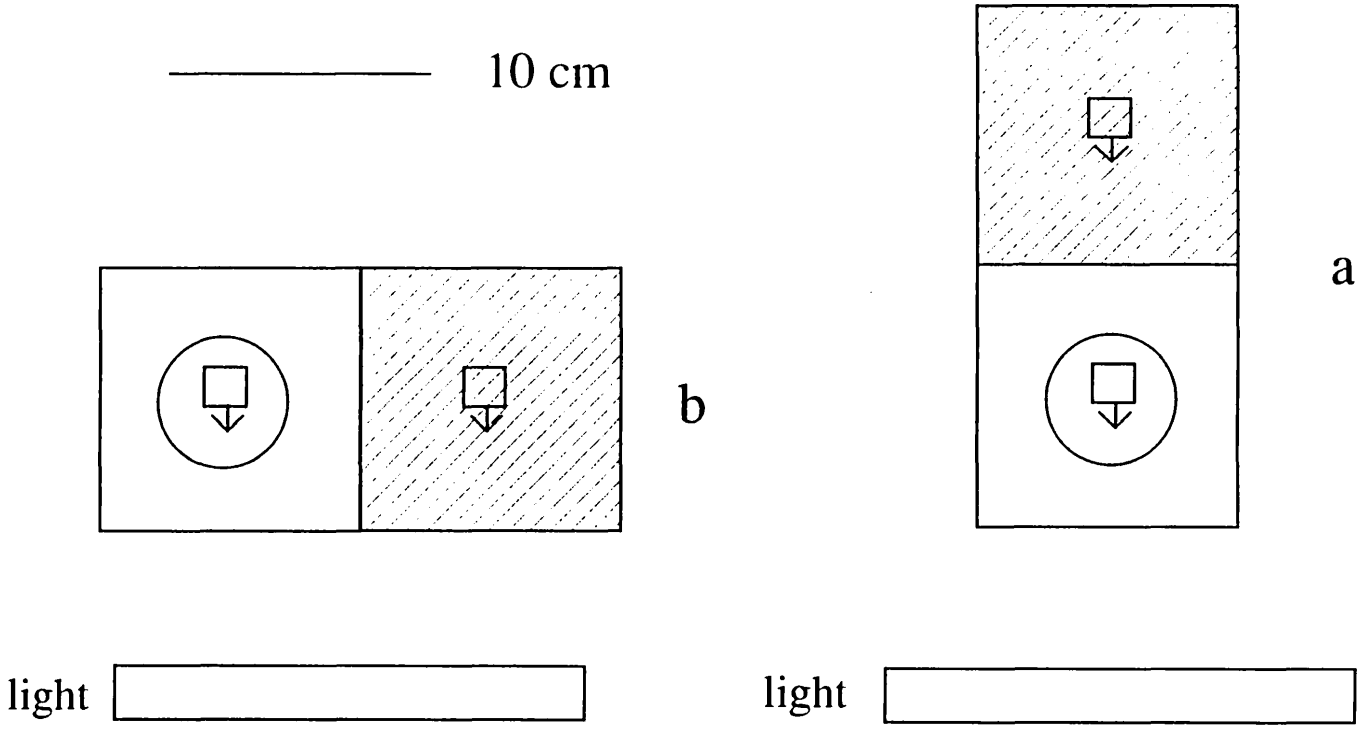


Figure 3.2 Orientation of bioassay arena relative to the fluorescent light source (a) perpendicular and (b) parallel, also showing position (square) and orientation (arrow) of photocell when taking light intensity measurements.

3.2.4 Distribution of cockroaches between shaded and illuminated sections of the arena

3.2.4.1 Effect of illumination of both chambers of arena on subsequent distribution

The black tape and paper were removed from the covered chamber and the arenas orientated parallel to the source and 10 cm from it. Cockroaches (10 per replicate) were anaesthetised and placed in one chamber and allowed to recover in the dark for 10 min. The arena was then illuminated and the cockroaches were given access to the alternative chamber. Distribution within the arena was noted at 30 min.

3.2.4.2 Effect of initial position of cockroaches in arena prior to illumination on subsequent distribution

Batches of 10 cockroaches were anaesthetised and placed either in the chamber of the arena which was subsequently illuminated when the arena was illuminated or in the chamber which was subsequently shaded when the arena was illuminated. Following 10 min recovery in the dark the

arena was illuminated, the card separating the chambers removed, and the distribution of the cockroaches recorded after 30 min. The experiment was repeated twice, once with the arena parallel to the light source and once with it perpendicular, with 5 replicates for each experiment.

3.2.4.3 Effect of light intensity and orientation of arena with respect to light source

The effects of varying light intensity (distances of source from arena) in chambers of the arena on the distribution of cockroaches in the arena were investigated. The arena were orientated either parallel or perpendicular to the light source, and the chambers of the arena were blocked with a card. Experiments were carried out using 10 cockroaches in each arena and with 8 replications for each bioassay conditions in a darkened room maintained at 28-30°C. The insects were discarded after each experiment.

(1) The arena was placed with its long axis perpendicular to the light source [Figure 3.2 a]. Cockroaches were allowed to recover from CO_2 anaesthetisation in either dark or light for 10 min in the chamber which was subsequently illuminated after the cockroaches were given access to the shaded chamber. The distribution of the cockroaches

between the two chambers was recorded after 30 min.

This experiment was repeated with the source at various distances from the front end of the uncovered chamber of the arena.

(2) Conditions were as in (1), except that the arena was turned through 90° so that it was parallel to the light source [Figure 3.2 b], increasing the difference in light intensity between the two halves of the arena. Cockroaches were placed in the illuminated chamber and allowed to recover from anaesthetisation in the dark for 10 min before illuminating the arena. Cockroach distribution was determined 30 min after illuminating the arena with the distance of the light source from the arena varied. Five replicates of the experiment were carried out for each distance of the light source from the arena.

3.2.4.4 Effect of varying holding period in illuminated chamber prior to permitting choice between illuminated and shaded chambers

The distribution of the cockroaches was also determined at 30 min after light exposure periods of 5, 10, 15, 20, 25, 30 or 60 min and with the light source at 10 cm from and parallel to the long axis of the arena (5 replicates for each light exposure period).

3.2.4.5 Effect of continuous light or continuous dark exposure prior to release into arena

Cockroaches were exposed to continuous dark or continuous light for 36 h (with food and water *ad lib.*) prior to the experiments and their reaction to the light source compared with that of cockroaches taken from the stock culture maintained in a 12:12 photocycle. The experiments on the effect of time in the light/dark cycle on distribution in the arenas were started at the beginning of the photophase. The orientation of the arena was parallel to the light source at 10 cm. Cockroaches were anaesthetised prior to transfer to the arenas and allowed to recover for 10 min in the dark before the card was removed to give the cockroaches access to the shaded chamber. Distribution between the chambers was recorded over the subsequent 24 h in a 12:12 (L:D) cycle started from the beginning of the photophase. Five replicates of 10 insects were used for each test.

3.2.4.6 Effect of carbon dioxide anaesthetisation

Cockroaches in a culture tin were anaesthetised with carbon dioxide. Fifty males were removed, and transferred to the illuminated chamber of five arenas, 10 to each arena. The distribution of cockroaches

following 10 min dark recovery, illumination of the light source, and removal of the card separating the chambers was investigated as in 3.2.4.2 with the arena parallel to the light source at 10 cm. Thirty min after the first anaesthetisation the cockroaches remaining in the culture tin were again anaesthetised with carbon dioxide, and the procedure repeated. The process of re-anaesthetisation was continued, so that the final group of cockroaches tested had been anaesthetised 5 times with intervening 30 min recovery periods prior to testing.

A trial with unanaesthetised cockroaches was also carried out. The access hole to the light chamber of an arena was covered with a piece of polythene and a small (2 1b) breeding tin with its base removed placed on top of it. Cockroaches (10) were anaesthetised with carbon dioxide and transferred to the tins together with a source of food and water, and allowed to recover for 24 h. The cockroaches were then given access to the light chamber by sliding the polythene sheet from under the tin, and were forced into the chamber using a close fitting plunger which was slowly pushed down the interior of the breeding tin. The top of the light chamber was then re-covered with black card, and the experiment continued as previously. The experiment was replicated 5 times.

3.2.4.7 Division of experimental population into "light preferring" and "dark preferring" groups

The long axis of the arena was parallel to and at 10 cm distance from the light source. Cockroaches (10) were introduced into each arena without carbon dioxide anaesthetisation as in 3.2.4.6, and Experiment 3.2.4.3 (2) then repeated. At the end of the 30 min light exposure period the cockroaches which were resting in the dark and light halves of the arena were collected separately into two tins, and the experiments repeated using either all "dark- preferring" or "light-preferring" insects in an arena. Fifteen experiments were carried out to accumulate populations of "dark- preferring" and "light-preferring" insects, and 10 replications were then performed with "dark-preferring" insects and 3 with "light-preferring" insects to determine their subsequent distribution within the arena using the method of 3.2.4.3 (2).

3.2.5 Effect of permethrin deposits on the distribution between illuminated and shaded parts of arena

3.2.5.1 Bioassay for intrinsic toxicity of permethrin deposits

Filter papers (12 cm dia.) were sprayed with permethrin 25% WP from a Potter Tower and air-dried for 1 h. Batches of 10 cockroaches were anaesthetised with carbon dioxide and transferred to the filter paper under a 10 cm diameter glass funnel. Water-sprayed filter paper was employed as the control. The wall of the funnel was painted with a PTFE emulsion ("Fluon") to constrain the cockroaches to move on the filter paper. Five replicates were carried out for each concentration of permethrin (2.32, 3.94, 4.66, 5.41, 6.44, 9.27, 21.92, 30.36, 41.27 and 51.20 mg a.i./m^2). After 30 min exposure, the cockroaches were transferred to an untreated filter paper by sliding the funnel carefully with the cockroaches underneath. The number of knocked-down cockroaches was recorded at the end of the exposure period and mortality recorded after 24 hours.

3.2.5.2 Bioassay arena for illuminated/shaded area choice tests

The same arenas were used as described in 3.2.3, but the base of the illuminated or shaded half of the arena was covered with a piece of

Whatman's No 1 filter paper (10 cm sq) which had been previously sprayed with the diluted permethrin WP from a Potter Tower and dried for 1 h. The treated paper was changed after each experiment. The cockroaches were constrained to move on this paper by painting the top of each petri dish with a PTFE emulsion ("Fluon"). The arenas were arranged so that their long edges were parallel to and 10 cm from the light source.

3.2.5.3 Distribution of cockroaches in arenas half-treated with permethrin deposits

A test arena was prepared by placing a piece of filter paper sprayed with 25% WP permethrin at a concentration of 1.25 mg/m² on the base of either the illuminated or shaded chamber of the arenas, and a piece of water-sprayed filter paper on the base of the alternative half. The two pieces of paper were accurately cut so that they butted together at the join. A control arena was prepared using two water-sprayed filter papers as the base of each chamber. The arenas were orientated parallel to the light source at 10 cm. Ten cockroaches were anaesthetised, transferred to the illuminated chamber (either treated or untreated) and held in this chamber for 30 min in the dark before illuminating it and giving the cockroaches access to the shaded chamber.

Three observation times (30, 60 min and 24 hours after the cockroaches were given access to the shaded chamber) were made separately for each of the 2 treatments (illuminated chamber treated, shaded chamber treated) and for the control.

The experiment was designed as a randomised complete block design. Each block was run concurrently and consisted of two replicates for each of the treatments (illuminated side treated or shaded side treated) and two replicates of the the control. There were three observation times (independent experiments) in each block and four blocks.

3.2.5.4 Distribution of cockroaches pre-exposed to permethrin deposits between shaded and illuminated parts of the arena following treatment and after 24 and 48 h recovery periods

Filter papers were sprayed with permethrin WP at different concentrations and dried for 1 h in the laboratory. Batches of 10 cockroaches were exposed to the filter papers under a funnel for 30 min in the light (Section 3.2.5.2) and then anaesthetised with CO_2. Their response to illumination of the arena was then examined. The cockroaches were allowed to recover for 10 min in the dark before illuminating the arena and giving the cockroaches access to the shaded chamber. Their distribution was noted after 30 min and the cockroaches

then transferred to an untreated tin with water and food. The experiment was repeated using the same batches of cockroaches after 24 h and 48 h recovery from the initial exposure. Five replications of the experiment were carried out at concentrations of 1.0, 2.0, 3.4, 5.4 and 6.4 mg/m^2. Only survivors of each exposure were used for the examination of the light response in the arena and so fewer replicates were carried out for the 24 and 48 h observations at higher permethrin concentrations.

3.2.5.5 Exposure to permethrin deposits in a three-chambered arena

A 3 chambered arena was constructed using three 10 x 10 cm inverted plastic petri dishes butted end to end, with an 8 cm passage cut from the two end dishes to the central one. Three sides of the arena were covered with black tape on the outside and all were coated with PTFE emulsion on the inside. The central chamber had a 2 cm dia. hole in the top through which to transfer cockroaches to the arena. The arena was orientated with its long axis perpendicular to the light source and 10 cm from it (Section 3.2.3) and placed on a glass base. The chamber furthest from the light source was covered with black paper (Figure 3.3).

Filter papers were sprayed with permethrin WP at concentrations of 4.8,

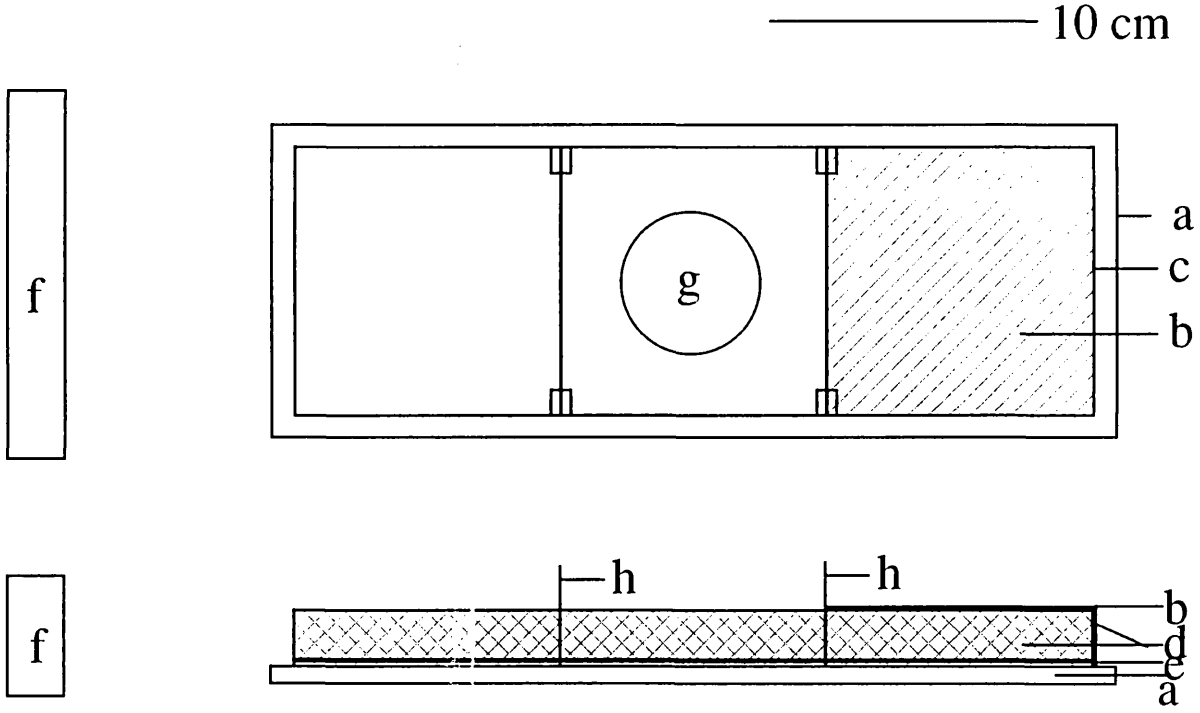


Figure 3.3 Arena (three chamber) for bioassay of resting position of B. germanica relative to a fluorescent light source.
a. glass base, b. black paper, c. petri dish,
d. black tape, e. filter paper, f. light source,
g. port for introducing cockroaches.
h. removable card separating chambers.

7.7, 8.0, 12.9, 13.6, 18.0, 22.6 and 33.3 mg/m² as in the previous bioassays (Section 3.2.5.1). Treated filter papers were placed under the central chamber and butted against untreated filter papers under the other two chambers. The arenas were set up in groups of 5 for each concentration.

Batches of 10 cockroaches were anasethetised with carbon dioxide and placed in the central exposure chamber. They were allowed to recover in the dark for 10 min with the access holes to the escape chambers blocked with pieces of card. The cards were then gently removed and the arena illuminated, the experiment being timed from this moment. The distribution of the cockroaches between the three chambers was recorded at 30 min from the point at which the card was removed. The experiment was replicated 5 times.

3.2.6 Data analysis

Proportions of cockroaches in any chamber of the arena were transformed to angles for statistical analysis but are expressed as percentages in the tables. Duncan's multiple range test (Duncan, 1951) was used to analyse experiments for which means of treatments were not identical at $p < 0.05$ using an implementation of the algorithm described by Gomez (1983) written in BBC BASIC.

Probit analysis was performed using an implementation of the algorithm described by Finney (1964) written in BBC BASIC to determine the toxicity of permethrin deposits to the cockroaches.

3.3 Results

3.3.1 Light intensity as a function of distance from the source

The fluorescent light source used in this work was a standard tube widely used domestically and in commercial buildings. The response of cockroaches to it was therefore considered to be relevant to their behaviour in their "natural" habitat.

The variation in light intensity with wavelength is shown in Table 3.1 and 3.2. The perspex used to construct the arenas was transparent (> 50% transmittance) down to 300 nm.

Relative light intensity at the centres of the two chambers of the bioassay arena when orientated parallel to the light source is shown as a function of the distance of the front edge of the arena from the

TABLE 3.1

Light intensity in illuminated and shaded chamber of arena orientatedparallely to and at different distances from the light source

distance (cm)	wavelengh (nm)	mean light intensity (W/m^2) light chamber	dark chamber	difference
10	420-680	2.063	0.043	2.020
	350-390	0.016	0.000	0.016
15	420-680	1.750	0.023	1.727
	350-390	0.010	0.000	0.010
20	420-680	1.375	0.014	1.361
	350-390	0.008	0.000	0.008
25	420-680	0.981	0.006	0.975
	350-390	0.006	0.000	0.006
30	420-680	0.844	0.004	0.840
	350-390	0.002	0.000	0.002

intensity difference = 4.983-1.265 ln(distance) r^2 = 0.809

TABLE 3.2

Light intensity in the illuminated and shaded chamber of arena orientated perpendicularly at different distances from the light source

distance (cm)	wavelengh (nm)	mean light intensity (W/m^2) light chamber	dark chamber	difference
10	420-680	2.250	1.019	1.231
	350-390	0.013	0.002	0.011
15	420-680	1.938	0.688	1.250
	350-390	0.008	0.001	0.007
20	420-680	1.500	0.568	0.932
	350-390	0.006	0.001	0.005
25	420-680	1.250	0.475	0.775
	350-390	0.004	0.000	0.004
30	420-680	1.075	0.413	0.662
	350-390	0.003	0.000	0.003

intensity difference = 1.870 exp(0.0344 distance) r^2 = 0.940

source in Table 3.1. The difference between the intensities (y) and the distance of the front of the arena from the light source (x) was best fitted by the linear relationship $y = 4.983 - 1.265 \ln(x)$ with a correlation coefficient (r^2) of 0.81.

The corresponding data when the bioassay arena was orientated perpendicular to the light source is shown in Table 3.2, the best fit regression being $y = 1.87 \exp(0.0344x)$ ($r^2 = 0.940$).

3.3.2 General observations on movement of cockroaches in the arena when exposed to illumination following the recovery period

Cockroaches recovering from carbon dioxide anaesthetisation showed a short period of hyperactivity. A 10 min recovery period was adequate to restore normal resting behaviour, with most cockroaches then stationary and within touching distance of each other or the walls of the recovery chamber. Slight vibration of the culture tins or bioassay arenas (entering room, closing door) did not cause the cockroaches to move from these resting positions but illumination of the recovery chamber activated the cockroaches and they then moved rapidly around the arena, resting for progressively longer intervals so that 2-5 min later most or all had come to rest. The orientation of the resting cockroaches with respect to the light source appeared random. Gentle

removal of the card separating the two chambers in dim light did not activate the cockroaches from their resting positions

3.3.3 Effect of illumination of both chambers on subsequent distribution of cockroaches

The cockroaches were allowed to recover for 10 min in the dark in one chamber and then given access to the alternative chamber when both were illuminated. The proportion of the cockroaches in the two chambers 30 min later is shown in Table 3.3. The null hypothesis of a 1:1 distribution between the two chambers was not rejected ($p > 0.05$).

3.3.4 Effect of position of cockroaches in arena prior to illumination on subsequent distribution

Results are shown in Table 3.4. When the arena was parallel to the light source the cockroaches showed a preference for resting in the shaded chamber regardless of whether they were in the shaded or illuminated chamber when the light source was switched on although the effect was not significant at $p < 0.05$ (chi-square, null hypothesis 1:1 distribution) in either case. Unquantified observations indicated that

TABLE 3.3

Number of cockroaches located in alternative chamber following 10 min dark recovery period in one chamber and then illumination of both chambers[1]

number[2] of cockroaches in alternative chamber replication	mean (s.e.)	p[3]
4 4 4 5 5	4.4 (0.6)	n.s.

[1] orientated parallel to the source at 10 cm
[2] number out of 10
[3] hypothesis of 1:1 ratio between the two chambers

TABLE 3.4

Effect of initial position of cockroaches in arena during dark recovery period prior to illumination and being given access to both chambers

orientation of arena/chamber for dark recovery	number[1] of cockroaches in shaded half at 30 min replication					mean (s.e)	p[2]
parallel/ illuminated	4	6	4	6	8	5.6 (1.7)	n.s.
parallel/ shaded	6	6	6	8	7	6.6 (0.9)	n.s.
perpendicular/ illuminated	6	4	5	4	4	4.6 (0.9)	n.s.
perpendicular/ shaded	6	6	8	3	5	5.6 (1.8)	n.s.

[1] out of 10
[2] hypothesis of 1:1 ratio between the two halves

the cockroaches were not as activated by the sudden illumination if they were resting in the dark chamber as if they were resting in the illuminated chamber.

Results when the arena was orientated perpendicular to the light source and the cockroaches could see the source from both chambers indicated no preference for the chamber furthest from the light source when the cockroaches were initially positioned in the chamber closest to the source and only a marginal preference when they were positioned in the further chamber ($p > 0.05$ in both cases).

3.3.5 Effect of light intensity and orientation of arena with respect to light source

Interaction between distance of light source from arena and orientation of the arenas to the light source was not significant ($p > 0.05$, ANOVA, Appendix 3.2). This permitted an unbiased examination of the main effects of light intensity and orientation of the arenas on cockroach distribution.

With the arena arranged with its long axis perpendicular to the light source and the cockroaches allowed to recover from anaesthetisation in the light, there was no significant difference between the proportions of cockroaches in the two chambers and that expected on the basis of a

1:1 distribution at any distance of the arenas from the light source. The same result was obtained with the arena parallel to the light source when the cockroaches were allowed to recover in the dark prior to illumination of the arena. When the areas were orientated perpendicular to the light source and cockroaches recovered in the dark, significantly more cockroaches remained in the illuminated half of the arena when it was placed 30 cm from the light source, but not for distances closer than this ($p < 0.05$, Duncan's multiple range test; Table 3.5).

3.3.6 Effect of varying holding period in illuminated chamber prior to permitting choice between illuminated and shaded chambers

Cockroaches were allowed to recover from anaesthetisation for 10 min in the dark after being placed in the unshaded chamber and then exposed to illumination for varying periods of time before being given access to the shaded chamber. The arena was orientated parallel to the light source. The results of the experiment are shown in Table 3.6.

There was no evident correlation between time of illumination and mean proportion of cockroaches in the shaded half of the arena following removal of the dividing card ($r^2 = 0.12$). At all exposure times the mean proportion of cockroaches in the shaded half of the arena was

TABLE 3.5

Mean (s.e.) % of cockroaches in shaded chamber at 30 min following 10 min recovery period in uncovered chamber which was subsequently illuminated (n = 8).

	arena perpendicular to source				arena parallel to source	
distance (cm)	cockroaches recovered in the light	p[1]	cockroaches recovered in the dark	p	cockroaches recovered in the dark	p
10	46.3 (17.7) b[2]	n.s	47.5 (12.8) b	n.s	63.8 (9.2) a	n.s
15	48.8 (12.5) ab	n.s	46.3 (10.6) b	n.s	60.0 (7.6) a	n.s
20	45.0 (9.3) b	n.s	42.5 (15.8) b	n.s	57.5 (12.8) a	n.s
25	48.8 (12.5) ab	n.s	38.8 (12.5) b	n.s	55.0 (5.4) a	n.s
30	53.8 (17.7) b	n.s	33.8 (10.6) c	n.s	62.5 (12.8) a	n.s

[1] chi-square test for hypothesis of 1:1 distribution

[2] means followed by same letter are not significantly different ($p > 0.05$) (Duncan's multiple range test).

TABLE 3.6

Comparison between % of cockroaches in illuminated and shaded chambers of arenas after 10 min dark recovery period followed by variable periods of exposure to illumination prior to being given access to shaded chamber.

exposure period (min.)	mean (s.e) % cockroaches in shaded chamber	illuminated chamber	p[1]
5	66.0 (5.5)	34.0 (5.5)	n.s.
10	62.0 (13.0)	38.0 (13.0)	n.s.
15	66.0 (11.4)	34.0 (11.4)	n.s.
20	78.0 (13.0)	22.0 (13.0)	**
25	76.0 (11.4)	24.0 (11.4)	*
30	64.0 (13.4)	36.0 (13.4)	n.s.
60	58.0 (8.4)	42.0 (8.4)	n.s.

[1] chi-square test for hypothesis of 1:1 ratio between illuminated and shaded chamber (n.s. $p > 0.05$; * $0.01 < p < 0.05$; ** $p < 0.01$).

greater 30 minutes after removing the card than would be expected on the basis of a 1:1 distribution, but only following the 20 and 25 min exposures was this difference significant at $p < 0.05$ (chi-square).

3.3.7 Effect of continuous light or continuous dark exposure prior to release into arena

Cockroaches were kept in culture tins either in a normal 12:12 (L:D) photocycle, a (24:0) photocycle or a (0:24) photocycle for a period of 36 h, the exposure being started at the end of a dark phase of the normal 12:12 photocycle. Each group of cockroaches was then anaesthetised, split into replicate populations (5 per treatment) and transferred to the arenas (parallel orientation). Recovery in the illuminated chamber (10 min) was in the dark if they had been previously held in the dark, and in the light if previously light-exposed. Following removal of the card separating the two chambers the distribution of the cockroaches was noted at intervals up to 23 h later with continuous illumination of the arena. The results are shown in Table 3.7.

In none of the treatments was there a correlation between the proportion of cockroaches resting in the dark chamber and the time of observation. (r^2 = 0.37, 0.02, 0.00 for 12:12, 24:0, and 0:24 L:D

TABLE 3.7

Distribution of cockroaches in the illuminated/shaded choice boxes at 30 min light exposure after long term pre-exposure to light or dark

time from beginning of photophase (h)	mean (s.e.) percentage in shaded chamber					
	12:12 (L:D)	p[1]	24:0 (L:D)	p	0:24 (L:D)	p
0.5	64.0 (11.4) b	n.s	80.0 (12.2) a	**	90.0 (7.1) a	**
1.5	56.0 (8.9) c	n.s	88.0 (4.5) a	**	70.0 (10.0) b	n.s
4.5	54.0 (15.2) b	n.s	78.0 (11.0) a	*	80.0 (7.1) a	**
6.5	58.0 (8.4) b	n.s	74.0 (11.4) a	n.s	74.0 (5.5) a	n.s
9.5	50.0 (10.0) b	n.s	82.0 (8.4) a	**	80.0 (14.1) a	**
11.5	56.0 (11.4) b	n.s	78.0 (16.4) a	**	66.0 (11.4) b	n.s
12.5	64.0 (11.4) c	n.s	88.0 (4.5) a	**	72.0 (8.4) b	n.s
13.5	68.0 (16.4) a	n.s	78.0 (8.4) a	**	78.0 (11.0) a	**
15.5	68.0 (13.0) b	n.s	84.0 (5.5) a	**	80.0 (7.1) a	**
18.5	74.0 (15.2) a	*	82.0 (8.4) a	**	80.0 (10.0) a	**
21.5	76.0 (13.4) a	*	78.0 (8.4) a	*	82.0 (4.5) a	**
23.5	62.0 (16.4) b	n.s	78.0 (8.4) a	*	80.0 (7.1) a	**

[1] chi-square test for 1:1 distribution between chambers; n.s $p>0.05$; * $0.01 < p < 0.05$; ** $p < 0.01$.

[2] means in row followed by same letter are not significantly different ($p < 0.05$) by Duncan's multiple range test

cycles respectively). Cockroaches exposed to the normal 12:12 cycle showed a slight preference for the shaded chamber at all observations times (mean proportion in shaded chamber > 0.5) but this preference was only significant at $p < 0.05$ for 2 out of the 12 observations. In the case of the cockroaches conditioned to either a 24:0 L:D or a 0:24 L:D cycle the results indicate a definite preference for the shaded chamber, mean proportions in this area being > 0.70 in all cases and the number of observations at which the null hypothesis of a 1:1 distribution was not consistent with the data being 11 out of 12 for the 24:0 L:D cycle and 8 out of 12 for the 0:24 L:D cycle (chi-square).

3.3.8 Effect of carbon dioxide anaesthetisation

Cockroaches which had been anaesthetised 5 times within 2.5 h appeared no different in the extent to which they were activated by illumination or in the period required for them to settle down after they had been activated following the normal 10 min recovery period than cockroaches which had been anaesthetised 24 h earlier, transferred to the arena without anaesthetisation, and acclimatised for 10 min in the dark. There was no clear correlation between the proportion of cockroaches preferring to settle in the shaded chamber and the number of anaesthetisations ($r^2 = 0.43$). In all cases the mean proportion of cockroaches in the shaded chamber was greater than 0.60, but overall

the proportions were only significantly different from a 1:1 distribution ($p < 0.05$, Table 3.8) for cockroaches which had not been anaesthetised and those which had been anaesthetised 4 times.

The experiment established that there was no significant difference between the proportion of unanaesthetised cockroaches in the shaded chamber and the proportion of cockroaches given the standard single anaesthetisation used in the other experiments described in this section.

3.3.9 Division of experimental population into "light preferring" and "dark preferring" groups

The results of this experiment are analysed in Table 3.9. There appeared to be no significant difference between the proportion of "light preferring" and "dark-preferring" cockroaches in the shaded chamber following isolation and trial of these two groups ($p > 0.05$, Student's t test) although only 3 replicates of the experiment for the light-seeking group were carried out and the result must be viewed with caution.

TABLE 3.8

The effect of carbon dioxide anaesthetisation on the distribution of cockroaches in arenas

no. of CO_2 anaesthetisations	mean (s.e.) cockroaches in shaded chamber (%)		p^2
0	84.0 (5.5)	a[1]	**
1	72.0 (11.0)	ab	n.s
2	68.0 (11.0)	ab	n.s
3	68.0 (14.8)	ab	n.s
4	62.0 (11.0)	b	**
5	72.0 (11.0)	ab	n.s

[1] means followed by the same letter are not significantly different (Duncan's multiple range test) at $p < 0.05$.

[2] chi-square for hypothesis of 1:1 ratio in number of cockroaches between illuminated half and shaded half of arena.

TABLE 3.9

Effect of separation of cockroaches into two populations (light-prefering and dark-prefering) on subsequent distribution in light/dark choic eboxes.

population	mean (s.e.) % cockroaches in dark chamber	p^1
initial population	85.79 (14.02)	
light-prefering insects	90.00 (12.47)	n.s
dark-prefering insects	80.00 (26.46)	

[1] t test for hypothesis that means are not significantly different between "light-preferring" and "dark-preferring"

3.3.10 Effect of permethrin deposits on the distribution between illuminated and shaded parts of the arena

3.3.10.1 Bioassay for intrinsic toxicity of permethrin deposits

Probit analysis of 24 h mortality following a 30 min exposure to deposits of 25% WP permethrin are shown in Table 3.10. The LC50 was 4.6 mg/m² with a log(dose)/probit(mortality) regression of y = 1.43 + 5.37 x. The EC50 (30 min knockdown) was 40 mg/m² with a log(dose)/probit(knockdown) regression of y = 3.68 + 0.82 x.

3.3.10.2 Distribution of cockroaches in arenas half-treated with permethrin deposit

The experiment was designed so that either the cockroaches recovered in the dark in the untreated unshaded chamber and were then given access to the treated shaded chamber following illumination of the arena, or they recovered in the dark in the treated unshaded chamber and were then given access to the untreated shaded chamber. The exposure concentration (1.3 mg/m²) was sublethal (< LC5) for a 30 min exposure. In the control experiment both chambers were untreated. The results (Table 3.11 and ANOVA, Appendix 3.10) show no significant interaction

TABLE 3.10

Toxicity of permethrin 25% WP to cockroaches (two week old male adults; 30 min exposure on filter paper)

24 h mortality

regression equation

$y = 1.427 + 5.369x$

chi-square = 0.474 d.f.= 5 P = 0.993

	mg/m²	(95% fiducial limits)
ED95	9.4	11.5 - 7.7
ED50	4.6	5.1 - 4.2
ED5	2.3	2.9 - 1.8

30 min knockdown

regression equation

$y = 3.684 + 0.817x$

chi-square = 22.452 d.f.= 8 P=0.004
(data hetereogeneous)

	mg/m²	(95% fiducial limits)
ED95	4254.6	52378.8 - 439.6
ED50	40.7	79.3 - 22.1
ED5	0.4	1.5 - 0.1

between treatments (half-treated illuminated, half-treated shaded, control) and observation times following access to the shaded chamber.

Clear differences were apparent between, on the one hand, the proportion of cockroaches in the dark chamber for the control group and the group which recovered on the permethrin deposits in the illuminated chamber, and on the other, the group exposed to the deposits in the shaded chamber. The control group overall showed the same preference for resting in the shaded chamber as in previous experiments (mean proportion in shaded chamber > 0.5 at 0.5, 1 and 24 h) but the mean proportion was not as great and the standard error of the proportions was larger than for previous control groups. The reason for this effect was unknown, since the cockroaches were treated identically in this experiment as in previous ones. Cockroaches allowed to recover from anaesthetisation in the untreated illuminated chamber and then given access to the shaded treated chamber showed a clear preference for the illuminated chamber. The hypothesis of a 1:1 distribution of these cockroaches between the two chambers was rejected at the 0.05 level at 0.5 h and 24 h but not at 1 h for cockroaches which spent their 10 min dark recovery period in the untreated illuminated chamber. Cockroaches which spent the 10 min dark recovery period on the deposit and were then activated by illuminating this chamber showed a slight preference for resting in the illuminated chamber at 30 min (mean proportion, 0.41 in shaded chamber) but the hypothesis of a 1:1

distribution was not rejected at $p < 0.05$ at all 3 observation times (Table 3.11).

3.3.10.3 Distribution of cockroaches pre-exposed to permethrin deposits between shaded and illuminated parts of arena following treatment and after 24 and 48 h recovery periods

Permethrin 25% WP was sprayed onto filter paper at doses corresponding to a range from sublethal (< LC5) to an LC75 (30 min exposure, 24 h mortality, Section 3.3.10.1). The cockroaches were exposed to the deposit for 30 min (in the light), and then anaesthetised with carbon dioxide, transferred to the arena and allowed to recover for 10 min in the dark prior to illuminating the recovery chamber. Control groups were exposed to untreated filter paper. At the end of the 30 min observation period all the cockroaches exposed to a particular concentration were transferred to a holding container, maintained with food and water for 24 h, and survivors then retested using the same procedure. The same procedure was then again adopted with survivors after a further 24 h. No survivors were available for experimentation at 24 or 48 h following exposure at 6.4 mg/m^2.

The proportion of surviving cockroaches in the shaded chambers at 30 min, 24 h and 48 h is shown in Table 3.12. This proportion appeared

TABLE 3.11

Distribution of cockroaches in half-treated illuminated/shaded choice arena with 25% WP permethrin deposite at 1.25 mg/m²

treated chamber	mean (s.e.) % in shaded chamber 30 min	p[2]	60 min	p	24 h	p
illuminated chamber	41.3 (34.4) a[1]	n.s	63.8 (29.2) a	n.s	62.5 (26.0) a	n.s
shaded chamber	11.3 (13.6) b	*	16.3 (20.0) b	n.s	7.5 (8.9) b	*
control	51.3 (34.4) a	n.s.	53.8 (38.5) a	n.s	66.3 (34.2) a	n.s

[1] means in columns followed by same letter are not significantly different (Duncan's multiple range test, $p < 0.05$).

[2] hypothesis of 1:1 distribution between two chamber. n.s $p > 0.05$, * $0.01 < p < 0.05$.

TABLE 3.12

Distribution of treated cockroaches following exposure to 25% WP permethrin deposit in arena

dose (mg/m²)	mean (s.e.) % of cockroaches in shaded chamber 30 min	24 h	48 h
0.0	66.0 (13.4)	66.0 (11.4)	66.0 (13.4)
1.0	84.0 (5.5)	96.0 (5.5)	96.0 (5.5)
2.0	80.0 (15.8)	96.0 (5.5)	96.0 (5.5)
3.4	56.0 (15.2)	88.0 (10.9)	98.0 (4.5)
5.4	40.0 (12.3)	73.3 (23.1)	100.0 (0.0)
6.4	16.0 (15.2)		

to decrease for exposures to increasing concentrations of permethrin deposit for groups tested 30 min after being given access to the shaded chamber ($y = 100 - 12x$, $r^2 = 0.97$) if the control treatment is omitted from the regression ($r^2 = 0.78$ if control included), and the same effect is evident in the 24 h data ($r^2 = 0.94$ without control, $r^2 = 0.00$ with control), but not for survivors tested at 48 h. More of the survivors tested at 24 and 48 h appeared to prefer to settle in the shaded chamber than the original group tested immediately after exposure at any dose level, but since the same cockroaches were tested at each observation time it was not possible to test this effect statistically. Similarly, the groups which survived treatment at any dose level appeared more likely to be found in the shaded chamber than in the illuminated one 24 and 48 h following exposure than groups unexposed to the permethrin deposits.

3.3.10.4 Exposure to permethrin deposits in a three-chambered arena

The three chambered arena was orientated with the long axis perpendicular to the light source. Light intensity measurements in the chamber closest to the source and the central chamber corresponded to those measured in Section 3.1 and Table 3.2. Intensity measurements were not made for the shaded chamber furthest from the light source but can be estimated approximately from the empirical relationship

established in Section 3.1. The chamber furthest from the light source was shaded.

The deposits of permethrin to which the cockroaches were exposed during the 10 min recovery period ranged from 4.8 mg/m² to 31.3 mg/m² but since many of the cockroaches were anaesthetised and on their backs at the beginning of the recovery period these concentrations cannot be related to LC or EC values using the probit regressions of Section 3.3.10.1. The proportions of cockroaches in the chamber closest the source, the central, and the shaded chambers at 30 min following illumination together with statistical analysis are shown in Table 3.13.

It is difficult to identify any clear trends from this experiment. The higher dose rates probably correspond approximately to those used in cockroach control operations, and the experiment was designed to identify any preference for illuminated or shaded environments following exposure under these conditions. The cockroaches showed no particular evidence of hyperactivity following the 10 min exposure period, and when illuminated and given access to the two escape chambers they ran around the arena and eventually settled in one of the chambers within the observation period of 30 min as in experiments in which they were not exposed to permethrin.

TABLE 3.13

Distribution of cockroaches in the three-chambered choice arena at 30 min observation period after 10 min exposure to the deposits of 25% WP permethrin on the base of central chamber at various concentrations before being given access to escape chambers

dose (mg/m^2)	mean[1] (s.e.) % of cockroaches illuminated chamber	shaded chamber	p[2]	central chamber
0	11.4 (10.7)	27.1 (18.0)	n.s.	61.4 (19.5)
4.8	20.0 (20.9)	13.3 (23.4)	n.s.	66.7 (27.3)
7.7	38.3 (19.4)	13.3 (10.3)	*	48.3 (24.8)
8.0	40.0 (11.0)	18.3 (21.4)	n.s.	41.7 (25.6)
12.9	25.0 (17.6)	26.7 (26.4)	n.s.	48.3 (22.3)
13.6	40.0 (36.9)	15.0 (12.3)	n.s.	45.0 (40.4)
18.0	15.0 (13.8)	13.3 (17.5)	n.s.	71.7 (22.3)
22.6	14.0 (16.7)	10.0 (10.0)	n.s.	78.0 (14.8)
31.3	30.0 (14.1)	6.0 (5.5)	*	64.0 (16.7)

[1] seven replications in control arena; six replications for concentrations of 4.8, 7.7, 8.0, 12.9, 13.6 and 18.0 mg/m^2; five replications for concentrations of 22.6 and 31.3 mg/m^2.

[2] t test for hypothesis that mean percentages (angles) between illuminated and shaded chambers are not significantly different. n.s $p > 0.05$, * $0.01 < p < 0.05$, ** $p < 0.01$.

A comparison between the proportion of cockroaches in the illuminated untreated chamber and in the shaded untreated chamber at 30 min following access to the escape chambers indicated a significantly greater number of cockroaches in the light chamber (Student's t test, $p < 0.05$) at 2 concentrations out of 8 tested; these 2 corresponded to the highest, and one just higher than LC50 for 30 min exposure and 24 h mortality. In the other cases, there was no significant difference between the proportions in the two chambers. There was no clear correlation between the proportion of cockroaches in any of the 3 chambers and the concentration of the permethrin deposit ($r^2 = 0.00$, 0.59, and 0.15 for the illuminated, shaded and central chamber respectively).

3.4 Discussion

The main pest species of cockroaches are reported to show a number of distinct reactions to light: they become disturbed when suddenly exposed to both long and short wavelength uv light following dark adaptation and show an increased level of activity (Wharton, 1971; Gingrich, 1973; Leppla et al., 1989); they are entrained to a diurnal rhythm of activity by suitable cycling of either long uv light (365 nm) or light from an incandescent lamp (Gunn, 1940, Driesig and Neilson, 1971; Leppla et al., 1989); and natural infestations have been reported

not to emerge from harbourages into a room ~~continually~~ continuously lit with an incandescent lamp during their dark active phase when alternative dark areas were available (Mellanby, 1940). Driesig and Nielson (1971) also reported that cockroaches held in continuous light for long periods tend to remain inactive.

While *B.germanica* possesses ocelli and photosensitive abdominal ganglia, the compound eye appears to be the primary photoreceptor (Brady, 1974). This organ has two kinds of photoreceptors: one detects long wavelength ultra-violet light with a peak sensitivity at 365 nm and the other blue-green light with peak sensitivity at 490 nm (Koehler et al., 1987). Leppla et al. (1989) suggested that uv light provides direct nervous stimulation whereas blue-green light requires central nervous system integration prior to entraining behavioural responses such as the circadian rhythm of activity. UV light below 300 nm is lethal to German and American cockroach nymphs, producing mortality up to 6 days after exposure (Cohen et al., 1973, Gingrich, 1973), presumably explaining the stimulation and avoidance reaction shown to it.

Gingrich (1973) found that avoidance of light by American cockroaches increased with growth stage and that adults given the choice between a dark environment and short wavelength uv, long wavelength uv (> 365 nm) or a white fluorescent lamp in small, two-chambered arenas showed a

significant preference for the dark chamber ($p < 0.05$) within 15 min of illumination in each case. The present author has also observed that *B. germanica* colonies allowed to harbour for a number of days in a dark area showed rapid movement towards the dark when part of the harbourage was suddenly illuminated during photophase. Bell *et al.* (1983) reported that individuals of the species *Blaberus craniifer* tended to turn away from a light beam if it was directed towards them. However, while the activation produced by sudden illumination was consistently obtained in the bioassay arenas described in this Chapter it proved remarkably difficult in these experiments to reproduce a light avoidance behaviour with *B. germanica* of the same magnitude as that reported by Gringrich.

The light-avoiding reaction has not been examined systematically in terms of the difference in intensity between the two halves of an arena, although Ballard *et al.* (1984) reported that a light intensity of 6.0 uE/m²/s in the light half of an Ebeling choice box (intensity not reported in dark half) did not result in a significantly different catch using traps in the light half of the chamber than when uniformly dark conditions were used, and Raucher (1985) showed a significant preference by *B. germanica* for the dark chamber of Ebeling choice boxes for light intensities of less than 5 uE/m²/s in the dark chamber and 6.5 - 7.5 uE/m²/s in the light chamber. The latter work demonstrated that the presence or absence of light in a chamber of the choice box

was the only factor (of food, water, insecticide, and light) which consistently influenced the distribution of German cockroaches in choice boxes.

The fluorescent light source used in the present work had a significant long wavelength uv component which was not filtered by the perspex arena and was similar in spectral characteristics to that used by Gingrich (1973) and by Miall and le Patourel (1986). The activation produced on illumination confirmed that the intensity of light in the arena was sufficient to be perceived by the 365 nm peak sensitivity photoreceptors.

The initial experiments reported here were designed to optimise the design of the bioassay arena and experimental procedure to obtain the maximum light avoidance reaction. Time did not permit full exploration of all variables, and it is thought that there remains considerable scope for experimentation.

The results obtained when the arena was orientated parallel to the light source (maximising the intensity difference between the two chambers) indicated a greater preference for resting in the shaded chamber than when the arena was orientated perpendicular and the source was visible to the cockroaches in either chamber. It was thought that the perpendicular arrangement of the arena would most

closely resemble the natural conditions under which cockroaches either approach or retreat from the entrance to a harbourage following day-time spraying, but the greater difference in light intensity in the parallel arrangement was presumably the cause of the greater light avoidance reaction and so this arrangement was adopted in most of the subsequent experiments. Neither absolute light intensity nor difference in intensity between the two chambers appeared to markedly affect the light avoidance response in the both orientations, and it is possible that the response is triggered at a threshold below any that were used in these experiments. The response was not large with the arena orientated parallel to the light source, and although the mean numbers in the shaded chamber were consistently greater than those in the illuminated chamber the asymmetry of distribution was not significant at any of the experimental distances of the arena from the source.

The possibility that the cockroaches might "condition" the chamber in which they spent the 10 min recovery period by depositing faeces and aggregation pheromone, thus making this chamber the preferred resting area after activation by illumination, was not supported by the experiment in which both chambers were equally illuminated and the experiment in which the cockroaches spent the 10 min recovery period in either shaded or illuminated chamber, since the resulting distribution of cockroaches was close to 1:1.

There appeared to be some indication that it was possible to increase the proportion of cockroaches choosing to rest in the shaded chamber if they were exposed to periods of illumination following recovery and before being given access to the shaded chamber. The asymmetry of distribution was not correlated with the length of light exposure (r^2 = 0.12), but was significant at $p < 0.05$ for exposures of 20 and 25 min. The experiment was intended to reveal any tendency for cockroaches to become increasingly stressed on exposure to light and to relieve such stress by moving to the shaded chamber when it was made available to them, but the results were inconclusive in this respect.

The experiment in which the photocycle was altered to continuous light or continuous dark pre-exposure produced significant results. The control cockroaches showed the normal slight preference for the dark chamber, while cockroaches either kept in continuous light or in continuous dark for the preceding 24 h showed a significant preference for the dark chamber. However, the explanation for the results is unclear.

The possibility that carbon dioxide anaesthetisation could have affected the preference for the dark was investigated. Carbon dioxide anaesthetisation produces hyperactivity on recovery and if repeated a number of times during the development of the insect can produce extra moults - symptomatic of stress conditions and also produced by poor

food or loss of appendages. Many experimenters allow longer periods of recovery from carbon dioxide prior to initiating behavioural studies, generally of the order of 1 h (Ballard *et al.*, 1984) and recovery time was an important variable not investigated in the present series of experiments. Examination of the behaviour of cockroaches following carbon dioxide recovery showed an initial period of hyperactivity similar to that shown after pyrethrin treatment. In a large arena (100 x 50 cm) where free running was possible cockroaches recovering from anaesthetisation generally showed brief intermittent periods of rest between bouts of rapid running, either spontaneous or when touched by another insect. This distinct phase lasted about 5-8 minutes, and was followed by apparently normal activity consisting of resting interrupted by brief periods of walking at a normal rate. These observations were consistent with those observed in the small arenas used in these tests.

Unanaesthetised cockroaches showed a significant ($p < 0.05$) preference for the shaded chamber whereas the corresponding preference of cockroaches anaesthetised once was not significant at this level. However there was no correlation between the preference of cockroaches for the shaded chamber and the number of times they were anaesthetised, and since cockroaches anaesthetised four times also showed a significant preference for the shaded chamber it was concluded that within this arena and under these experimental conditions the

anaesthetisation process had no marked effect on the light avoidance reaction.

An experiment was designed to establish whether or not the light avoidance reaction was an inherent characteristic of individual cockroaches, since the colony used in these experiments might have drifted genetically from the original field sample and the behavioural response lost from a proportion of the individuals. The results show clearly that this was not so, the proportion of "dark-seeking" individuals preferring the shaded chamber on re-exposure to light being approximately equal to that of the original sample from which these individuals were selected.

In spite of the rather poor light avoidance reaction obtained in these initial experiments, it was decided to test the effect of permethrin exposure on it. The LC50 value (30 min exposure) obtained with the 25% permethrin WP in continuous contact tests was comparable to that obtained by Chadwick (1971) and provided an index by which to gauge the toxicity of deposits to which *B. germanica* was exposed and their relationship to a field application rate.

The cockroaches were exposed to the permethrin deposit for 10 min during their recovery from anaesthetisation, the deposit corresponding to less than LC50 over a 30 min exposure. The results were difficult to

interpret, since cockroaches which were exposed in the chamber which was subsequently illuminated showed a significant preference ($p < 0.05$) for this chamber following lights-on, comparable to the response reported by Miall and le Patourel (1986), whereas the cockroaches which were exposed in the chamber which was subsequently shaded showed no greater difference in their preference for illuminated or shaded chamber than did untreated cockroaches. However, the experiment did appear to rule out the possibilities of (a) cockroaches preferring to harbour in the chamber in which they were exposed because of increased levels of aggregation pheromone during the initial phase of intoxication, and (b) cockroaches preferring to rest in the untreated chamber because of the excito-repellent properties of the permethrin deposit.

The experiment using the three chambered arena was similar to the one described by Miall and le Patourel (1986), giving cockroaches the option of resting either in an untreated shaded chamber or an illuminated one or remaining on the treated deposit in the illuminated central chamber. The arena was orientated perpendicular to the light source so that it was visible to the cockroaches from all three chambers. The tendency to accumulate in the illuminated chamber was evident in most of the tests and was significant at $p < 0.05$ in two of them, supporting the view that the reversal of light avoidance behaviour following exposure was a genuine effect, but also indicating

that it could not be produced consistently using this arena and bioassay method.

CHAPTER 4

HATCHING OF OOTHECAE FROM FEMALE *Blattella germanica* EXPOSED TO HYDRAMETHYLNON AND BORIC ACID BAITS

4.1 Introduction

Bait formulations of blatticides provide a useful alternative to the use of residual deposits for control of cockroach infestations, particularly in domestic and other sensitive situations where insecticidal sprays constitute an unacceptable toxic, electrical or other hazard. They can be distributed unobtrusively in tamper-proof containers, and provided the formulation is stable they can be used for long term control in complex environments, particularly where re-invasion makes eradication impossible. Baiting may also be useful as a follow-up treatment to selective spray operations with residual insecticides.

Boric acid has been widely used in bait formulations for cockroach control, sometimes mixed with other insecticides. It is slow-acting, but non-repellent to cockroaches (Ebeling *et al.*, 1967). The symptomology of poisoning suggests either direct or indirect action on the nervous system, but no cross-resistance has been recorded from populations resistant to other groups of neuroactive insecticides. An alternative commercial bait formulation (Maxforce®) is based on

hydramethylnon, an amidinohydrazone insecticide which suppresses mitochondrial respiration through the inhibition of the electron transport chain (Hollinghaus, 1987). The toxicity of this compound varies markedly with temperature, a 10 fold decrease in LC50 to *B. germanica* being observed on raising the treatment temperature from 27°C to 38°C (Hollinghaus and Little, 1984; Silverman and Shapas, 1986). The compound is rapidly broken down by light (Mallipudi *et al.*, 1986) and is formulated in a waxy paste within sealed opaque plastic bait stations. Trials using these baits suitably distributed at a rate of 12 stations per apartment have given good control of *B.germanica* populations in a residential complex in Florida for at least 6 months (Milio *et al.*, 1986) and similar results have been reported from Japan (Hayashi *et al.*, 1986).

The effectiveness of both bait formulations and residual deposits of insecticides for control of *Blattella germanica* (L.) depends in part on the fate of oothecae carried by females which succumb to the poison. Exposure to deposits of a variety of neuroactive insecticides causes females carrying oothecae to drop them prematurely, but many of these oothecae hatch normally when their development period is complete (Woodbury, 1938), the proportion increasing with the relative humidity of the environment (Barson and Renn, 1983). Hatch rates may be somewhat reduced if the ootheca is deposited on an insecticidal deposit, and the new nymphs may pick-up a lethal dose during the first few hours

following emergence (Harmon and Ross, 1987). However females ingesting slow-acting poisons at bait stations may die or shed their ootheca at locations well removed from the toxicant, and nymphs emerging from these oothecae have the potential to maintain an infestation.

Barson (1982) showed that female *B. germanica* exposed to both boric acid and iodofenphos baits drop their oothecae prematurely, and that there was a reduction in the number of ootheca which hatched following deposition by females poisoned with boric acid compared with those deposited by untreated females. No information is available on the fate of oothecae carried by females when poisoned by hydramethylnon.

While *B. germanica* breeds optimally at around 28°C it is not unusual to find cockroaches foraging away from locally warm habitats, and baits may be positioned in such areas. Also *B. germanica* tends to become disorientated and wander erratically when poisoned by boric acid (Barson, 1982), and such movement may result in cockroaches dying outside locally warm habitats. The toxic effects of boric acid and hydramethylnon bait formulations and the fate of oothecae of poisoned females were thus examined at both 20°C and 28°C.

4.2 Materials and Methods

4.2.1 Insects

New adults were separated from tins containing nymphs (VI) within 7 days of emergence and were kept together for a further 7 days. Females carrying oothecae were separated and either used directly (7-14 day old adults) or after a further 7 days (14-21 day old adults).

4.2.2 Baits and formulations

Maxforce® bait station formulations of hydramethylnon (1.65% w/w a.i.) were obtained from Wellcome Research Ltd., Crewe and were stored at 5°C in sealed bags prior to use. Boric acid was of British Pharmacopoeia grade and was purchased locally as finely powdered crystals. Bait formulations were prepared by mixing boric acid with powdered dog chow pellets in a 1 : 1 w/w ratio and packing 2.0 g of the mixture into carefully cleaned and dried Maxforce® bait stations from which the hydramethylnon formulation had been removed.

4.2.3 Experimental bioassay

The experimental arenas were polythene containers (30 x 30 x 16 cm) each containing a bait station containing either 1.4 g of the Maxforce® formulation of hydramethylnon at 1.65% w/w a.i. in a waxy substrate or 2.0 g of the boric acid/powdered dog chow mixture at the centre of the containers, an open petri dish containing whole dog chow pellets (10 g) on one side and a water reservoir on the other. The relative humidity (r.h.) in each container was maintained at 90% using a saturated solution of barium chloride suspended from the lid.

4.2.4 Exposure of oothecae

Fifty female cockroaches 2-3 weeks from adult emergence and carrying oothecae were transferred from the culture tins to polyethylene containers and incubated at 20°C or 28°C in a 12 : 12 L:D photocycle, the experiment being timed from the point of transfer. In the controls the cockroaches were provided with food and water but no bait station. Bait stations were placed at the centre of the box, with an open petri dish containing the food pellets on one side and the water reservoir on the other. Three replicate boxes were incubated at two temperatures and adult mortality was recorded at 24 h intervals. The experiment was repeated with female adults 1-2 week old from emergence and with

ootheca visible at the start of the experiment. Exposure was continued until all the cockroaches had died.

At the end of the exposure period the number of females which had deposited their oothecae, the number of these oothecae which had hatched following deposition, and the number of oothecae which had hatched while still attached to the dead female cockroach was counted. Unhatched oothecae deposited during the exposure period and dead cockroaches carrying oothecae were transferred to individual specimen tubes lightly plugged with cotton wool, returned to the containers and monitored daily. Oothecae were scored as viable if they retained a dark green stripe of meconium along the side of the case. Non-viable oothecae acquired a shrunken appearance, darkened, and one or more egg cells turned black; such oothecae were retained for periodic examination for 4 weeks following apparent loss of viability but never subsequently hatched. The total number of egg cells and the number of cells containing unhatched embryos were also recorded. The time course of deposition and hatch of oothecae from untreated batches of females of both age groups was determined in the same manner.

A second experiment investigated the cause of the reduced hatch of oothecae from 2-3 week old females exposed to hydramethylnon baits at 20°C. Groups of females were either (i) exposed to hydramethylnon baits as above; (ii) treated as the control groups above, but their

oothecae manually removed; or (iii) decapitated and the thorax sealed with paraffin wax at 10 days after exposed to hydramethylnon baits (the time for 50% of this batch of cockroaches to succumb to the hydramethylnon bait at 20°C), or (iv) treated as controls. Three replicates of each test were carried out, using 35, 40 and 50 females per replicate. Five more replications were carried out for each test using 12 females per replication. The same data as for the first experiment was recorded.

4.3 Results

4.3.1 Symptomology of poisoning

Symptoms of poisoning by hydramethylnon appeared in some cockroaches after the first day of exposure to the bait station, affected individuals remaining motionless for long periods with their legs splayed. Cockroaches showing obvious symptoms of poisoning generally died within the subsequent 48 h.

The initial symptoms of boric acid poisoning comprised an increase in the level of activity, generally of a discoordinated nature. During the first 36 h of exposure to the bait, when a large proportion of the

population was still apparently unaffected, some individuals showing symptoms of poisoning were subject to cannibalism, their heads and abdomens being partly eaten at death. Shortly before death many cockroaches became motionless, standing with legs extended vertically but with antennae moving normally.

4.3.2 Effect of temperature, age and sex on susceptibility of adults to hydramethylnon and boric acid baits

Fertilised females 14-21 days from emergence and carrying oothecae showed a positive temperature coefficient of tolerance to hydramethylnon baits with respect to lethal response time under the bioassay conditions. Figure 4.1 shows the cumulative mortality of such cockroaches exposed to the baits at 28°C and 20°C. At 20°C the estimated LT50 was 6 days while at 28°C it was 3 days; similarly the mean time for all individuals in the populations to succumb to the baits at 20°C was nearly twice that at 28°C. Fertilised females taken from the culture 7-14 days following adult emergence showed a similar pattern of susceptibility to the baits at the two temperatures (Figure 4.1). Boric acid also had a positive temperature coefficient of action but this was less marked than for hydramethylnon. The toxicity of the boric acid baits to 14-21 day old adult female populations was slightly greater than that of the hydramethylnon baits at both 28°C

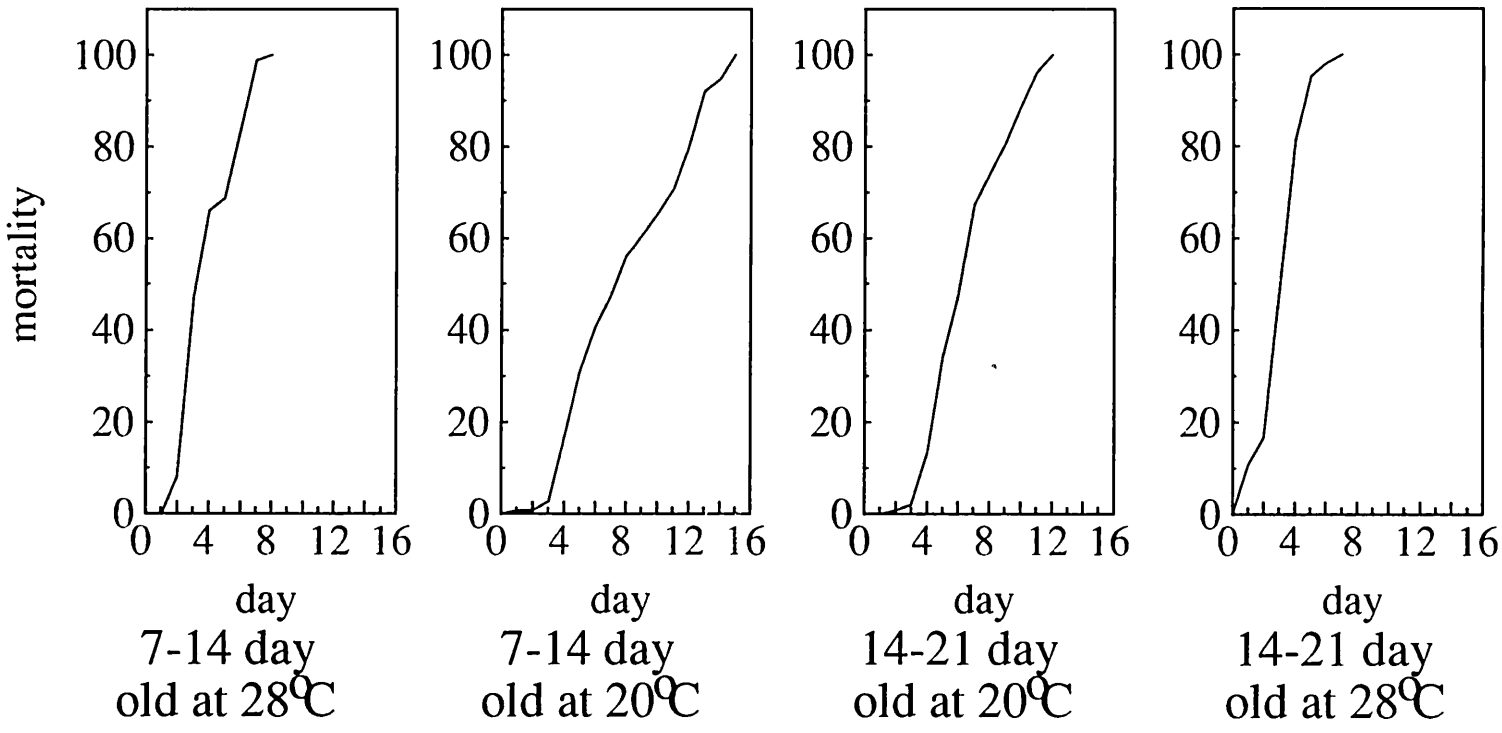


Fig.4.1 Cumulative mortality of B. germanica female adults exposed to hydramethylnon baits.

and 20°C as measured by time to 50% mortality, but the effect of temperature on the toxicity of the baits was not so great (Figure 4.2, estimated LT50's: 1-2 days at 28°C and 3-4 days at 20°C). Similar results were obtained for populations of 7-14 day old females exposed to boric acid baits.

The experiments were repeated ~~several~~ three times with different batches of cockroaches, and while some variability (1-2 days) in the time to 50% mortality was observed between batches the relative values at the different exposure temperatures and between the two bait formulations remained approximately constant.

4.3.3 Fate of oothecae

4.3.3.1 Untreated females

First oothecae were extruded from the genital pouch of females about 10 days after adult emergence, so that gravid females selected from 7-14 day old adult cultures carried oothecae that were up to 4 days old and those from 14-21 day old cultures carried oothecae that were between 4 and 11 days old. Untreated females deposited their oothecae either immediately before or during the hatching process. The

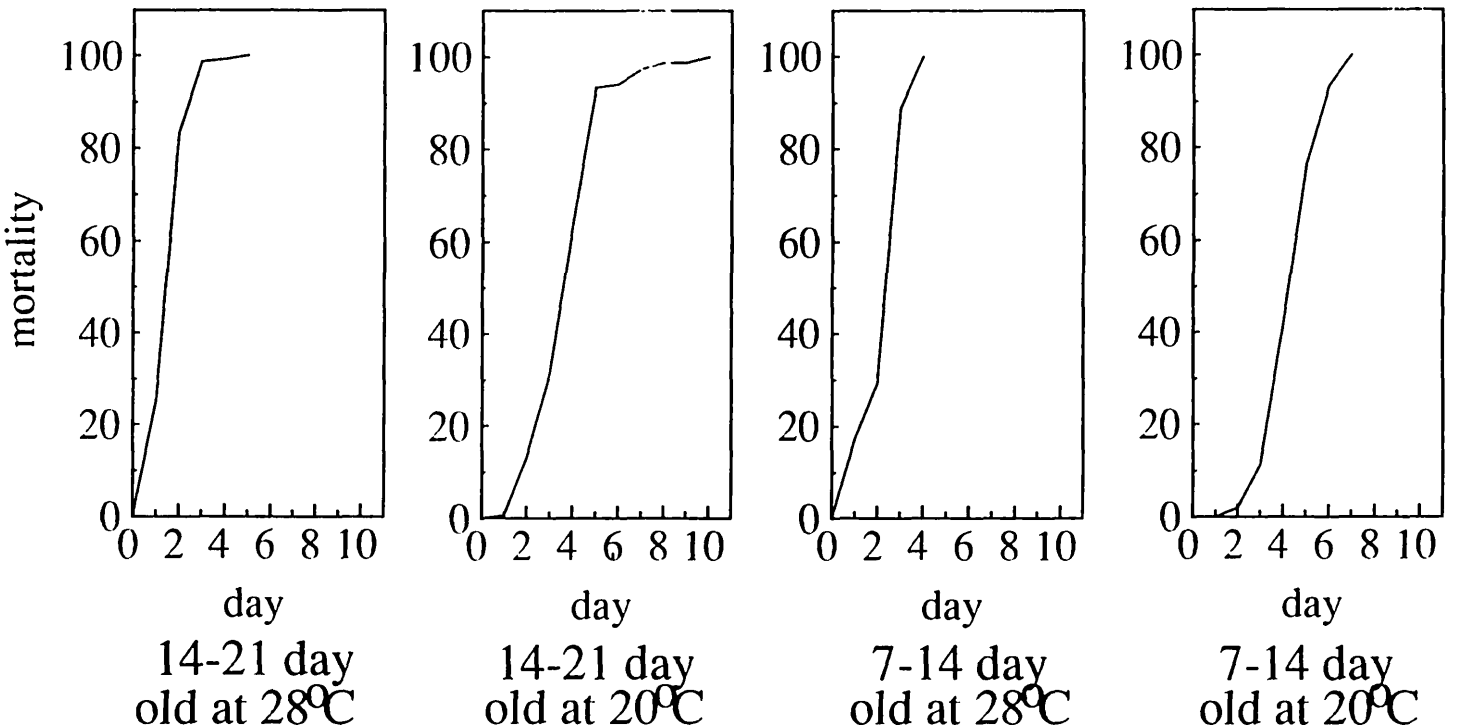


Fig. 4.2 Cumulative mortality of B. germanica female adults exposed to boric acid baits.

cumulative percentage hatch of oothecae from females of both age groups cultured at 28°C and then transferred to containers either at 20°C or 28°C is shown in Figure 4.3. Hatch of oothecae took place over a 15 day period with 96% hatch for the 14-21 day old adults and over a 14 day period with 90% hatch for the 7-14 day old adults at 28°C. Oothecae from 14-21 day old females transferred to containers maintained at 20°C started to hatch after 10 days, with all viable oothecae (87%) hatching within 43 days of transfer. Hatch of oothecae from 7-14 day old females started at 17 days after transfer and was complete by the 47th day, the mean hatch of oothecae from this group only reaching 52% (Figure 4.3).

4.3.3.2 Females exposed to hydramethylnon bait

Females (150 in three batches of 50 at 14-21 days from emergence) were exposed to hydromethylnon baits at 28°C until all had been killed (7 days). At the end of the exposure period 138 (92%) females had died with their oothecae still attached, all of these oothecae had not hatched but appeared viable; 12 females deposited their oothecae, 10 of them were viable (Table 4.1). The fate of all unhatched oothecae is shown in Figure 4.4. All had either hatched or lost viability within 14 days after the end of the baiting period; overall, 121 (88%) of the 138 oothecae ultimately hatched. Of the 12 oothecae deposited

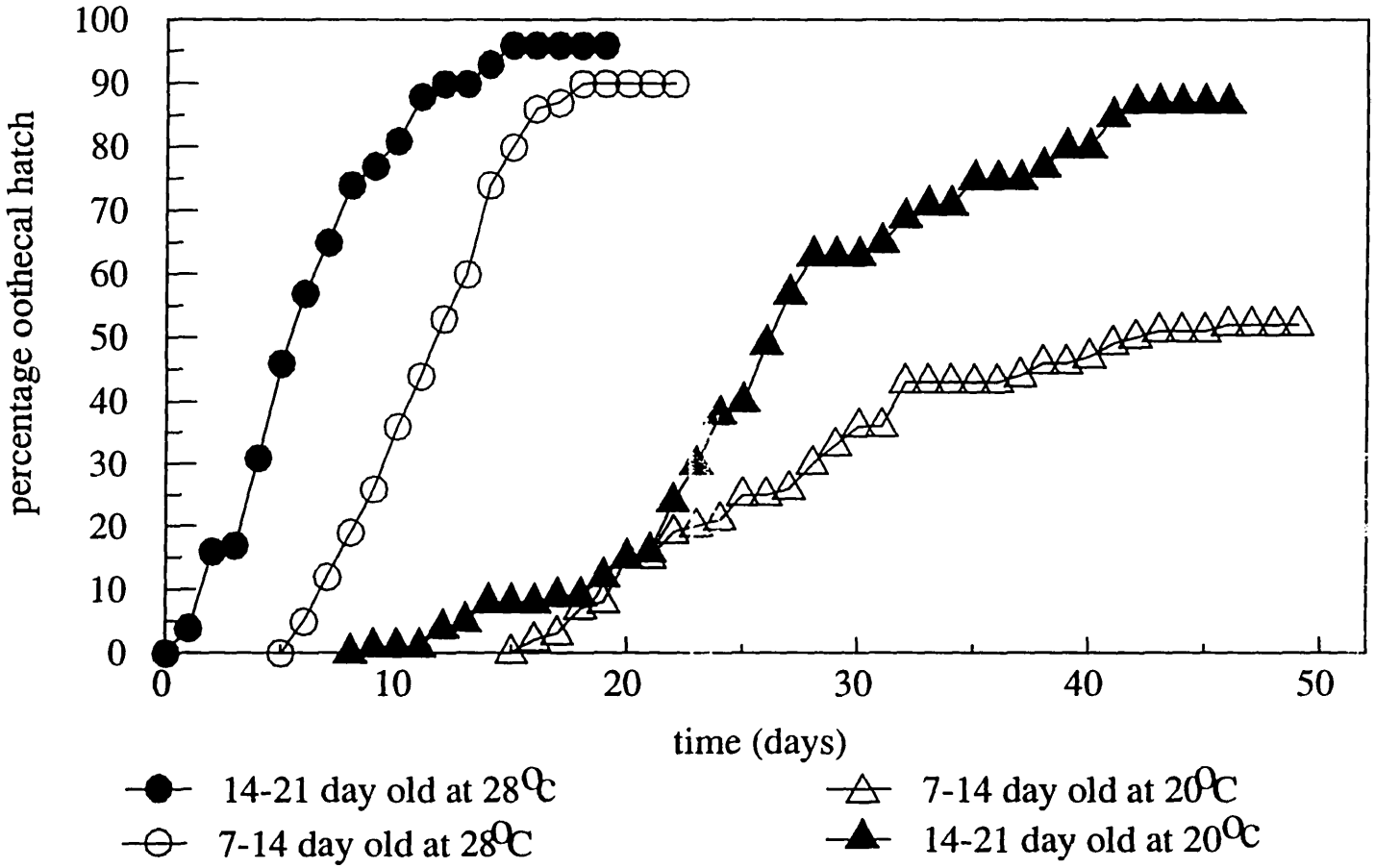


Figure 4.3 Cumulative hatch of oothecae carried by female adults of B. germanica (control group).

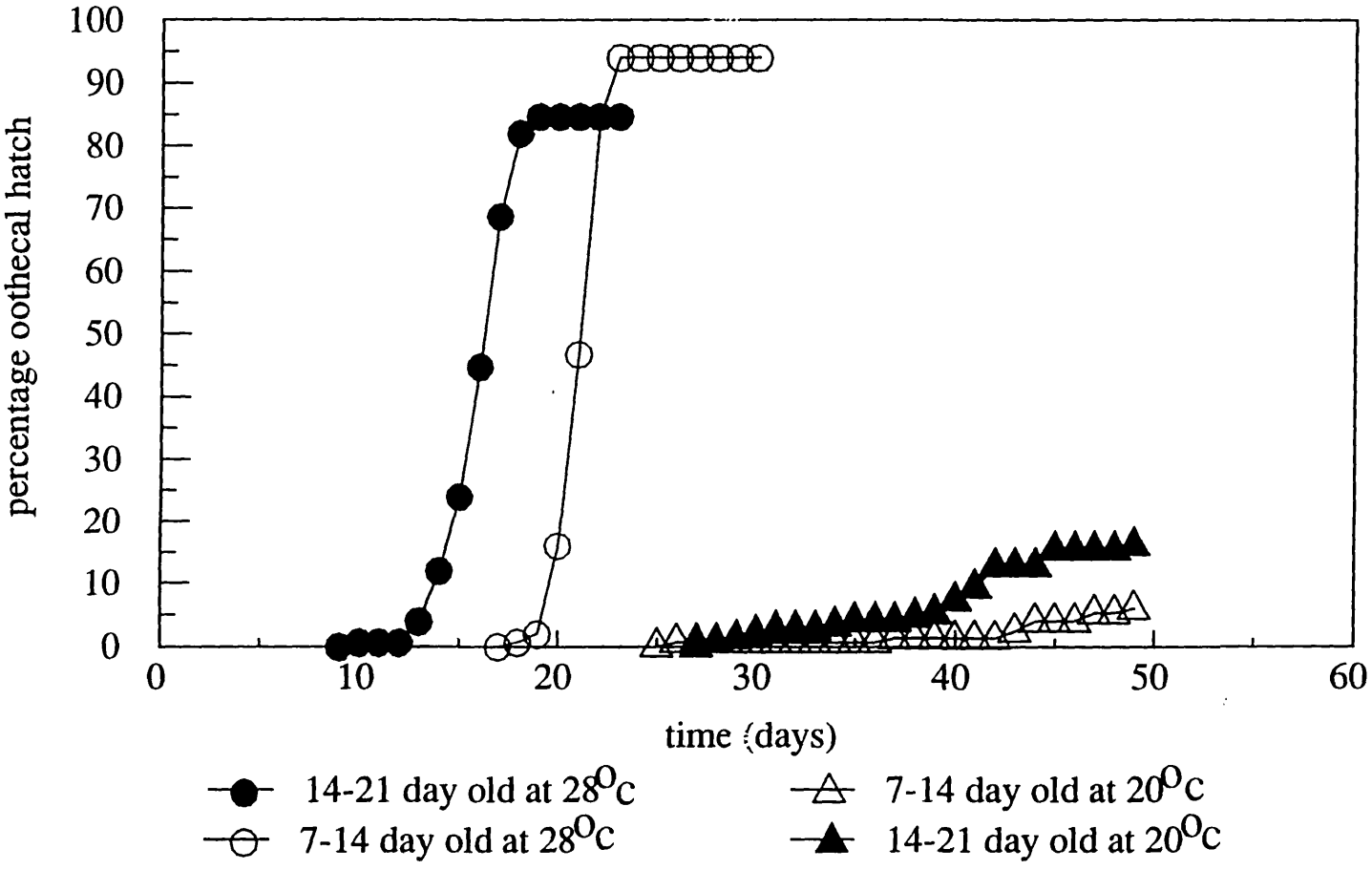


Figure 4.4 Cumulative hatch of oothecae carried by female adults of B. germanica exposed to hydramethylnon baits.

by the females prior to death 4 oothecae hatched, and the remainder of the oothecae desiccated during the experimental period (Table 4.2).

A period of 12 days exposure to the hydramethylnon baits was required to achieve complete mortality in populations of 14-21 day old females following removal from the culture and exposure at 20°C; the fate of all unhatched oothecae was determined within a further 39 days incubation at this temperature. The number of oothecae retained by the females at death (96%) was approximately the same as at 28°C, all were unhatched but apparently viable at the end of the baiting period (Table 4.1). However, the majority (83%) of these oothecae failed to hatch and subsequently desiccated. All the oothecae deposited by the females during the baiting period (6) lost viability either during this period or subsequently (Table 4.2).

Similar results were obtained for the 7-14 day old females (Table 4.1 and 4.2, Figure 4.4) at 28°C, the period of maximum ootheca hatch lagging that of the 14-21 day old group by about a week and 96% of the oothecae attached to the female ultimately hatched. Only 1 of the 4 oothecae deposited by the females prior to death subsequently hatched.

The time required to kill all the 7-14 day old females (15 days) at 20°C was similar to that of the older group (Figure 4.1). Most females (85%) retained their oothecae at death but few of these

TABLE 4.1

Fate of oothecae from adult female *B. germanica* exposed to boric acid or hydramethylnon baits

insecticide bait	temperature (°C)	age of adult pre-baiting (days)	number of oothecae						total oothecae tested
			attached to female			deposited prior to death			
			hatched	viable	non-viable	hatched	viable	non-viable	
boric acid	20	7-14	0	58	0	0	89	3	150
		14-21	0	78	0	0	60	3	141
	28	7-14	0	52	0	0	93	5	150
		14-21	0	83	0	0	59	3	145
hydramethylnon	20	7-14	0	127	0	0	16	7	150
		14-21	0	144	0	0	4	2	150
	28	7-14	0	146	0	0	3	1	150
		14-21	0	138	0	0	10	2	150

TABLE 4.2

Fate of oothecae from adult female *B. germanica* exposed to boric acid or hydramethylnon baits

insecticide bait	temperature (°C)	age of adult pre-baiting (days)	experimental period (days)	number of oothecae				total oothecae tested
				attached to female		deposited prior to death		
				hatched	non-viable	hatched	non-viable	
boric acid	20	7-14	40	0	58	2	87	150
		14-21	40	20	58	5	59	141
	28	7-14	26	41	11	50	48	150
		14-21	25	57	26	27	35	145
hydramethylnon	20	7-14	57	11	116	0	23	150
		14-21	51	24	120	0	6	150
	28	7-14	26	140	6	1	3	150
		14-21	21	121	17	6	6	150
control	20	7-14	47	0	0	52	48	100
		14-21	43	0	0	87	13	100
	28	7-14	19	0	0	90	10	100
		14-21	15	0	0	96	4	100

oothecae (7.3%) subsequently hatched irrespective of whether they were attached to the female body or had been deposited prior to death (Table 4.1 and 4.2, Figure 4.4).

The hydramethylnon treatment significantly reduced the hatch rate relative to the control for both age groups at 20°C (Table 4.3). However, the hydramethylnon treatment to the adult females did not significantly affect the hatch of oothecae of German cockroaches at 28°C for both age groups (Table 4.3).

4.3.3.3 Females exposed to boric acid

The total hatch of oothecae from the females poisoned with boric acid at 28°C was significantly lower than that of oothecae from untreated females at both temperatures and for both age groups (Table 4.3; Figure 4.5).

The boric acid baits acted more rapidly than the hydramethylnon baits and 100% mortality was achieved in all test populations within 5 days at 28°C and 10 days at 20°C. No hatch of oothecae was observed during baiting period whether attached to or separated from the female (Table 4.1). A small number (3 in both cases) of the oothecae were desiccated at both temperatures. The fate of unhatched oothecae of the 14-21 day

TABLE 4.3

Hatch of oothecae from adult female *B. germanica* exposed to boric acid or hydramethylnon baits compared with untreated females

insecticide bait	temperature (°C)	age of adult pre-baiting (days)	percentage of hatched oothecae (%)
boric acid	20	7-14	1.3
		14-21	1.8
	28	7-14	60.7
		14-21	57.9
hydramethylnon	20	7-14	7.3
		14-21	16.0
	28	7-14	94.0
		14-21	83.3
control	20	7-14	52.0
		14-21	87.0
	28	7-14	90.0
		14-21	96.0

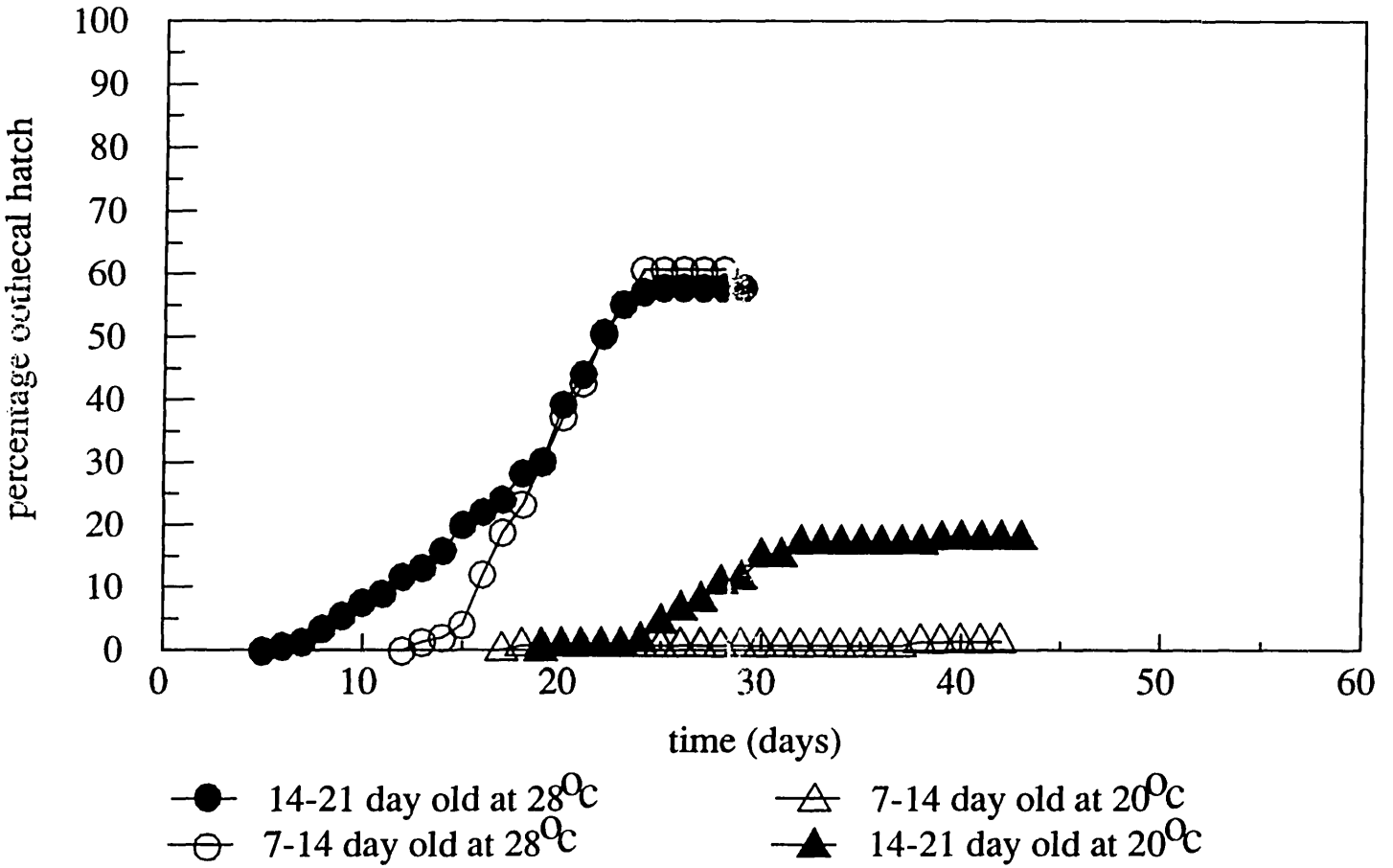


Figure 4.5 Cumulative hatch of oothecae carried by female adults of B. germanica exposed to boric acid baits.

old females, whether attached to the female or deposited, was determined over the subsequent 20 days (Figures 4.5); approximately equal numbers of the two types of oothecae ultimately becoming non-viable. Hatching of both types started 6-8 days after the baiting period and more attached oothecae hatched than oothecae which were deposited (69% vs. 44%; 14-21 day old females, 28° C)

A significantly greater proportion (42.8% of the 14-21 day adult females and 65.3% of the 7-14 day adult females) deposited their oothecae during exposure to the boric acid bait than during exposure to the hydramethylnon bait at 28° C, and similar results were obtained at 20°C. The numbers of oothecae hatching from the two age groups is shown in Table 4.2 and Figure 4.5. At 28°C the hatching success of oothecae deposited prematurely by females was significantly lower ($p < 0.01$ for both age groups) than for those retained by the female at death (51% vs. 79% for 7-14 day old females; 44% vs. 69% for 14-21 day old females); a similar result was obtained for the 14-21 day old group at 20°C ($p < 0.02$) but too few oothecae hatched from the 7-14 day old group to make a comparison.

Table 4.4 Mean (s.e) percentage hatch of oothecae from female *B. germanica* at the end of 30 day observation period under 20°C and 92.5% r.h. (n=8, 12-50 females each test)

control	oothecae manually detached on day 10	oothecae attached to dead females (hydramethylnon-treated)	oothcae attached to dead females (untreated, decapitated)
87.5 (6.2)	65.8 (16.8)	71.8 (16.8)	62.4 (20.5)

4.3.4 Comparison of hatching success of oothecae from hydramethylnon-treated, decapitated, or untreated females and of manually-detached oothecae.

The mean percentage hatch from the four groups of females and the range is shown in Table 4.4. Hatch was reduced significantly relative to the control in the three treated groups of females and the effects of the pre-treatments of the oothecae from 10 day old adult females of German cockroaches was significant ($p < 0.05$). Duncan's test indicated that there was no significant difference between the mean hatch of manually detached oothecae, oothecae attached to dead hydramethylnon-treated females, or oothecae attached to decapitated females ($p > 0.05$).

4.4 Discussion

4.4.1 Untreated cockroaches

The cockroach ootheca is a protective protein capsule enclosing the eggs which is extruded and carried externally by the female until or shortly before the young hatch (Ross, 1929; Roth and Willis 1955a). Oothecae are occasionally dropped prematurely, usually by virgin

females carrying oothecae containing infertile eggs (Roth and Stay, 1962) but under normal circumstances female *B. germanica* retain their oothecae until or shortly before the nymphs emerge. During the maturation process the oothecae remain water-permeable at their anterior end, absorbing water from the female through the genital pouch (Roth and Willis, 1955a). Barson and Renn (1983) showed that the hatch rate of manually-detached oothecae increased with increasing relative humidity, and under high humidity conditions (90% r.h.) more than 85% of oothecae were reported to hatch normally when detached from the female at any time later than 9 days after formation (Roth and Willis, 1955b).

4.4.2 Effect of hydramethylnon bait

Many insecticides which act on the insect nervous system, such as pyrethroid, carbamate and organophosphorus esters, cause female *B. germanica* to deposit their oothecae prematurely, even when applied at sub-lethal doses (Parker and Campbell, 1940; Van den Heuval and Shenker, 1965; Muller and Coch, 1975). Conversely, females poisoned by the respiratory inhibitors n-butyl carbitol thiocyanate (2-(2-butoxyethoxy)- ethylthiocyanate) and rotenone largely retain their oothecae at death (Parker and Campbell, 1940) as with hydramethylnon. It might be anticipated that hatch of oothecae

retained by females at death could be affected by toxicant diffusing from the female corpse, although in the case of females poisoned when their oothecae were still immature a reduction in hatch rate might also be attributed to loss of water transfer from the female. Parker and Campbell reported a 53% inhibition of hatch of oothecae from females poisoned with a dose of n-butyl carbitol thiocyanate sufficient to give 100% kill, but at an unstated temperature, humidity, and stage of oothecal development. Barson (1982) reported a 20% hatch of oothecae dropped by female *B. germanica* exposed to boric acid baits at 27°C and 45% r.h., an effect attributed to the increased likelihood of desiccation at this low r.h., but the fate of oothecae retained by females at death was not reported.

The reason for the reduced hatch of oothecae from females treated with hydramethylnon at 20°C but not at 28°C is unclear. The possibility that it is due to a greater accumulation of the compound in the body of the female at death at the lower temperature resulting in diffusion of the compound from the corpse to the ootheca is not supported by the lack of a significant difference between the hatch of these oothecae, oothecae attached to decapitated females, and oothecae manually detached from the females at the time when 50% of the females exposed to the bait had died. While excretion of hydramethylnon and its residues from topically-dosed *B.germanica* is directly related to environmental temperature, and insects treated with sub-lethal doses

have been shown to retain an appreciable fraction of the compound after 20 days at 16°C while eliminating most of it within the same period at 28°C (Silverman and Shapas, 1986), any diffusion of this accumulated material to the oothecae evidently does not affect their hatch rate at 20°C. An alternative explanation is that at the lower temperature the effect is due to lack of continued water exchange between adult and ootheca, although this would presuppose a marked temperature effect on the water transport process.

4.4.3 Effect of boric acid bait

In the case of oothecae from females poisoned with boric acid, the reduced hatch of prematurely-deposited oothecae at both temperatures is also unlikely to be due to transfer of toxicant since deposition of the oothecae occurred at the same time as the appearance of symptoms of intoxication, with little time for any substantial diffusion from adult to eggs. Increased likelihood of desiccation during the maturation process appears a more probable explanation of the effect (Barson and Renn, 1983). Only in the case of oothecae retained by the females was there the possibility of substantial diffusion of toxicant from female to eggs, but a greater proportion of these oothecae hatched than those prematurely deposited at both temperatures, suggesting that transfer of insecticide was not a significant factor

in reducing oothecal hatch.

Given the potential of oothecae of female *B. germanica* poisoned by insecticides to hatch up to six weeks later and reinfest a treated environment it is perhaps surprising that more attention is not given to determining their fate following the demise of the parent. Both hydramethylnon and boric acid in bait formulations appear able to reduce total oothecal hatch relative to controls at a temperature close to the lower end of the range within which *B. germanica* breeds, and in the case of boric acid the effect is also evident at the optimum breeding temperature, but there is little evidence from these results that transfer of insecticide from the female to the ootheca plays an important part in the process.

The toxic action and time course of poisoning produced by the boric acid baits at 28°C were similar to those reported by Barson (1982) using experimental arenas with a harbourage and alternative food source. Self-dosing at bait stations, particularly by females carrying oothecae, is known to be sporadic when a harbourage is provided (Silverman, 1986) and also depends on the attractiveness of the bait relative to alternative food sources. However, the absence of a harbourage and presence of alternative food in the experiments reported here did not appear to affect the time course of poisoning for this toxicant to any great extent.

The comparatively small effect of temperature on the toxicity of boric acid baits does not appear to have been reported previously and is in contrast to the large temperature coefficient of action for hydramethylnon noted both in experiments reported by Hollinghaus and Little (1984) in which the compound was applied topically and in these experiments.

The mode of action of boric acid has not been established with certainty, although the symptoms of poisoning suggest an effect, direct or indirect, on the nervous system.

CHAPTER 5

ACTION OF AMORPHOUS SILICA DUSTS ON *Blattella germanica*

5.1 Introduction

Amorphous silica dusts and products based on them have been used commercially for cockroach control for at least 30 years. Ebeling (1971) has produced good evidence that amorphous silicas manufactured either by drying silica hydrogels (precipitated silicas) or by burning silicon tetrahalides in oxygen (pyrogenic silicas) act as desiccant insecticides towards many insect species, adsorbing lipid from the surface of the cuticle as insects crawl through loose deposits. Removal of the waterproof lipid layer makes the insect vulnerable to rapid water loss. The potential advantages of desiccant silica insecticides include negligible toxicity to mammals and prolonged persistence of action of undisturbed deposits (Ebeling, 1971).

Because silica deposits are visible and easily disturbed silicas have been used largely to prevent infestations developing in void areas such as hollow walls, behind panelling and in false roofing, rather than as general purpose blatticides. They can be applied using standard or improvised dusting equipment, and provide long-lasting protection against infestation of inaccessible harbourages. When incorporated into buildings at the construction stage they deprive cockroaches of

potential harbourages and the ability to move freely within the structure via ducting and wall-voids (Ebeling *et al.*, 1969).

Silica and silica-based formulations have not proved as generally popular as synthetic organic insecticides for routine disinfestation of premises, for reasons which may include their relatively slow action (Cornwell, 1976) and the "repellency" of treated surfaces to cockroaches (Ebeling *et al.*, 1967, 1971).

The need for an integrated approach to cockroach population management in complex environments has been emphasised by a number of workers, and in view of the limited number of insecticides approved for use as blatticides, available materials need to be examined to determine how they can be used to optimum effect. Amorphous silicas could be used alone or together with conventional insecticides in areas where people may contact surfaces immediately after treatment or where cockroaches have developed resistance to organic insecticides, but their limitations need to be clearly defined.

The first part of this chapter describes the effects of contact time and relative humidity on the action of two untreated amorphous silicas identified as lethal to *Blattella germanica* in contact tests, and the effect of "repellency" on the toxicity of deposits to cockroaches provided with a choice of treated and untreated surfaces. Experiments

were devised to investigate dose/response relationships for some commercial amorphous silica products covering a range of physical properties and the behaviour of the cockroaches on dusted surfaces.

It appeared likely that the behaviour of cockroaches constrained to remain on dusted surfaces would differ from that on untreated surfaces, and therefore that locomotory and grooming activity would influence pick-up as much as dust type and surface concentration. Three bioassay methods were devised to test the "intrinsic" toxic effect of different dust types: in the first, the insect briefly contacted a treated surface and was then held in an untreated arena for determination of mortality after a fixed period - this method was intended to simulate the lethal effect of a dust following a brief encounter with a deposit, with the cockroach able to groom and remove any dust picked up; in the second the cockroach was held in a small cell on a treated surface - the cockroach could groom but continually recontaminated itself with silica deposit - so that pick-up was not influenced by changes in locomotory activity; in the third the cockroach was provided with a choice between a treated and an untreated surface in a large darkened choice chamber.

Silicas have been used experimentally and commercially as carriers for conventional organic insecticides. It has been claimed that dust formulations of this type can be more effective against some insects than would be expected on the basis of simple additive action of the

two toxicants (Shawir et al., 1989). A commercial formulation of synergised pyrethrins adsorbed on silica (Drione, Wellcome Ltd.) has been extensively used in the United States for cockroach control, and this prompted an investigation into the comparative effectiveness of alternative residual pyrethroids formulated on a sorptive silica and talc when tested against *B. germanica*.

5.2 Materials and Methods

5.2.1 Chemicals and formulations

The pyrethroids cypermethrin, alpha-cypermethrin, deltamethrin, permethrin and cyfluthrin were purchased as analytical standards (> 98% w/w) from British Greyhound Chromatography, Birkenhead.

The Gasil series of precipitated silicas was from Crossfield Silicas, Warrington and Sipernat 22 was from Degussa, Frankfurt. The pyrogenic silicas HDK H20 and N20 were from Wacker-Chemie GMBH, Munich, and the Cab-O-Sil series from the Cabot Corporation, Boston.

Silica products were: Crossfield silicas - Gasil HP25. Gasil 23F, Gasil 114, Gasil HP37, Gasil GM2 and Gasil 200; Wacker - HDK H20; Cabot

Carbon - Cab-O-Sil M-7D; Degussa - Sipernat 22. Oil absorption capacities of silicas were determined by equilibrating a sample (3 g) with a mixture of Risella 17 oil : diethyl ether (1:3 v/v, 100 ml), filtering under vacuum, air-drying and determining the weight of oil retained.

5.2.2 Bioassays

Cockroach cultures were maintained in a constant environment room at 40% r.h. outside the culturing tins (unknown humidity within the tins), a temperature of 30° C and a 12:12 (L:D) photophase from 8.00 a.m. to 8.00 p.m. As far as possible, bioassays were set up to commence at around 10.00 a.m., 2 h into the photophase and during the quiescent period of the diurnal activity cycle.

5.2.3 Bioassays with continuous exposure to silica deposits

The bioassay arena was a modified plastic petri dish 9 cm dia. and 1.5 cm high. Amounts of silicas varying from 0.6 - 400 mg were weighed into the dishes and spread as evenly as possible over the floor of the petri dish using a fine brush. The base of the dish was divided into 10 equal sements using a card insert (cell divider).

Batches of 10 *B. germanica* male adults 2-3 week old were taken from the culture and cooled to 0°C. Immobilised cockroaches were placed in an upright position in individual cells within the dish which was then covered with the open mesh lid and placed in a sealed translucent polythene box (30 x 30 x 15 cm). Dishes stacked in the box were separated by spacers (1 cm high). The box contained a saturated NaCl solution to maintain a relative humidity of 75% and was placed in the constant environment room at 28° C. Cockroaches took approximately 2 minutes to recover normal condition at this temperature but were more active than normal immediately following recovery. The size of the exposure cell made it difficult for the cockroach to move or to fall on its back during the exposure period, although most did so at death. Thus differences between pick-up of the products due to movement of the cockroach over the treated surface, abdomen dragging, or knockdown were minimised in these tests, as was the removal of deposits by grooming. The mortality of the cockroaches was monitored after 24 hours. There was no control mortality within this time in any of the experiments. Log(dose)/probit (response) regressions and associated statistics were determined by probit analysis.

5.2.4 Bioassays with short exposure to silica deposits

A square plastic dish (10 x 10 cm) was modified so that the halved lid could be moved from one side of the dish to the other without disturbing dust deposits on the base. One half of the base of each dish, separated from the other by a 1 mm thin card strip, was dusted with various concentrations of either Gasil HP37 (precipitated silica) or HDK N20 (pyrogenic silica). Batches of 10 male German cockroaches 2-3 weeks following adult emergence were taken from the culture, placed in a container and cooled to 0°C. Immobilised cockroaches were placed in batches of 10 in the untreated half of the dish and allowed to recover for 30 min. They were moved carefully to the treated half of the dish by sliding the lid across, held there for the period of 1.5, 5, 30 and 60 minutes, and then moved back to the untreated half. Forty dishes were set up simultaneously for the two silica dusts at five concentrations for each of four exposure periods. The dishes were then maintained in sealed polythene boxes containing a saturated sodium chloride solution to maintain a relative humidity of 75%. The boxes were kept at 30°C and mortality assessed at 24, 48 and 72 h.

5.2.5 Pick-up of silica from treated surfaces

Silicas of Gasil HP37, Sipernat 22 and Wacker HDK N20 were dyed with

Rhodamine B at 50 mg/g and dusted onto one half of the split bioassay arenas described above at 2 g/m². Batches of 5 cockroaches were cooled to 0°C, transferred to the untreated half of the arena, and allowed to recover for 30 min. They were then exposed to the silica deposits for periods of 1.5, 5, 30, 60, 90, 120 and 180 minutes, then moved back to the untreated side of the chamber, and rapidly chilled to 0°C. Immobilised cockroaches were picked out and extracted with 5 ml ethanol : toluene (1:1 v/v), dye concentrations being estimated at 556 nm and dust weights correspondingly determined. Dust pick-up experiments were replicated 5 times at each exposure interval, and were carried out at 30°C and 40% relative humidity.

5.2.6 Toxicity of silica deposits in choice boxes

Choice boxes (Figure 5.1) were of wooden construction with a base area of 100 x 50 cm and sides of height 5 cm. Holes in the base accommodated saturated salt solutions to modify the relative humidity in the box, and the base was covered with a sheet of filter paper. A PTFE-painted block served as the central partition, with gaps of 5 cm at either end to allow the cockroaches to move between the two halves of the box. The glass top was entirely covered with single thickness red cellophane enabling observations to be made without any apparent disturbance of the cockroaches. The walls were lined with PTFE-painted glass inserts

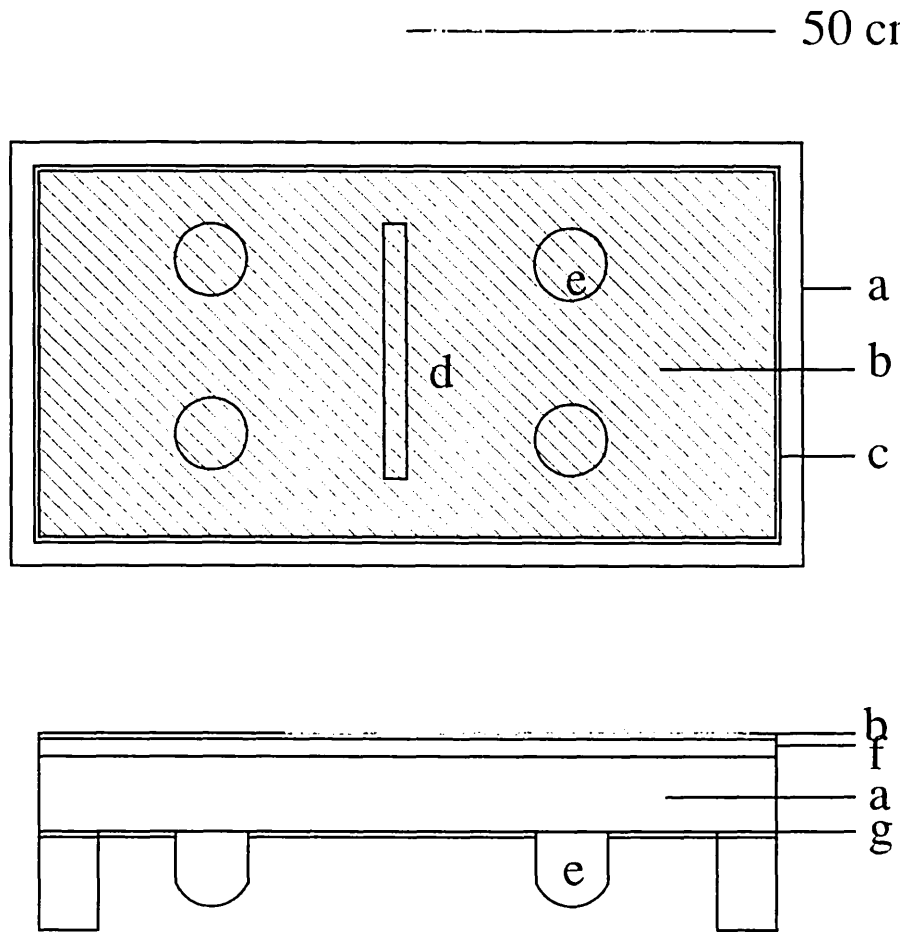


Fig. 5.1 Large scale arena for bioassay of resting behaviour of B. germanica in the presence of silica dusta.
a. wood support, b. red cellophane film, c. glass wall, d. centre barrier, e. cup with saturated salt solution, f. glass cover, g. filter paper.

to confine the cockroaches to the floor of the boxes; these were cleaned and re-painted and the filter paper base replaced prior to each experiment.

5.2.6.1 Effect of pre-treatment of cockroaches

Batches of 20 cockroaches were anaesthetised with CO_2 and kept in a glass jar for 24 h either with food and water provided *ad lib.* or with no food or water. Groups from both types of pre-treatment were transferred to the untreated half of choice boxes maintained at 75% r.h. and either allowed immediate access to the treated half (silica HP37 dusted at 1.15 g/m^2) or confined to the untreated half of the box for a period of 2 hours prior to removal of an internal barrier which allowed access to the treated half. Timing for both experiments was from the point at which the insects were allowed access to the treated half. Nine replications were carried out for each of two types of pre-treatment under each of two exposure conditions.

5.2.6.2 Effect of relative humidity and presence of free water on toxicity of silica deposits

Batches of 20 cockroaches were anaesthetised with CO_2 and kept without

food or water for 24 h. They were transferred to the untreated side of a choice box treated on the alternative side with silica HP37 at 1.15 g/m², and allowed immediate access to the silica deposit. In three of the four choice boxes the relative humidity was controlled by saturated salt solutions (58%, 85% and 95% r.h.) while the fourth box was provided with 2 water dispensers (2 cm³ glass vials with cotton wicks) located at the corners of the treated side (relative humidity undetermined, variable within the box). The experiment was a randomised block design with four replicates for each test condition.

5.2.6.3 Toxicity of pyrethroid/silica combinations against the German cockroach

Solutions (10 ml) of pyrethroids at known concentrations in hexane : acetone (24:1 v/v) were added to 5.0 g silica and mixed thoroughly. The mixtures were air-dried for 24 h, vacuum-dried (2 h) at room temperature, and sieved through an 85 mesh sieve. Samples were stored in the dark at 4°C. Silica/pyrethroid mixtures containing 0.1%, 0.05% and 0.01% w/w of pyrethroid on silica were prepared.

Samples (1 g/m²) of dust were weighed on a micro-balance and spread as evenly as possible on the treated side of split-area arenas (5.2.4). Ten adult male cockroaches, 2-3 weeks from the last nymphal moult, were

anaesthetised with carbon dioxide in culture tins, removed and retained in the untreated side of the arena for 1 h at 30°C. They were then moved to the treated side, held there for 3 min., and moved back to the untreated side. The arenas were transferred to sealed polythene containers over a saturated salt solution to maintain 75% r.h. The cockroaches were not supplied with water and food during the experimental period (24 h). Mortality was assessed at 24 h following exposure, the bioassays being conducted at 30°C. Experiments were started 1-2 h into the photophase of the 12:12 photocycle.

5.3 Results

5.3.1 Bioassays with continuous exposure to silica deposits

The results are shown in Table 5.1 together with some physical properties of the silica dusts. Only one of the bioassays gave data which was a poor fit to the probit(mortality)/log (dose) regression ($P < 0.05$, chi square test), this being apparently due to scatter rather than a consistent trend away from linearity. Toxicity to *B. germanica* as estimated by the LC50 generally increased with increasing oil adsorption capacity of the two dust types, while specific surface area

TABLE 5.1

Physical properties and toxicity of some silica dusts to *Blattella germanica* at at 30°C and 75% r.h. (continuous exposure for 24 h)

silica	surface area (m^2/g)[1]	oil absorption (g/100 g)	LC50 (g/m^2)	confidence interval (95%)	regression	p
Gasil 23F	290	343.6(4.5)	0.23	0.39-0.10	y = 0.97 + 1.51 x	0.25
Gasil HP37	280	311.7(19.3)	0.32	0.44-0.22	y = 1.17 + 2.37 x	0.06
HDK H20	170	456.9(6.4)	0.53	0.64-0.43	y = 1.05 + 3.76 x	0.001*
BH5	390	739.0(9.6)	0.56	1.00-0.11	y = 0.78 + 3.08 x	0.38
H5	325	734.4(21.4)	0.58	0.90-0.25	y = 0.95 + 4.01 x	0.79
HDK N20	200	730.0(3.9)	0.66	0.94-0.39	y = 1.03 + 5.73 x	0.74
Gasil HP25	400	198.4(3.8)	0.74	0.99-0.44	y = 0.46 + 3.58 x	0.84
Gasil 114	320	244.4(8.4)	0.92	2.05-0.38	y = 0.14 + 3.73 x	0.44
Gasil AF	320	210.1(3.4)	1.61	3.98-0.66	y = -0.53 + 2.55 x	0.41
M-7D	200	554.7(2.4)	2.41	3.12-1.88	y = -0.90 + 2.37 x	0.45
Sipernat 22	190	290.2(1.3) >	7.80	-	-	-
Gasil GM2	750	55.4(3.6) >	7.80	-	-	-
Gasil 200	750	69.3(1.1) >	7.80	-	-	-

[1] manufacturer's data

as estimated by nitrogen adsorption (B.E.T. method, manufacturers' data) was a poor predictor of lethal effect. Of 13 silica dusts tested in the experiments, 6 having oil adsorption capacities from 3000 g/kg to 7400 g/kg gave LC50 values between 0.23 and 0.66 g/m², and three having oil adsorption capacities from 2000 to 2440 g/kg gave LC50 values between 0.74 and 1.61 g/m². One pyrogenic silica (Wacker M-7D) with a relatively high oil adsorption capacity (5540 g/kg) had an anomalously low toxicity with an LC50 of 2.41 g/m². Three of the silicas proved non-toxic to the cockroaches under the conditions of the test and had an LC50s greater than 7.8 mg/m². Two of these (Gasil GM2 and Gasil 200) had low oil adsorption capacities (550 and 690 g/kg, respectively) but had high specific surface areas (750 m²/g). The low pore volumes of these materials (0.4 ml/g) may limit their ability to adsorb lipid. The third (Sipernat 22) had an oil adsorption capacity comparable to that of the more toxic silicas but a bulk density closer to that of the pyrogenic silicas.

Pyrogenic silicas were less toxic than would have been expected on the basis of their oil adsorption capacities relative to those of the precipitated silicas.

5.3.2 Bioassays with short exposure to silica deposits

Although trends are evident in the effects of surface concentration of silica, exposure time and post-exposure holding time on mortality (Table 5.2), the mortality in these experiments was considerably more variable than for the continuous exposure bioassays.

For both types of silica cockroach mortality increased up to 72 h after removal from the dust deposit, even after brief exposures. It was not possible to extend the holding time beyond 72 h in the absence of free water without producing control mortality due to natural desiccation. There did not appear to be clear differences in the mortality produced by a given surface concentration at any of the three post-exposure holding periods for exposures of 5 min or more, but a 1.5 min exposure to either dust gave a lower kill at 72 h post-treatment than longer exposure periods. For any given exposure time and post-exposure holding period, cockroach mortality generally showed an increasing trend with surface concentration of either of the two silicas, but the variability of the mortality data was too great to attempt a probit analysis.

TABLE 5.2

Toxicity of silica dusts in discontinuous contact bioassays

surface conc. (mg/m^2)	exposure time (min.)	no. dead (out of 10) at 24	48	72h	surface conc. (mg/m^2)	exposure time (min)	no. dead (out of 10) at 24	48	72h
	Gasil HP37					Wacker HDK N20			
0.22	60	0	0	1	0.25	60	0	0	1
0.50		6	6	8	0.51		0	0	2
0.86		6	7	7	0.82		0	0	0
1.12		6	6	8	1.15		1	4	7
1.46		4	4	6	1.40		10	10	10
0.22	30	0	0	0	0.22	30	0	0	0
0.52		0	0	2	0.51		1	5	8
0.88		1	1	2	0.81		0	3	8
1.16		3	7	9	1.11		10	10	10
1.38		7	10	10	1.41		2	9	10
0.22	5	0	0	0	0.22	5	0	0	0
0.52		0	0	2	0.51		1	4	6
0.90		4	7	9	0.80		3	8	10
1.20		3	5	8	1.15		4	8	10
1.42		10	10	10	1.40		0	1	6
0.20	1.5	0	0	0	0.22	1.5	0	0	2
0.52		0	0	0	0.52		0	0	0
0.78		0	0	0	0.81		0	1	1
1.14		0	0	3	1.13		3	8	10
1.44		1	3	8	1.44		0	2	3

5.3.3 Pick-up of silicas from treated surfaces

The mean weight of dust picked-up by the cockroaches is shown in Table 5.3 and Figure 5.2. Aggregations of silica particles accumulated on the tarsi and to a lesser extent on the tip of the abdomen. Saturation of these accumulation sites was apparently complete within approximately 90 s for the Sipernat 22 and HDK N20, and 90 min for the more toxic silica dust Gasil HP37. Thereafter the weight of dust remained more or less constant for exposure periods up to 3 hours. There was a clear difference between the toxic Gasil HP37 and the less toxic Sipernat 22 and Wacker HDK N20 in the amount of dust picked up by the cockroaches during the exposure period. The difference was apparent from exposure periods of 5 minutes up to 3 hours, the cockroaches picking up significantly more Gasil HP37 than Sipernat 22 and Wacker HDK N20. These results are in accordance with the toxicity of these dusts to the German cockroaches in continuous exposure experiments (Table 5.1).

5.3.4 Effect of pre-treatment of cockroaches on toxicity of silica in choice boxes

On being allowed access to the treated area of the choice boxes the cockroaches initially showed sporadic locomotory activity (unquantified) regardless of pre-treatment, and distributed themselves

TABLE 5.3

Pick-up of silica dusts by male German cockroaches from a petri dish dusted at 2 g/m²

exposure time (min)	mean (s.e.) amount of dust (mg/insect) Gasil HP37	Wacker HDK N20	Sipernat 22
1.5	0.094(0.030)	0.043(0.008)	0.045(0.001)
5	0.196(0.052)	0.023(0.007)	0.044(0.001)
30	0.189(0.028)	0.022(0.005)	0.041(0.001)
60	0.258(0.047)	0.032(0.008)	0.046(0.002)
90	0.524(0.139)	0.041(0.011)	0.042(0.001)
120	0.487(0.146)	0.122(0.024)	0.041(0.001)
180	0.520(0.158)	0.150(0.032)	0.043(0.001)

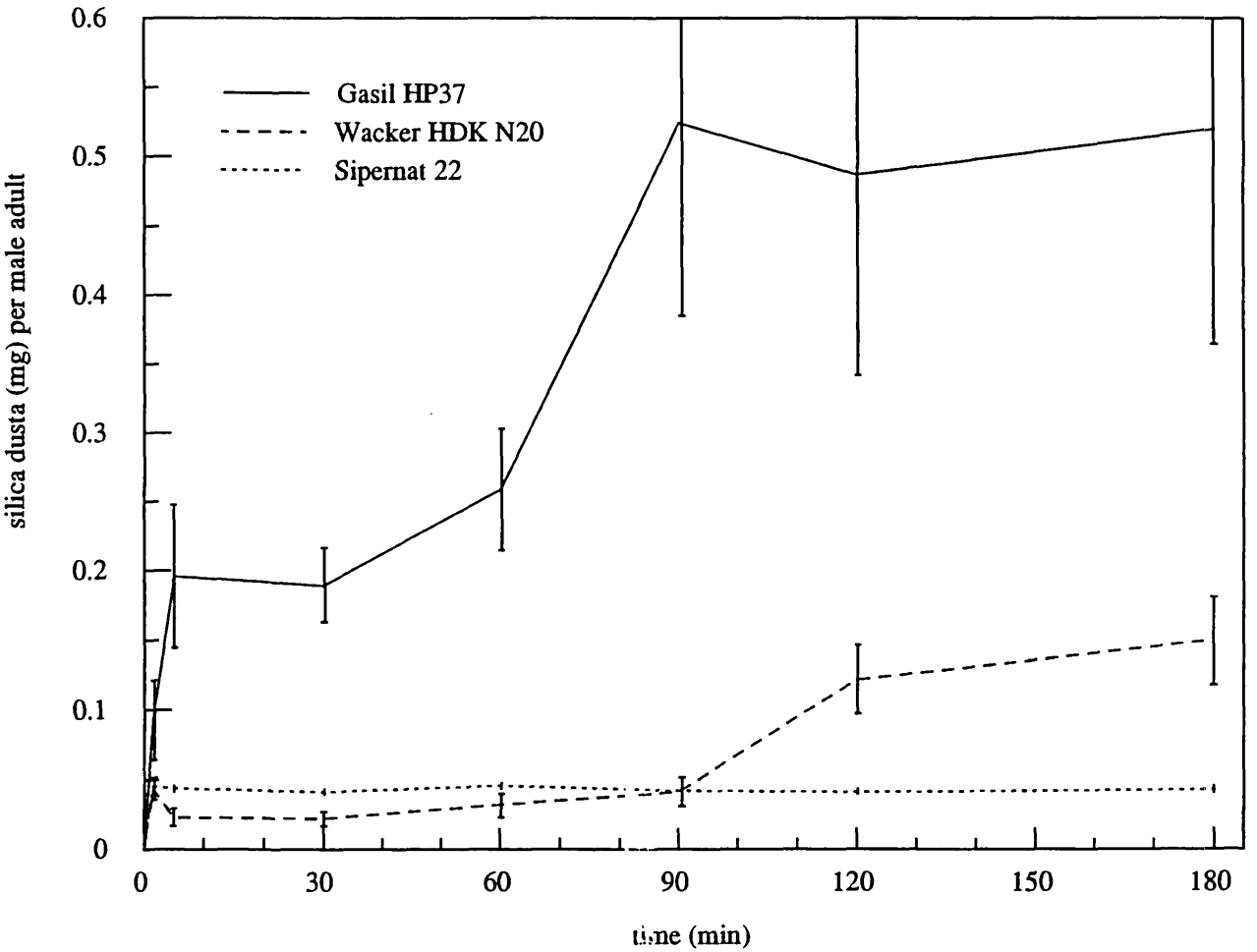


Figure 5.2 Pick-up of silica deposits by B. germanica with time in petri dish chamber.

apparently randomly over both treated and untreated surfaces. There was no evidence of turning movements or change in walking speed when entering the treated side of the boxes from the untreated side. After about 20 min a tendency for the cockroaches to accumulate on the untreated side of the box was apparent, the activity level dropped on the untreated side, and many of the insects remained stationary, removing dust from accumulation sites on the antennae and front two pairs of legs by passing them through the mouth parts, and from the hind legs by rubbing them on the rear of their abdomens. Grooming activity by insects on the silica deposits was also observed, but the insects did not settle and apparently normal walking activity followed within a short time.

Mortality of the cockroaches in the choice boxes is shown in Table 5.4 for observation times up to 24 h, as is the proportion of surviving cockroaches in the untreated half of the choice box at these observation times in Table 5.5. There was an apparent trend towards a greater mean mortality among cockroaches deprived of food and water for 24 h (Table 5.4), but prolongation of the exposure decreased the difference between the mean mortalities due to increasing mortality in the groups provided with food and water prior to testing (Table 5.4). Mean mortality of cockroaches which had been allowed a 2 h acclimation period in the untreated side was not significantly different from that of cockroaches allowed immediate access to the deposit. However, for

TABLE 5.4

Mortality of German cockroaches in choice boxes half-treated with Gasil HP37 at 1.15 mg/m² over a 24 h exposure period (n=9)

pre-treatment	exposure period (h)					
	1	2	4	6	12	24
starved conditioned[1]	1.2	4.6	4.9	12.6	23.2	82.2
starved unconditioned	0.0	2.8	13.0	27.7	59.3	98.3
unstarved conditioned	0.0	11.1	22.8	22.8	25.6	35.8
unstarved conditioned	0.0	0.0	1.1	1.1	2.2	46.5

[1] Cockroaches held for 24 h following removal from culture tin either without (starved) or with (unstarved) food and water. Cockroaches were either held for 2 h in untreated half of choice boxes prior to being given access to treated half (conditioned) or allowed immediately access to treated half following addition to untreated half of boxes.

each pre-treatment and at all observation times significantly more of the surviving cockroaches were found resting on the untreated side of the boxes; the lowest proportion was 0.84 (Table 5.5).

5.3.5 Effect of relative humidity and presence of free water on the toxicity of silica deposits in choice boxes

Mean mortality of the cockroaches exposed to Gasil HP37 at 1.15 mg/m² in choice boxes at different relative humidity is shown as a function of exposure time in Table 5.6. The toxicity of the deposits decreased markedly with increase of relative humidity in the choice boxes at each exposure time over 24 hours, and significantly more cockroaches were located in the untreated chamber of treated choice boxes relative to the number of cockroaches on the corresponding chamber of untreated choice boxes (Table 5.7).

Cockroaches released into a choice box with water dispensers also showed apparently random activity between the two sides of the box, but when their antennae contacted the dispenser wick they immediately stopped to drink. Their subsequent distribution and behaviour was similar to that of cockroaches deprived of water, but grooming of appendages was more prolonged and the appearance and movement of the cockroaches which survived the treatment was closer to that of controls

TABLE 5.5

Mean proportion of total live 2-3 wk male adults *B. germanica* from adult emerging in untreated (UT) chamber of choice boxes half-treated with Gasil HP37 at 1.15 mg/m²

	1 h	2 h	4 h	6 h	12 h	24 h
starved:						
conditioned[1]	0.96 (0.06)	0.99 (0.03)	0.98 (0.04)	0.95 (0.09)	0.84 (0.17)	0.98 (0.03)
unconditioned	0.96 (0.06)	0.98 (0.05)	0.91 (0.11)	0.96 (0.07)	0.87 (0.25)	-
unstarved:						
conditioned	0.91 (0.12)	0.93 (0.13)	0.98 (0.03)	0.99 (0.04)	0.96 (0.05)	0.99 (0.02)
unconditioned	0.96 (0.07)	0.96 (0.09)	0.95 (0.11)	0.96 (0.11)	0.88 (0.12)	0.93 (0.10)

[1] as Table 5.4

TABLE 5.6

Mean mortality of male adult German cockroaches in choice boxes half-treated with Gasil HP37 at 1.15 mg/m² at various relative humidities in the choice boxes[1]

relative humidity	arena	exposure period (h) 1	2	4	6	12	24
58%	treated	2.5	8.8	40.0	47.5	50.0	81.3
	untreated	0.0	0.0	0.0	0.0	0.0	3.8
85%	treated	0.0	2.6	15.4	32.1	53.6	70.0
	untreated	0.0	0.0	0.0	0.0	0.0	0.0
95-100%	treated	0.0	1.3	1.3	1.3	1.3	18.9
	untreated	0.0	0.0	0.0	0.0	0.0	8.3

[1] cockroaches starved for 24 h before being introduced into untreated half of choice boxes and allowed access to treated half immediately after placing in the choice boxes.

TABLE 5.7

Effect of relative humidity on the percentage of male adult German cockroaches[1] in treated half of choice boxes half-treated with Gasil HP37 at 1.15 mg/m²

relative humidity (%)		exposure period (h) 1	2	4	6	12	24
58	treated	3.3	6.1	1.3	0.6	0.0	0.0
	untreated	6.8	6.2	6.0	5.5	33.0	28.5
85	treated	3.2	1.4	0.0	2.5	2.3	14.0
	untreated	7.0	9.4	6.1	1.3	10.4	14.1
95	treated	1.7	0.7	0.7	1.4	9.8	0.0
	untreated	10.3	11.9	12.1	11.9	7.3	17.7

[1] as Table 5.6

with water provided.

The presence of water dispensers in the treated side of the choice boxes had a significant effect on mortality of the cockroaches in the boxes (Table 5.8). The proportion of live cockroaches in the untreated half of the boxes was significantly greater than that in the treated half at all observation times for cockroaches both with and without water (Table 5.9).

5.3.6 Toxicity of pyrethroid/silica combinations to the German cockroach

The surface concentration of Gasil HP25 used in these experiments was calculated from earlier bioassays to be just below that which would produce toxicity as a result of desiccant action under these bioassay conditions. The control results (untreated silica) demonstrate that this rate was achieved in practice, the mean control mortality being 8.7% at the concentration used (Table 5.10). Talc alone was not toxic to the cockroaches at any realistic surface concentration under these conditions. The results from the bioassays with pyrethroids adsorbed on Gasil HP25 indicate that formulation on silica produces a greater toxic effect under these conditions than when the same amount of pyrethroid is adsorbed on talc and applied at the same surface

Table 5.8

Effect of presence of water dispensers in treated half of choice boxes on mortality of cockroaches (n=4)

	Mean mortality (%)					
Exposure time	1 h	2 h	4 h	6 h	12 h	24 h
without water dispensers	2.72	17.72	47.79	68.09	95.81	100.0
with water dispensers	0.00	1.25	5.00	7.50	8.75	21.25

TABLE 5.9

Mean proportion of live male adults[1] in untreated chamber of choice boxes half-treated with Gasil HP37 at 1.15 mg/m² (n=4)

	mean (s.e.) proportion					
exposure time	1 h	2 h	4 h	6 h	12 h	24 h
no water dispensers	0.77 (0.11)	0.74 (0.10)	0.53 (0.11)	0.75 (0.29)	–	–
with water dispensers	0.81 (0.14)	0.79 (0.19)	0.85 (0.11)	0.85 (0.07)	0.78 (0.08)	0.91 (0.14)

[1] cockroaches starved for 24 h before being introduced into choice boxes and allowed access to treated half of the choice boxes.

TABLE 5.10

Mean number of dead cockroaches out of 10 (n=5) at 24 hours following 3 min exposure to formulations of pyrethroids on Gasil HP25 and talc at 1 g/m²

combination	concentration (w/w) 0.1%	0.05%	0.01%
deltamethrin plus			
talc	-	8.0 (1.9)	0.0 (0.0)
silica	-	10.0 (0.0)	4.0 (4.7)
alphamethrin plus			
talc	-	0.8 (0.8)	0.0 (0.0)
silica	-	9.8 (0.5)	6.4 (2.6)
cyfluthrin plus			
talc	3.4 (1.3)	0.6 (0.9)	0.0 (0.0)
silica	10.0 (0.0)	5.8 (1.9)	1.6 (2.3)
cypermethrin plus			
talc	2.6 (2.3)	0.0 (0.0)	0.0 (0.0)
silica	9.2 (1.3)	5.4 (3.1)	1.4 (2.2)
permethrin plus			
talc	0.4 (0.9)	0.0 (0.0)	0.0 (0.0)
silica	3.2 (2.3)	1.8 (3.0)	1.2 (2.7)
control			
talc	0.2 (0.5)	0.0 (0.0)	0.0 (0.0)
silica	1.0 (1.4)	1.4 (1.5)	0.2 (0.5)

concentration.

Two aspects of the results are significant: deltamethrin was the most toxic pyrethroid under these conditions, the silica/deltamethrin producing significant mortality at the lowest concentration of 0.01% deltamethrin on silica; adsorption of alphamethrin onto silica produced the greatest increase in toxicity of the pyrethroid relative to that when adsorbed on talc (from 8% on talc to 98% on silica at 0.05% pyrethroid on carrier and an application rate of 1 g/m^2). In all cases, formulation of the pyrethroids on silica produced more effective deposits than those resulting from the same concentration of the pyrethroid on talc and applied at the same surface concentration.

5.4 Discussion

Cockroaches confined on deposits of silicas and with only a few mm of free movement gave homogeneous log(dose)/probit(response) regression lines. The LC50's thus gave a good comparative estimate of the intrinsic desiccating potential of the different silicas. In general, high insecticidal activity was associated with high oil adsorption capacity, an effect previously noted for stored product insects with a similar series of silica products (le Patourel and Singh, 1984). The fumed silicas and Wessalon S were not as toxic as would have been

expected on the basis of this physical property however, an effect possibly associated with their lower bulk density. The homogeneity of continuous contact bioassay results together with the small variation in the amount of dust accumulating on the cockroaches observed in the pick-up studies suggests that much of the variability in mortality of the discontinuous contact study was due to grooming, silica aggregates being either knocked off or ingested. Desiccant silicas are non-toxic to *B.germanica* by ingestion (Ebeling *et al.*, 1975).

The lethal effect of a silica as a result of a brief contact with a treated surface is presumably a function of its intrinsic ability to adsorb cuticular lipid, the total weight of dust picked up during the exposure period, and the extent to which aggregates on the cockroach can be subsequently removed by grooming. The results using dyed dusts illustrate two features of dust pick-up: accumulation sites on the insects are rapidly saturated, the maximum weight being picked-up within 90 s for the less toxic silica dust and 90 min for the more toxic dust; and the weight of dust particles picked-up differs significantly depending on the physical characteristics of the silica. Lewis and Hughes (1957) demonstrated a rapid and reversible accumulation of dye particles by blowflies walking on dusted surfaces, the process being essentially complete within a few seconds, while rice weevils moving through grain dusted with a toxic silica aerogel continued to accumulate the particles for up to 3 h (le Patourel *et*

al., 1989). The weight of silica picked up by *B. germanica* under the test conditions may be a function of its bulk density, there being a fixed volume of accumulation sites on the insect body. This would account for the greater pick-up of Gasil HP37 relative to Sipernat 22 and Wacker N20, both of which are highly dispersed, low bulk density products, and also suggests an explanation for the lower than expected toxicity of the pyrogenic silicas and Sipernat 22.

Choice boxes containing food and water were used by Ebeling *et al.* (1967) in their studies on the "repellent" effects of a number of insecticides including Dri-die 67, a precipitated silica containing 4.7% w/w ammonium fluorosilicate, and Silikil, an untreated silica. The treated half of their box was darkened relative to the untreated side (which contained food and water), providing the cockroaches with an incentive to sample the dusted surface as a result of their tendency to retreat into a dark harbourage. The results of these tests were similar to the present ones at high relative humidity or where free water was provided. Ebeling *et al.* interpreted the low mortality under these circumstances as due to the repellency of the deposits, cockroaches repeatedly picking up sub-lethal doses and possibly modifying their behaviour through a learning process. However, it seems more likely that the results were due to the greatly decreased toxicity of silica dusts when free water is provided in the choice box.

The use of the term repellency to explain the accumulation of cockroaches in the untreated half of the choice box seems inappropriate and gives a misleading impression of the effect of silica deposits on the insects. An alternative interpretation of the accumulation of cockroaches on the untreated side of the choice chamber is that the normal settling response of the cockroach following exploration of unfamiliar surroundings is inhibited on a dust-treated surface. This effect may be due to adsorption of lipid from touch receptors on the tarsi, or to adsorption of aggregation pheromone by the dust. A silica deposit might thus be described as a locomotor stimulant, in the terms of Dethier *et al* (1960), although there is no implication that its action is similar to that of a deposit of a neuroactive insecticide. Conversely, stasis on the untreated side of the choice box is re-enforced both by grooming behaviour following pick-up of dust aggregates and by normal conditioning of the surface with faeces and aggregation pheromone.

The effect of relative humidity on the lethal effect of the silica was as would be expected given its mode of action. The rapid fall-off in mortality at relative humidity above 85% and when free water was provided confirms that unmodified silicas have little potential as control agents where cockroaches pick-up dust and harbour in high humidity environments or have ready access to free water. Some trial results using such silicas tend to confirm this view (Eastin and

Burden, 1960).

The potential of desiccant silicas as carriers for conventional insecticides for cockroach control has not been extensively investigated. Dri-Die 67 was initially chosen for commercial development from the screening program initiated by Ebeling (1959) to identify active desiccant dusts on the basis of its enhanced knock-down and kill characteristics over other products, and the possibility that this was due as much to the presence of up to 4.7% ammonium fluorosilicate adsorbed onto the product as to the toxicity of the silica appears to have been considered only after the event (Ebeling, 1971). Ammonium hexafluorosilicate, like other complex fluoride salts, has a high toxicity to insects as a stomach poison. From results obtained in the present work, it would appear that much, if not all, of the toxicity of the Dri-Die formulation at high humidities or where cockroaches have access to free water must derive from the ammonium fluorosilicate component rather than the desiccant action of the silica.

Tarshis (1962, 1963) studied the use of Dri-Die 67/dibrom combinations for use as animal ectoparasiticides, the organophosphorus component being added to improve the speed of initial knockdown and detachment. The formulations were reported to be successful in this respect, but do not appear to have been developed commercially. The most successful

development in the area of silica/organic insecticide combinations resulted from a formulation of Dri-Die 67 with synergised natural pyrethrum. A formulation containing 1.0% w/w pyrethrins, 10.0% w/w piperonyl butoxide, 40.0% amorphous silica gel and 49% w/w petroleum distillate called Drione has been widely marketed in the U.S., and more recently in the U.K. for cockroach control by crack-and-crevice treatment (aerosol pack) and dusting. The status of ammonium fluorosilicate in this formulation is unclear; the original product was based on Dri-Die 67, which contains 4.7% w/w of the chemical (Ebeling, 1971), but an enquiry to the current manufacturer of the product in the U.K. revealed that ammonium fluorosilicate was no longer included in the formulation (G.Dodd, Wellcome Ltd., personal communication to G.le Patourel). The knock-down and lethal characteristics of Drione as originally formulated to *B.germanica* have been reported by Tarshis (1967) and Ebeling (1971) and were regarded as outstanding at the time.

The use of a pyrethroid additive to silica appeared a promising line of investigation, and the results reported in this work indicate a substantial enhancement of the biological activity of a number of pyrethroids when formulated on a desiccant silica over that obtained when formulated on the non-toxic talc carrier. The reason for this effect is unclear. Le Patourel and Singh (1984) and Shawir *et al.* (1988) reported that the toxicity of pyrethroid/silica or organophosphate/silica combinations gave greater than additive toxic

action to the rice weevil *Sitophilus oryzae* and the grain beetle *Tribolium castaneum* when admixed with grain and suggested enhanced pick-up and/or penetration of pyrethroids through de-waxed cuticle as possible reasons for the toxicity enhancement. A number of pyrethroids are currently being developed for cockroach control work (Fuchs, 1989), and it would appear from the present study that silica-based dust formulations might be worthy of investigation as a treatment for undisturbed areas.

SUMMARY

1. A culture method for *B. germanica* is described in which insects were reared in cohorts hatching in the same week at 28 ± 2° C.

2. Productivity of females from the culture was examined either when caged with males throughout reproductive life or when separated from males after first mating. Females separated from males produced fewer oothecae on average, but the number of egg cells and hatch was not significantly different, and neither was female longevity.

3. The effect of population density on the rate of development of nymphal stages and adult recruitment was studied: density did not affect development rate; density-dependent mortality appeared to be largely due to cannibalism during or immediately after moulting; and there was a linear dependence of final adult density on initial nymphal density over the range investigated. Presence of adults had little effect on rate of adult recruitment from the nymphal stages. Daily carbon dioxide anaesthetisation significantly reduced development times for nymphal stages.

4. The effect of a domestic fluorescent light source on the resting behaviour of *B. germanica* was examined using a 2 chamber (shaded/unshaded) perspex arena. Cockroaches were anaesthetised,

transferred to one chamber of the arena, allowed to recover for 10 minutes, allowed access to the alternative chamber, and their distribution recorded after 30 min. Bioassay conditions (absolute light intensity, light intensity difference between chambers, period of illumination prior to being given access to shaded chamber, change of photocycle prior to bioassay, extent of anaesthetisation with carbon dioxide, and division of cockroaches into "dark-preferring" and "light-preferring" groups) had little effect on the proportion of cockroaches preferring to rest in the shaded arena, and although the cockroaches showed a significant preference for the shaded arena over a number of trials, the effect was not always evident in individual replicates. The light-avoidance reaction obtained in these trials was not as great as has been reported in the literature for *B. germanica*.

5. The two chamber arena was used to investigate the resting behaviour of *B. germanica* during and after exposure to sub-lethal deposits of permethrin WP in either the shaded or illuminated chamber. Cockroaches given access to the treated shaded chamber following recovery in the untreated and subsequently illuminated chamber showed a preference for the illuminated chamber, while the hypothesis of a 1:1 distribution was not rejected for cockroaches which recovered in treated and subsequently illuminated chamber and were then given access to the untreated shaded chamber.

6. The experiments were repeated with cockroaches pre-exposed to permethrin WP deposits at doses ranging from an LC5 to an LC75 (30 min exposure, 24 h mortality). Cockroaches which recovered in the subsequently illuminated chamber showed a tendency to remain in the illuminated chamber which increased with permethrin deposit concentration.

7. A linear three chamber arena (illuminated escape; illuminated and treated recovery; shaded and untreated escape) was also used to test the light-avoidance behaviour of *B. germanica* following exposure to permethrin WP deposits. The results showed no clear trend towards a greater or a lesser light avoidance reaction with increasing permethrin concentration, there being evidence for a reversal of the normal photophobic response at some concentrations but not at others. Further work in this area requires the normal light avoidance behaviour observed in field infestations to be more closely simulated within a bioassay arena. Disturbance of the cockroaches and carbon dioxide anaesthetisation may possibly reduce the response.

8. Adult females carrying oothecae were confined in polythene cages with bait formulations of boric acid (1:1 w/w boric acid : powdered dog chow pellets) or a 1.65% w/w formulation of hydramethylnon (both in Maxforce® bait stations) at 90% r.h. and either 20° or 28° C until all had been killed. Mortality, apparent viability of oothecae, and

oothecal hatch were monitored at 24 h intervals thereafter until all oothecae had either hatched or appeared non-viable.

9. Females poisoned with hydramethylnon retained their oothecae at death, while a considerable proportion of those killed by boric acid deposited their oothecae prematurely. Oothecae from females taken from the culture both 7-14 and 14-21 days from adult emergence and poisoned with hydramethylnon showed reduced hatch relative to controls at 20°C but not at 28° C, while oothecae from both groups of females poisoned with boric acid at both temperatures showed reduced hatch relative to controls, and the proportion of those which did hatch was higher in the group retained by the female at death than in the group prematurely deposited. The results taken together with those from experiments examining the % hatch of oothecae either manually removed from females or allowed to remain in the genital pouch of the females until hatch suggested that for neither compound was transfer of toxicant from female to oothecae an important factor in controlling oothecal hatch following the demise of the parent.

10. Adult males were exposed to deposits of amorphous silicas in continuous and discontinuous contact bioassays. Homogeneous bioassay results were generally obtained in the continuous contact bioassays and the LC50's of silicas generally increased with oil adsorption capacity of the dusts as measured by the ability to adsorb Risella 17

oil. Discontinuous contact bioassays (1.5 - 60 min) showed greater variability, apparently due to the ability of cockroaches to remove the deposit from their body and appendages by grooming and ingestion. Pick-up of 3 of the dusts by cockroaches in small arenas over a 3 h period was also related to their relative toxicities.

11. The toxicity of silica dusts in half-treated choice boxes under red light varied with relative humidity and was effectively eliminated at 95% r.h. or when free water was provided in the arenas. Cockroaches tended to accumulate in the untreated side of the choice box within 1 h of being introduced into the arena.

12. The relative toxicity (24 h) of a number of pyrethroid insecticides to adult male cockroaches was determined when the compounds were formulated either on one of the toxic (desiccant) silicas or on talc, using a discontinuous contact bioassay with 3 min exposure. The silica-based formulations were appreciably more toxic than the talc-based ones at a dust concentration of 1 g/m^2. The suitability of silica-based dusts for cockroach control is discussed.

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APPENDIX 2.1

Hatch of oothecae produced by females caged with males throughout their life time

ootheca	time to hatch[1]	eggs/ootheca	nymphs/ootheca
1st	28 27 29 27 29 30	52 48 44 50 46 40	52 48 44 49 40 40
2nd	24 25 23 23 23 29	44 46 46 44 44 36	44 45 44 41 44 35
3rd	24 26 27 25 25 24	45 46 42 40 40 35	45 45 42 40 37 35
4th	29 29 27 27 28 28	40 38 34 40 39 30	38 37 32 40 39 30
5th	30 34 28 29 28	30 28 32 40 36	29 27 31 36 34

[1] number of days from adult emergence or from hatch of previous oothecae to day on which the oothecae hatched.

APPENDIX 2.2

Hatch of oothecae produced by females caged with males until first ootheca visible

ootheca	time to hatch	eggs/ootheca	nymphs/ootheca
1st	28 26 26 27 30 27	52 48 44 44 46 42	48 48 42 43 43 37
2nd	25 25 25 25 29 24	50 42 38 38 42 44	48 40 36 37 42 44
3rd	23 23 24 24 26 25	48 44 34 36 38 36	43 37 34 34 37 34
4th	27 27 30 30 32 27	34 40 34 24 30 34	30 37 31 21 28 16
5th	30 35 41	28 28 28	26 27 28

APPENDIX 2.3

Size of oothecae produced by females caged with males either until first ootheca visible or throughout life time

ootheca	length (width) (mm)					
	oothecae from females caged with males until first ootheca visible			oothecae from females caged with males throughout life		
1st	8.0 (2.0)	8.0 (2.0)	7.5 (2.5)	9.0 (2.0)	7.5 (2.5)	8.0 (2.5)
	7.5 (2.5)	8.0 (2.0)	7.0 (2.0)	8.0 (2.5)	7.0 (2.0)	7.0 (2.5)
2nd	7.5 (2.5)	7.0 (2.5)	6.5 (2.0)	8.0 (2.0)	7.0 (2.0)	7.5 (2.5)
	7.0 (2.0)	6.5 (2.0)	7.0 (2.0)	7.0 (2.5)	7.0 (2.0)	7.0 (2.0)
3rd	8.0 (3.0)	7.5 (2.0)	6.5 (2.5)	7.8 (5.0)	7.5 (2.5)	8.0 (2.5)
	6.0 (2.0)	6.5 (2.5)	7.0 (2.0)	7.5 (2.5)	6.5 (2.5)	6.5 (2.5)
4th	7.0 (2.5)	7.0 (2.0)	6.0 (2.0)	7.0 (2.0)	6.5 (2.5)	7.0 (2.0)
	5.0 (2.0)	5.0 (2.5)	7.0 (2.0)	7.5 (2.5)	7.0 (2.0)	5.0 (2.0)
5th	6.0 (2.0)	5.0 (2.0)	5.0 (2.0)	6.0 (2.0)	5.0 (2.0)	6.0 (2.0)
				6.5 (2.5)	6.0 (2.0)	

APPENDIX 2.4

Time to moult (days) from hatch of ootheca in synchronous populations of different densities

initial density[1]	moult 1st		2nd		3rd		4th		5th		6th	
10	7	8	13	13	18	17	26	25	33	31	38	36
	8	6	12	13	17	16	27	27	34	35	37	41
	7		14		17		27		35		43	
20	7	7	12	12	17	17	24	25	31	31	38	43
	7	7	12	13	17	17	23	23	30	31	43	43
	7		13		17		24		31		39	
35	6	7	13	12	18	19	25	25	32	32	40	42
	7	7	12	12	18	18	25	24	31	31	43	38
	7		13		17		23		31		38	
50	6	7	13	11	18	16	23	21	30	28	40	39
	7	7	12	12	18	18	23	24	29	31	40	41
	7		11		18		23		30		42	

[1] number of cockroaches kept in jar (360 cm^2) with food and water throughout development period (no carbon dioxide used).

APPENDIX 2.5

Mean (s.e.) number of nymphs of *B. germanica* as a function of initial number of first stage nymphs at ten day intervals during nymphal development (n = 5)

days following egg hatch	mean (range) number of nymphs surviving population						
	1	2	3	4	5	6	7
0	11.6(1.1)	20.8(1.8)	34.4(0.9)	50.4(2.9)	39.0(1.4)	170.5(17.7)	331.5(30.4)
10	11.6(1.1)	19.8(2.6)	30.8(2.7)	49.0(4.4)	36.5(2.1)	152.0(8.5)	255.0(14.1)
20	11.0(1.0)	19.2(2.7)	29.4(3.1)	48.0(4.7)	36.0(4.2)	147.0(1.4)	241.0(29.7)
30	10.0(1.2)	18.8(2.1)	29.2(3.4)	43.0(6.7)	32.5(5.0)	138.0(1.0)	205.5(77.1)
40	10.0(1.2)	18.6(2.3)	28.2(2.6)	39.4(4.9)	31.5(3.5)	116.5(3.5)	124.0(2.8)
50	10.0(1.2)	18.4(2.0)	27.4(2.7)	38.0(5.4)	30.0(2.8)	104.0(2.8)	121.0(5.7)
60	10.0(1.2)	18.4(2.0)	27.4(2.7)	36.6(5.0)	29.0(1.4)	101.5(0.7)	108.0(7.1)

APPENDIX 2.6

Number of nymphs of *B.germanica* as a function of initial number of first stage nymphs at ten day intervals during nymphal development

days following egg hatch	number of nymphs surviving population						
	1	2	3	4	5	6	7
0	13 12 11 12 10	24 20 20 20 20	33 35 34 35 35	50 55 47 50 50	38 40	158 183	310 353
10	13 12 11 12 10	24 19 17 20 19	29 31 28 35 31	50 55 43 50 47	38 35	158 146	265 245
20	12 10 11 12 10	24 19 17 17 19	27 30 26 34 30	50 54 41 48 47	33 39	148 146	262 220
30	9 10 9 12 10	22 19 17 17 19	26 30 26 34 30	33 50 40 45 47	29 36	131 145	260 151
40	9 10 9 12 10	22 19 16 17 19	25 30 26 31 29	32 44 37 42 42	29 34	119 114	126 122
50	9 10 9 12 10	21 19 16 17 19	25 30 24 29 29	32 44 33 39 42	28 32	106 102	125 117
60	9 10 9 12 10	21 19 16 17 19	25 30 24 29 29	32 42 33 34 42	28 30	101 102	103 113

APPENDIX 2.7

Mean (s.e., n = 4) number of nymphs surviving when caged with adult populations

days from hatching	mean number surviving (s.e.) of nymphs			
	N	NM	NF	NFM
0	34.3 (1.0)	39.5 (4.0)	37.8 (2.5)	39.0 (3.5)
10	30.8 (3.1)	36.8 (4.9)	32.8 (2.9)	37.3 (5.1)
20	29.3 (3.6)	34.3 (3.9)	32.0 (2.2)	36.0 (5.7)
30	29.0 (3.8)	32.3 (2.2)	31.3 (1.7)	33.8 (5.4)
40	28.0 (2.9)	32.3 (2.2)	30.3 (2.1)	33.3 (6.4)
50	27.0 (2.9)	32.3 (2.2)	29.0 (2.2)	32.8 (6.0)
60	27.0 (2.9)	32.3 (2.2)	28.8 (2.2)	31.8 (5.7)

N	nymphs alone
NM	nymphs with 10 male adults
NF	nymphs with 10 female adults
NFM	nymphs with 5 male and 5 female adults

APPENDIX 2.8

Number of nymphs surviving when caged with adult populations

days from hatching	mean number surviving (s.e.) of nymphs			
	N	NM	NF	NFM
0	33 35 34 35	43 39 34 42	38 41 37 35	40 34 40 42
10	29 31 28 35	39 35 31 42	35 35 29 32	39 30 38 42
20	27 30 26 34	38 34 29 36	34 33 29 32	39 28 36 41
30	26 30 26 34	33 33 29 34	31 33 29 32	38 26 34 37
40	25 30 26 31	33 33 29 34	31 32 28 30	38 24 34 37
50	25 30 24 29	33 33 29 34	30 31 28 29	36 24 34 37
60	25 30 24 29	33 33 29 34	30 31 26 28	36 24 31 36

APPENDIX 2.9

Effect of carbon dioxide anaesethetisation on nymphal maturation period

adult emergence	treatment	days adult emergence noted replication	mean (s.e.)
1st adult	with CO_2	56 60 54 55 50	55.0 (3.6)
	without CO_2	36 36 37 35 37	36.2 (0.8)
all adult	with CO_2	67 68 66 63 67	66.2 (1.9)
	without CO_2	47 45 48 46 47	46.6 (1.1)

APPENDIX 2.10

Development of "undisturbed"[1] nymphal population of *B. germanica*.

days after egg hatch	number of cockroaches					
	replication 1		replication 2		replication 3	
	nymphs	adults	nymphs	adult	nymphs	adults
0	38	0	35	0	40	0
35	30	0	31	0	34	2
36	27	3	31	0	33	3
37	27	3	29	2	31	5
38	26	4	29	2	23	13
39	15	15	28	3	18	16
40	12	18	25	6	17	17
41	7	23	22	9	16	18
42	5	25	17	14	14	20
43	3	27	15	16	11	23
44	1	29	13	18	7	27
45	0	30	10	21	2	32
46			7	24	0	34
47			5	26		
48			0	31		

[1] nymphal populations allowed to develop with no anaesthetisations with carbon dioxide.

APPENDIX 3.1

Number of cockroaches in shaded chamber at 30 min following 10 min recovery period in the dark or light

distance from source (cm)	arena perpendicular to source		arena parallel to sourec, cockroaches in unilluminated arena during recovery
	cockroaches in illuminated arena during recovery	cockroaches in unilluminated arena during recovery	
10	4 4 3 3 7 3 7 6	4 5 4 4 3 6 5 7	6 8 5 6 6 6 7 7
15	4 3 5 7 4 5 6 5	4 6 6 4 5 3 4 5	6 6 7 6 5 7 6 5
20	5 4 6 5 4 3 5 4	3 4 6 2 3 4 6 6	7 4 6 4 5 6 7 7
25	5 4 4 5 5 7 6 3	4 2 5 3 3 6 4 4	6 6 6 5 5 6 5 5
30	5 4 5 5 3 8 8 5	3 4 4 5 2 2 3 4	7 5 7 4 7 6 6 8

APPENDIX 3.2

Analysis of variance of data from the experiment of effect of orientation and distance of the boxes from the light source on the proportion of cockroach in the shaded chamber of the two choice boxes (RCB design with equal number of replications in each treatment)

source of variance	degrees of freedom	sum of squares	mean square	p
replication	7	736.48	105.21	n.s.
treatment	14	2962.67	211.62	**
orientation (A)	2	2312.97	1156.48	**
distance (B)	4	154.17	38.54	n.s.
A x B	8	495.54	61.94	n.s.
error	98	4989.06	50.91	
Total	120	8688.21		

c.v.= 1.4%

n.s. $p > 0.05$; * $0.01 < p < 0.05$; ** $p < 0.01$.

APPENDIX 3.3

Distribution of 10 cockroaches in arena orientated parallel at 10 cm from light source (cockroaches recovered from anaesthetisation in the dark)

exposure time (min)	number of cockroaches in shaded chamber replication					mean	s.e.
5	7	7	6	6	7	6.6	0.6
10	8	6	5	5	7	6.2	1.3
15	5	6	7	7	8	6.6	1.1
20	7	9	8	9	6	7.8	1.3
25	8	7	8	6	9	7.6	1.1
30	7	5	7	5	8	6.4	1.3
60	7	6	6	5	5	5.8	0.8

APPENDIX 3.4

Effect of long-term exposure to light or dark on distribution of 10 cockroaches in illuminated/shaded arena

test time from beginning of photophase (h)	number (s.e.) of cockroaches in shaded chamber 12:12 (L:D)					12:0 (L:D)					0:12 (L:D)				
0.5	6	5	7	6	8	8	8	6	9	9	9	9	8	10	9
1.5	5	5	7	5	6	9	9	9	9	8	7	6	8	8	6
4.5	7	4	7	4	5	8	6	8	9	8	8	7	9	8	8
6.5	5	6	7	5	6	6	7	8	7	9	7	7	7	8	8
9.5	6	5	6	4	4	9	7	9	8	8	9	9	7	6	9
11.5	7	5	6	6	4	9	8	8	5	9	8	5	6	7	7
12.5	8	6	7	5	6	9	9	8	9	9	8	7	6	7	8
13.5	8	9	6	5	6	7	8	8	9	7	7	7	9	7	9
15.5	5	6	7	8	8	9	8	9	8	8	7	8	8	8	9
18.5	6	9	6	9	7	8	7	8	9	9	9	7	7	9	8
21.5	7	7	9	6	9	8	7	9	8	7	8	8	8	9	8
23.5	7	5	7	4	8	7	9	8	7	8	8	9	7	8	8

APPENDIX 3.5

Effect of CO_2 anaesthetisation on distribution of cockroaches in illuminated/shaded choice box.

number of CO_2 anaesthetisation	number of cockroaches in shaded chamber replications					mean	s.e.
0	8	9	8	8	9	8.4	0.6
1	7	6	7	7	9	7.2	1.1
2	6	6	8	8	6	6.8	1.1
3	6	5	7	7	9	6.8	1.5
4	7	5	7	5	7	6.2	1.1
5	7	6	7	7	9	7.2	1.1

APPENDIX 3.6

Effect of separation of cockroaches into two populations ("light-prefering" and "dark-prefering") on subsequent distribution in illuminated/shaded arena

treatment	number in shaded chamber replication				
initial experiment (unsorted cockoaches)	7/81	7/10	9/10	9/10	9/10
	8/9	5/11	9/12	9/10	12/12
	10/10	9/10	8/10	10/10	9/10
second trial (insects seeking dark chamber)	10/10	7/10	7/10	10/10	10/10
	9/10	8/10	9/10	10/10	10/10
second trial (insects seeking light chamber)	10/10	9/10	5/10		

[1] number of cockroaches in shaded chamber/total number of cockroaches in the arena.

APPENDIX 3.7

Toxicity of permethrin 25% WP to cockroaches (two week old male adult; 30 min exposure on filter paper; 24 h mortality)

dose (mg/m^2)	number tested	responsed number	expected number	chi-square
24 h mortality				
2.3	50	3	2.7	0.04
3.9	50	17	17.7	0.04
4.7	50	24	25.3	0.14
5.4	50	33	32.1	0.07
6.4	50	40	39.0	0.12
9.3	50	47	47.4	0.05
21.9	50	50	50.0	0.01
30 min knockdown				
2.3	50	0	7.7	9.14
3.9	50	13	10.2	0.98
4.7	50	14	11.0	1.02
5.4	50	14	11.8	0.51
6.4	50	17	12.8	1.83
9.3	50	18	15.0	0.87
21.9	50	12	20.7	6.17
30.4	50	20	22.9	0.69
41.3	50	29	25.1	1.22
51.2	50	27	26.6	0.01

APPENDIX 3.8

Knockdown (30 min) and mortality (24 h) of the cockroaches exposed to 25% WP permethrin deposits for 30 min on treated filter paper

dose (mg/m^2)	number[1] knockdown					mean (s.e)	number killed					mean (s.e.)
2.3	0	0	0	0	0	0.0 (0.0)	0	3	0	0	0	0.6 (1.3)
3.9	5	4	3	1	0	2.6 (2.1)	4	1	4	5	3	3.4 (1.5)
4.7	3	2	4	2	3	2.8 (0.8)	1	6	5	6	6	4.8 (2.2)
5.4	2	2	5	3	2	2.8 (1.3)	6	6	6	8	7	6.6 (0.9)
6.4	4	4	3	4	2	3.4 (0.9)	9	8	9	8	6	8.0 (1.2)
9.3	4	3	4	4	3	3.6 (0.6)	10	10	10	8	9	9.4 (0.9)
21.9	1	2	2	4	3	2.4 (1.1)	10	10	10	10	10	10.0 (0.0)
30.4	5	3	6	2	4	4.0 (1.6)	-	-	-	-	-	- -
41.3	5	7	7	2	8	5.8 (2.4)	-	-	-	-	-	- -
51.2	5	3	7	6	6	5.4 (1.5)	-	-	-	-	-	- -

[1] out of 10

APPENDIX 3.9

Number (of 10) cockroaches in the illuminated chamber of half-treated choice arena following 10 min dark habituation in shaded chamber

treatment of arena	observation time (h)	number of cockroaches block (two replicates) 1		2		3		4	
illuminated half treated	0.5	5	0	3	6	2	8	9	0
shaded half untreated	1	5	9	4	8	1	6	9	9
	24	7	9	5	8	2	3	8	8
illuminated half untreated	0.5	1	1	1	0	0	0	2	4
shaded half treated	1	1	2	0	0	2	0	2	6
	24	0	1	3	0	1	2	0	0
both halves untreated	0.5	1	7	8	1	1	8	8	7
	1	9	8	9	3	9	8	6	0
	24	10	9	9	9	2	7	6	1

APPENDIX 3.10

Analysis of variance of effect of surface treatment of one half of arenas on the distribution of cockroaches at various times

source of variance	degrees of freedom	sum of squares	mean square	p[1]
replication	7	3201.89	457.41	n.s.
treatment	8	19992.10	2499.01	**
surf. treatment (A)	2	17408.33	8704.17	**
observ. time (B)	2	989.11	494.56	n.s.
A x B	4	1594.66	398.67	n.s.
error	56	25146.81	449.05	
total	72	48340.80		

c.v.= 56.9%
n.s. P > 0.05; * 0.01 < p < 0.05; ** p < 0.01

APPENDIX 3.11

The distribution of cockroaches following treatment with deposits of 25% WP permethrin in the illuminated/shaded arena

dose (mg/m²)	number[1] in shaded chamber														
	0 h					24 h					48 h				
0.0	6	5	6	8	8	5	8	7	6	7	5	6	6	8	8
1.0	8	8	9	9	8	10	9	9	10	10	9	10	10	10	9
2.0	8	10	6	7	9	9	10	10	10	9	9	10	10	10	9
3.4	6	4	7	4	7	10	9	7	9	9	10	9	10	10	10
5.4	4	4	3	3	6	6	10	6[2]	–	–	10	10	10[2]	–	–
6.4	1	2	0	1	4	–	–	–	–	–	–	–	–	–	–

[1] out of 10
[2] only survivors

APPENDIX 3.12

Distribution of cockroaches in the three-chambered choice arena at 30 min observation period after 10 min exposure to the deposits of 25% WP permethrin on the base of central chamber at varies concentrations before given access to escape chambers

dose (mg/m^2)	replications																					
	illuminated chamber							shaded chamber							central chamber							
0	0	0	2	2	2	0	2	2	1	4	2	3	6	1	8	9	4	6	5	4	7	
4.8	2	0	1	2	1	6		1	0	0	1	6	0		7	10	9	7	3	4		
7.7	5	4	7	3	2	2		0	1	3	1	1	2		5	5	0	6	7	6		
8.0	4	4	4	3	3	6		3	0	1	1	1	2		0	6	5	6	6	2		
12.9	0	4	4	4	2	1		7	1	3	2	3	0		3	5	3	4	5	9		
13.6	0	0	7	5	9	3		1	1	1	4	1	1		9	9	2	1	0	6		
18.0	0	1	1	1	4	2		3	0	0	0	1	4		7	9	9	9	5	4		
22.6	4	1	0	0	2			0	2	2	0	1			6	7	8	10	8			
31.3	4	4	1	2	4			0	1	0	1	1			6	5	9	7	5			

APPENDIX 4.1

Fate of oothecae following death of 7-14 day old *B.germanica* females resulting from exposure to 50% boric acid bait at 20°C and 92.5% r.h. (controlled with saturated $BaCl_2$ solution) (total of 3 replicates)

day	adult mortality	oothecae remaining attached to body of female			oothecae deposited by female during baiting period		
		apparently viable	non-viable	hatched	apparently viable	non-viable	hatched
50% w/w boric acid bait							
1	0.0						
2	2.0						
3	11.3						
4	41.3						
5	76.0						
6	93.3						
7	100.0	58	0	0	89	3	0
8		58	0	0	89	0	0
9		58	0	0	89	0	0
10		58	0	0	89	0	0
11		58	0	0	89	0	0
12		58	0	0	89	0	0
13		58	0	0	89	0	0
14		58	0	0	89	0	0
15		58	0	0	89	0	0
16		58	0	0	89	0	0
17		58	0	0	89	0	0
18		58	0	0	89	0	0
19		58	0	0	86	2	1
20		58	0	0	86	2	1
21		58	0	0	86	2	1
22		57	1	0	81	7	1
23		56	2	0	77	11	1
24		53	5	0	76	12	1
25		53	5	0	75	13	1
26		49	9	0	73	15	1
27		43	15	0	73	15	1
28		37	21	0	67	21	1

APPENDIX 4.1 (CONTINUED)

Fate of oothecae following death of 7-14 day old *B.germanica* females resulting from exposure to 50% boric acid bait at 20°C and 92.5% r.h. (controlled with saturated $BaCl_2$ solution) (total of 3 replicates)

day adult mortality	oothecae remaining attached to body of female			oothecae deposited by female during baiting period		
	apparently viable	non-viable	hatched	apparently viable	non-viable	hatched
29	32	27	0	58	30	1
30	26	33	0	53	35	1
31	21	38	0	47	41	1
32	21	38	0	40	48	1
33	13	46	0	37	51	1
34	13	46	0	33	55	1
35	11	48	0	29	59	1
36	4	55	0	28	60	1
37	3	56	0	17	71	1
38	1	57	0	10	78	1
39	0	58	0	5	82	2
40	0	58	0	0	87	2

APPENDIX 4.2

Fate of oothecae following death of 14-21 day old *B. germanica* females resulting from exposure to 50% boric acid bait at 20°C and 92.5% r.h. (controlled with saturated $BaCl_2$ solution) (total of 3 replicates)

day	adult mortality (%)	oothecae remaining attached to body of female at death			oothecae deposited by female during baiting period		
		viable	non-viable	hatched	viable	non-viable	hatched
50% boric acid							
1	0.7						
2	13.3						
3	31.3						
4	63.3						
5	93.3						
6	94.0						
7	[illegible]						
8	98.7						
9	98.7						
10	100.0	78	0	0	60	3	0
11		78	0	0	60	3	0
12		78	0	0	60	3	0
13		78	0	0	60	3	0
14		78	0	0	60	3	0
15		78	0	0	60	3	0
16		78	0	0	58	5	0
17		78	0	0	56	7	0
18		78	0	0	56	7	0
19		78	0	0	56	7	0
20		77	0	1	56	7	0
21		77	0	1	56	7	0
22		76	1	1	56	7	0
23		76	1	1	55	8	0
24		75	2	1	52	10	1
25		70	4	4	49	12	2
26		67	5	6	48	12	3
27		64	6	8	48	13	3
28		59	7	12	46	15	3

APPENDIX 4.2 (CONTINUED)

Fate of oothecae following death of 14-21 day old *B. germanica* females resulting from exposure to 50% boric acid bait at 20°C and 92.5% r.h. (controlled with saturated $BaCl_2$ solution) (total of 3 replicates)

day	adult mortality (%)	oothecae remaining attached to body of female at death			oothecae deposited by female during baiting period		
		viable	non-viable	hatched	viable	non-viable	hatched
29		58	7	13	45	16	3
30		51	10	17	43	17	4
31		48	13	17	40	20	4
32		44	15	19	31	28	5
33		37	22	19	25	34	5
34		30	29	19	17	42	5
35		27	32	19	14	45	5
36		24	35	19	10	49	5
37		11	48	19	8	51	5
38		7	52	19	7	52	5
39		3	55	20	2	57	5
40		0	58	20	0	59	5

APPENDIX 4.3

Fate of oothecae following death of 7-14 day old *B.germanica* females resulting from exposure to 50% boric acid bait at 28°C and 92.5% r.h. (controlled with saturated $BaCl_2$ solution) (total of 3 replicates)

day	adult mortality	oothecae remaining attached to body of female			oothecae deposited by female during baiting period		
		apparently viable	non-viable	hatched	apparently viable	non-viable	hatched
50% w/w boric acid bait							
1	17.3						
2	29.3						
3	88.7						
4	100.0	52	0	0	93	5	0
5		52	0	0	93	5	0
6		52	0	0	93	5	0
7		52	0	0	93	5	0
8		52	0	0	93	5	0
9		52	0	0	93	5	0
10		52	0	0	93	5	0
11		52	0	0	90	8	0
12		52	0	0	90	8	0
13		52	0	0	90	8	0
14		51	1	0	83	13	2
15		48	3	1	80	16	2
16		46	3	3	79	16	3
17		43	3	6	69	17	12
18		37	4	11	63	18	17
19		32	4	16	57	22	19
20		26	4	22	52	23	23
21		22	4	26	41	27	30
22		18	4	30	36	28	34
23		11	6	35	18	39	41
24		9	6	37	11	41	46
25		2	9	41	1	47	50
26		0	11	41	0	48	50

APPENDIX 4.4

Fate of oothecae following death of 14-21 day old *B.germanica* females resulting from exposure to 50% boric acid bait at 28°C and 92.5% r.h. (controlled with saturated $BaCl_2$ solution) (total of 3 replicates)

day	adult mortality (%)	oothecae remaining attached to body of female			oothecae deposited by female during baiting period		
		apparently viable	non-viable	hatched	apparently viable	non-viable	hatched
50% w/w boric acid bait							
1	25.3						
2	83.3						
3	98.7						
4	99.3						
5	100.0	83	0	0	59	3	0
6		82	0	1	58	4	0
7		81	0	2	56	6	0
8		79	0	4	54	7	1
9		78	0	5	50	9	3
10		76	0	7	47	11	4
11		75	0	8	45	12	5
12		72	1	10	40	15	7
13		66	6	11	34	20	8
14		63	6	14	32	21	9
15		59	6	18	28	23	11
16		56	6	21	28	23	11
17		50	11	22	23	26	13
18		43	14	26	19	28	15
19		39	16	28	15	31	16
20		29	17	37	11	31	20
21		23	17	43	8	33	21
22		13	21	49	3	35	24
23		9	21	53	0	35	27
24		3	24	56	0	35	27
25		0	26	57	0	35	27

APPENDIX 4.5

Fate of oothecae following death of 7-14 day old *B.germanica* females resulting from exposure to hydramethylnon bait at 20°C and 92.5% r.h. (controlled with saturated $BaCl_2$ solution) (total of 3 replicates)

day	adult mortality (%)	oothecae remaining attached to body of female			oothecae deposited by female during baiting period		
		apparently viable	non-viable	hatched	apparently viable	non-viable	hatched
hydramethylnon							
1	0.7						
2	0.7						
3	2.7						
4	16.7						
5	30.7						
6	40.7						
7	47.3						
8	56.0						
9	60.7						
10	65.3						
11	70.7						
12	80.0						
13	92.0						
14	94.7						
15	100.0	127	0	0	16	7	0
16		127	0	0	16	7	0
17		127	0	0	16	7	0
18		127	0	0	16	7	0
19		127	0	0	16	7	0
20		127	0	0	16	7	0
21		127	0	0	14	9	0
22		127	0	0	14	9	0
23		127	0	0	14	9	0
24		127	0	0	14	9	0
25		127	0	0	14	9	0
26		127	0	0	14	9	0
27		126	0	1	14	9	0
28		126	0	1	14	9	0

APPENDIX 4.5 (CONTINUED)

Fate of oothecae following death of 7-14 day old *B.germanica* females resulting from exposure to hydramethylnon bait at 20°C and 92.5% r.h. (controlled with saturated $BaCl_2$ solution) (total of 3 replicates)

day	oothecae remaining attached to body of female			oothecae deposited by female during baiting period		
adult mortality (%)	apparently viable	non-viable	hatched	apparently viable	non-viable	hatched
29	126	0	1	14	9	0
30	126	0	1	14	9	0
31	126	0	1	14	9	0
32	126	0	1	14	9	0
33	124	2	1	12	11	0
34	121	5	1	10	13	0
35	121	5	1	10	13	0
36	121	5	1	10	13	0
37	117	9	1	6	17	0
38	116	9	2	6	17	0
39	114	11	2	4	19	0
40	112	11	2	3	20	0
41	105	18	2	0	3	0
42	105	18	2	-	-	-
43	104	19	2	-	-	-
44	100	23	4	-	-	-
45	93	30	6	-	-	-
46	85	38	6	-	-	-
47	79	44	6	-	-	-
48	64	59	8	-	-	-
49	57	66	8	-	-	-
50	41	81	9	-	-	-
51	28	93	10	-	-	-
52	23	98	10	-	-	-
53	17	104	10	-	-	-
54	11	110	10	-	-	-
55	5	116	10	-	-	-
56	4	116	11	-	-	-
57	0	116	11	-	-	-

APPENDIX 4.6

Fate of oothecae following death of 14-21 day old *B.germanica* females resulting from exposure to hydramethylnon bait at 20°C and 92.5% r.h. (controlled with saturated $BaCl_2$ solution) (total of 3 replicates)

Day		oothecae remaining attached to body of female			oothecae deposited by female during baiting period		
	adult mortality (%)	apparently viable	desiccated	hatched	apparently viable	desiccated	hatched
hydramethylnon							
1	0.0						
2	0.7						
3	2.0						
4	13.3						
5	21.0						
6	47.3						
7	67.3						
8	74.0						
9	80.7						
10	88.7						
11	96.0						
12	100.0	144	0	0	4	2	0
13		144	0	0	4	2	0
14		144	0	0	4	2	0
15		144	0	0	4	2	0
16		144	0	0	4	2	0
17		144	0	0	4	2	0
18		144	0	0	4	2	0
19		144	0	0	4	2	0
20		144	0	0	4	2	0
21		144	0	0	4	2	0
22		144	0	0	4	2	0
23		144	0	0	4	2	0
24		144	0	0	4	2	0
25		144	0	0	4	2	0
26		144	0	0	4	2	0
27		144	0	0	4	2	0
28		143	1	0	3	3	0

APPENDIX 4.6 (CONTINUED)

Fate of oothecae following death of 14-21 day old *B.germanica* females resulting from exposure to hydramethylnon bait at 20°C and 92.5% r.h. (controlled with saturated $BaCl_2$ solution) (total of 3 replicates)

Day adult mortality (%)	oothecae remaining attached to body of female			oothecae deposited by female during baiting period		
	apparently viable	desiccated	hatched	apparently viable	desiccated	hatched
29	141	2	1	2	4	0
30	140	2	2	2	4	0
31	139	2	3	1	5	0
32	138	2	4	1	5	0
33	137	3	4	0	6	0
34	137	3	4	-	-	-
35	136	3	5			-
36	135	3	6	-	-	
37	135	3	6	-	-	
38	135	3	6	-	-	
39	134	3	7	-	-	
40	107	29	8	-	-	
41	105	31	11	-	-	
42	102	31	14	-	-	
43	97	31	19	-	-	
44	97	31	19	-	-	
45	82	46	19	-	-	
46	62	62	23	-	-	
47	51	73	23	-	-	
48	43	81	23	-	-	
49	29	95	23	-	-	
50	14	109	24	-	-	
51	0	120	24	-	-	

APPENDIX 4.7

Fate of oothecae following death of 7-14 day old *B.germanica* females resulting from exposure to hydramethylnon bait at 28°C and 92.5% r.h. (controlled with saturated $BaCl_2$ solution) (total of 3 replicates)

day	adult mortality (%)	oothecae remaining attached to body of female			oothecae deposited by female during baiting period		
		apparently viable	non-viable	hatched	apparently viable	non-viable	hatched
hydramethylnon bait							
1	0.0						
2	8.0						
3	46.7						
4	66.0						
5	68.7						
6	84.0						
7	98.7						
8	100.7	146	0	0	3	1	0
9		146	0	0	3	1	0
10		146	0	0	3	1	0
11		146	0	0	3	1	0
12		146	0	0	3	1	0
13		146	0	0	2	2	0
14		146	0	0	2	2	0
15		146	0	0	2	2	0
16		146	0	0	2	2	0
17		146	0	0	2	2	0
18		146	0	0	2	2	0
19		146	0	0	1	2	1
20		144	0	2	1	2	1
21		123	0	23	1	2	1
22		77	0	69	1	2	1
23		20	0	126	1	2	1
24		6	0	140	1	2	1
25		3	3	140	1	2	1
26		0	6	140	0	3	1

APPENDIX 4.8

Fate of oothecae following death of 14-21 day old *B. germanica* females resulting from exposuer to hydramethylnon bait at 28°C and 92.5% (controled with saturated $BaCl_2$ solution) total of 3 replicates)

day	adult mortalt (%)	oothecae remaining attached to body of female			oothecae deposited by female during baiting period		
		apparently viable	non-viable	hatched	apparently viable	non-viable	hatched
hydramethylnon bait							
1	10.7						
2	16.7						
3	47.3						
4	91.3						
5	95.3						
6	98.0						
7	100.0	138	0	0	10	2	0
8		138	0	0	10	2	0
9		138	0	0	10	2	0
10		138	0	0	10	2	0
11		137	0	1	10	2	0
12		137	0	1	10	2	0
13		137	0	1	10	2	0
14		132	0	6	10	2	0
15		121	0	18	10	2	0
16		103	0	36	10	2	0
17		74	0	65	8	2	2
18		39	0	100	5	2	3
19		22	0	117	2	2	6
20		4	13	121	0	4	6
21		0	17	121	0	4	6

APPENDIX 4.9

Hatch of pre-treated oothecae from 10 day old adult females at the end of 30 day observation period at 20°C and 92.5% r.h. (controlled with saturated $BaCl_2$ solution)

day	Control								Decapted							
initial number	12	12	12	12	12	35	40	50	12	12	12	12	12	35	40	50
1	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0
4	0	0	1	1	0	0	0	11	0	0	1	0	0	0	0	0
5	1	0	1	2	0	0	0	21	0	2	1	0	1	0	0	0
6	2	1	2	2	0	0	0	23	0	2	1	0	1	0	0	0
7	3	1	2	2	0	1	0	23	0	2	1	0	1	0	0	2
8	3	1	2	2	1	1	0	25	1	2	1	1	1	0	0	2
9	3	1	2	3	1	4	0	30	3	3	3	1	1	1	0	2
10	4	1	2	4	1	5	0	32	3	3	3	2	2	1	0	7
11	4	1	3	6	1	7	0	32	3	3	3	2	3	4	0	9
12	4	2	3	6	2	7	0	32	4	3	4	2	3	6	0	16
13	5	4	3	6	3	7	0	32	4	3	4	2	3	6	0	17
14	5	4	3	6	3	9	0	33	5	3	4	3	4	9	0	20
15	6	4	3	6	3	10	1	33	5	4	8	3	5	11	0	26
16	6	5	5	6	3	12	5	33	5	5	8	5	6	11	1	27
17	7	7	5	6	6	13	11	34	6	5	8	7	6	14	1	29
18	7	7	5	6	7	16	14	34	6	5	9	7	6	17	1	30
19	7	7	7	7	8	19	20	34	8	6	10	7	7	17	3	32
20	7	7	7	7	9	22	22	34	8	7	10	7	8	18	3	33
21	8	8	7	7	9	25	25	34	8	7	10	7	9	18	3	33
22	8	9	7	7	9	25	25	34	8	7	10	7	9	19	3	33
23	8	9	8	7	10	25	27	34	9	7	10	7	9	21	4	33
24	10	11	10	7	10	26	29	35	9	7	10	7	9	23	4	34
25	10	11	11	8	10	26	31	36	9	7	10	7	9	24	4	34
26	10	11	11	8	11	26	31	37	9	7	10	7	9	24	4	34
27	10	11	11	9	11	26	33	37	9	7	10	7	9	24	4	34
28	10	11	11	10	11	27	33	37	9	7	10	7	9	24	4	34
29	10	11	11	10	11	29	37	37	9	7	10	7	9	24	5	34
30	10	11	11	10	11	32	37	37	9	7	10	7	9	24	5	34

APPENDIX 4.9 (continued)

Hatch of pre-treated oothecae from 10 day old adult females at the end of 30 day observation period at 20°C and 92.5% r.h. (controlled with saturated $BaCl_2$ solution)

day	Manully removed								Hydramethylnon treated						
initial number	12	12	12	12	12	35	40	50	12	12	12	12	12	35	40
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	2	0	0	2	0	0	0	0	0	0	0	0	0	0
6	0	2	0	0	2	0	0	0	0	0	0	0	0	0	0
7	0	2	2	2	4	0	0	1	0	0	0	0	0	1	0
8	0	2	2	2	4	2	0	5	0	0	0	0	0	1	0
9	0	2	2	2	4	2	0	5	1	1	3	0	3	2	0
10	1	3	2	3	4	7	0	8	1	1	6	0	3	2	0
11	5	6	3	3	6	7	0	9	3	1	6	0	3	7	0
12	6	6	4	3	6	11	0	13	3	1	7	1	6	9	0
13	6	6	4	3	6	11	0	15	3	1	7	2	6	10	0
14	6	7	5	4	7	14	0	16	3	1	7	3	6	15	0
15	7	7	6	4	7	18	0	17	5	1	7	4	7	20	1
16	9	7	6	7	11	20	1	18	5	1	7	6	9	20	2
17	10	9	6	7	11	23	1	19	6	1	9	7	9	23	2
18	10	9	6	7	11	23	1	20	7	2	9	7	9	24	3
19	10	9	6	7	11	24	2	22	9	3	10	9	10	24	3
20	10	9	6	7	11	24	2	24	9	4	10	9	10	24	9
21	10	9	6	7	11	25	3	25	9	4	10	10	10	25	10
22	10	9	6	7	11	25	9	25	9	5	10	10	10	25	13
23	10	9	6	7	11	26	13	25	9	5	10	10	10	26	15
24	10	9	6	7	11	26	14	25	9	5	10	10	10	28	19
25	10	9	6	7	11	26	14	26	9	5	10	10	10	29	19
26	10	9	6	7	11	26	16	26	9	5	10	10	10	30	19
27	10	9	6	7	11	26	16	27	9	5	10	10	10	30	19
28	10	9	6	7	11	26	16	27	9	5	10	10	10	30	20
29	10	9	6	7	11	26	16	27	9	5	10	10	10	30	20
30	10	9	6	7	11	26	16	27	9	5	10	10	10	30	20

APPENDIX 4.10

Deposition and hatch of oothecae from 14-21 day old female *B. germanica* at two temparetures and 92.5% r.h. (controlled with saturated $BaCl_2$ solution)

	28°C		20°C	
day	cumulative number deposited	cumulative number hatched	cumulative number deposited	cumulative number hatched
1	0 4	0 4	0 0	0 0
2	0 16	0 16	1 0	0 0
3	0 17	0 17	2 0	0 0
4	2 30	1 30	2 0	0 0
5	5 41	5 41	2 0	0 0
6	13 44	13 44	2 0	0 0
7	20 45	20 45	2 0	0 0
8	28 46	28 46	2 0	0 0
9	29 48	29 48	2 0	0 0
10	32 49	32 48	2 1	0 1
11	40 49	40 48	2 1	0 1
12	42 49	42 48	2 1	0 1
13	43 49	40 48	2 4	0 4
14	48 50	45 48	2 5	0 5
15	50 50	48 48	2 8	0 8
16	- -	- -	2 8	0 8
17	- -	- -	2 8	0 8
18	- -	- -	2 9	0 9
19	- -	- -	3 9	0 9
20	- -	- -	3 12	0 12
21	- -	- -	3 15	0 15
22	- -	- -	3 20	0 16
23	- -	- -	3 26	0 24
24	- -	- -	3 35	0 30
25	- -	- -	5 35	3 35
26	- -	- -	5 37	3 37
27	- -	- -	16 38	12 37
28	- -	- -	21 40	17 40
29	- -	- -	25 45	23 40
30	- -	- -	25 46	23 40

APPENDIX 4.10 (CONTINUED)

Deposition and hatch of oothecae from 14-21 day old female *B. germanica* at two temparetures and 92.5% r.h. (controlled with saturated $BaCl_2$ solution)

	28° C		20° C	
day	cumulative number deposited	cumulative number hatched	cumulative number deposited	cumulative number hatched
31	-	-	26 45	23 40
32	-	-	29 48	25 40
33	-	-	30 48	29 40
34	-	-	33 50	31 40
35	-	-	33 50	31 40
36	-	-	37 50	35 40
37	-	-	37 50	35 40
38	-	-	37 50	35 40
39	-	-	40 50	37 40
40	-	-	45 50	40 40
41	-	-	45 50	40 40
42	-	-	49 50	45 40
43	-	-	50 50	47 40

APPENDIX 4.11

Deposition and hatch of oothecae from 7-14 day old female *B. germanica* at two temparetures and 92.5% r.h. (controlled with saturated $BaCl_2$ solution)

	28°C		20°C	
day	cumulative number deposited	cumulative number hatched	cumulative number deposited	cumulative number hatched
1	0 0	0 0	0 0	0 0
2	0 0	0 0	0 0	0 0
3	0 0	0 0	0 0	0 0
4	0 0	0 0	0 0	0 0
5	0 0	0 0	0 0	0 0
6	0 0	0 0	0 0	0 0
7	0 5	0 5	2 0	0 0
8	0 12	0 12	3 0	0 0
9	0 19	0 19	3 0	0 0
10	0 27	0 26	3 0	0 0
11	6 34	5 31	3 0	0 0
12	11 34	11 33	3 0	0 0
13	20 36	19 34	3 0	0 0
14	24 40	24 36	3 0	0 0
15	37 40	37 37	3 0	0 0
16	43 43	43 37	3 2	0 0
17	46 45	46 40	3 3	0 2
18	47 46	47 40	3 3	0 3
19	50 50	50 40	3 7	0 7
20	- -	- -	3 8	0 8
21	- -	- -	3 16	0 15
22	- -	- -	3 16	0 15
23	- -	- -	3 20	0 19
24	- -	- -	3 20	0 20
25	- -	- -	3 21	0 21
26	- -	- -	4 25	0 25
27	- -	- -	4 26	0 25
28	- -	- -	4 26	0 26
29	- -	- -	4 31	0 30
30	- -	- -	4 33	0 33

APPENDIX 4.11 (CONTINUED)

Deposition and hatch of oothecae from 7-14 day old female *B. germanica* at two temparetures and 92.5% r.h. (controlled with saturated $BaCl_2$ solution)

	28°C		20°C	
day	cumulative number deposited	cumulative number hatched	cumulative number deposited	cumulative number hatched
31	- -	- -	5 35	1 35
32	- -	- -	5 37	1 35
33	- -	- -	5 42	1 42
34	- -	- -	5 46	1 42
35	- -	- -	5 46	1 42
36	- -	- -	7 46	1 42
37	- -	- -	7 46	1 42
38	- -	- -	11 47	2 42
39	- -	- -	16 47	4 42
40	- -	- -	16 47	4 42
41	- -	- -	20 47	5 42
42	- -	- -	23 47	7 42
43	- -	- -	25 48	8 42
44	- -	- -	26 48	9 42
45	- -	- -	32 49	9 42
46	- -	- -	32 50	9 42
47	- -	- -	32 50	10 42

APPENDIX 5.1

Number of dead cockroaches (2-3 wk old male adults) after continuous exposed to various concentrations of silica dusts for 24 hours in petri dishes

silica dust	number (dose mg/m²) of dead cockroaches out of 10													
Gasil 23F	4 (0.09)	4 (0.19)	6 (0.28)	4 (0.38)	7 (0.49)	5 (0.59)	6 (0.61)	10 (0.63)	9 (0.82)	9 (1.09)	9 (1.10)	10 (1.62)	8 (1.64)	
Gasil HP37	3 (0.13)	1 (0.16)	7 (0.35)	6 (0.36)	9 (0.46)	5 (0.53)	5 (0.61)	5 (0.64)	10 (0.83)	7 (0.85)	10 (1.18)	9 (1.20)	10 (1.54)	10 (1.57)
Gasil HP25	1 (0.35)	4 (0.69)	7 (0.80)	7 (0.94)	6 (1.09)	8 (1.34)	9 (1.59)							
Gasil 114	1 (0.38)	4 (0.69)	3 (0.94)	7 (1.26)	9 (1.59)									
Gasil AF	2 (0.83)	3 (1.10)	7 (1.62)	5 (2.34)	8 (3.11)									
Cab-O-Sil M-7D	5 (1.43)	3 (1.45)	4 (1.59)	4 (1.60)	2 (1.86)	2 (1.90)	6 (2.39)	6 (2.41)	4 (2.66)	3 (2.67)	5 (3.14)	7 (3.19)	8 (3.76)	8 (3.77)
Cab-O-Sil H5	2 (0.35)	6 (0.63)	7 (0.93)	9 (1.24)	10 (1.57)									
Cab-O-Sil HH5	3 (0.33)	5 (0.63)	6 (0.90)	10 (1.23)	9 (1.57)									
Wacker HDK H20	1 (0.16)	0 (0.17)	3 (0.31)	3 (0.35)	6 (0.47)	4 (0.52)	1 (0.60)	2 (0.63)	9 (0.76)	10 (0.80)	9 (1.07)	9 (1.12)	10 (1.57)	10 (1.60)
Wacker HDK N20	0 (0.31)	5 (0.60)	8 (0.94)	9 (1.26)	10 (1.60)									

APPENDIX 5.2

Amount of silica dust picked up by male adult German cockroaches during 3 minute exposure to the dusts at 2 g/m² in petri dishes

Gasil HP37

dyed dust (mg)	0.108	0.232	0.415	0.646	0.830	1.036
O.D.(5 ml sol.)	0.05	0.12	0.20	0.31	0.46	0.55

O.D = 0.032 + 1.821 (dust weight) r^2 = 0.992

exposure time (min)	1.5	5	30	60	90	120	180
O.D./insect	0.03	0.07	0.11	0.16	0.24	0.35	0.34
	0.02	0.07	0.08	0.12	0.39	0.32	0.34
	0.06	0.07	0.07	0.10	0.21	0.22	0.22
	0.04	0.11	0.08	0.10	0.21	0.18	0.14
	0.02	0.13	0.09	0.14	0.30	0.18	0.30

Wacker HDK N20

dyed dust (mg)	0.20	0.42	0.62	0.82	1.04
O.D.(5 ml sol.)	0.14	0.29	0.40	0.56	0.68

O.D. = 0.001 + 1.473 (dust weight) r^2 = 0.997

exposure time (min)	1.5	5	30	60	90	120	180
O.D./insect	0.022	0.012	0.018	0.016	0.024	0.070	0.120
	0.032	0.020	0.016	0.026	0.039	0.080	0.124
	0.027	0.020	0.010	0.018	0.021	0.104	0.090
	0.036	0.010	0.010	0.028	0.021	0.064	0.100
	0.026	0.012	0.016	0.016	0.030	0.092	0.072

Sipernat 22

dyed dust (mg)	0.05	0.14	0.22	0.46	0.77
O.D.(5 ml sol.)	0.10	0.30	0.46	1.09	1.91

O.D. = 0.024 + 0.394 (dust weight) r^2 = 0.999

exposure time (min)	1.5	5	30	60	90	120	180
O.D./insect	0.050	0.049	0.041	0.059	0.044	0.044	0.045
	0.054	0.051	0.038	0.056	0.044	0.043	0.049
	0.055	0.056	0.047	0.046	0.044	0.040	0.047

APPENDIX 5.3

Mortality of 2-3 wk adult male *B. germanica* in choice boxes half-treated with HP37 at 1.15 g/m²)

treatment	exposure period (h)						total no. tested
	1	2	4	6	12	24	
starved:							
conditioned	0 0 0 0	0 0 0 0	0 0 0 3	0 0 0 3	0 0 2 7	6 4 18 20	20 20 20 20
	0 0 0 2	0 2 0 6	0 5 0 7	0 7 0 8	4 13 2 9	20 20 20 19	20 20 20 19
	0	0	4	4	4	19	19
unconditioned	0 0 0 0	0 1 2 0	2 7 2 0	5 11 3 0	18 17 5 1	18 20 20 17	18 20 20 20
	0 0 0 0	0 0 0 2	2 1 0 2	5 5 0 7	14 13 2 14	20 20 20 19	20 20 20 19
	0	0	7	13	20	20	20
unstarved:							
conditioned	0 0 0 0	0 7 9 0	0 18 17 0	0 18 17 0	0 19 17 1	0 19 17 1	20 19 17 20
	0 0 0 0	1 0 0 0	1 0 0 0	1 0 0 0	2 0 2 0	6 2 10 0	17 19 19 20
	0	1	1	1	1	5	20
unconditioned	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 1 13 4	20 20 20 20
	0 0 0 0	0 0 0 0	0 2 0 0	0 2 0 0	0 3 1 0	15 20 14 10	20 20 20 20
	0	0	0	0	0	6	20

starved: held for 24 h without food + water following removal from culture tin
unstarved: held for 24 h with food + water following removal from culture tin
unconditioned: allowed immediate access to treated half of chamber following addition to choice box
conditioned: held for 2 h in untreated half of choice box prior to being given access to treated half

APPENDIX 5.4

Number of live 2-3 wk male adult *B. germanica* in treated (T) and untreated (UT) halves of choice boxes half-treated with Gasil HP37 at 1.15 mg/m²

treatment	exposure period 1 h T	UT	2 h T	UT	4 h T	UT	6 h T	UT	12 h T	UT	24 h T	UT	total in box
starved:													
conditioned	0	20	0	20	2	18	0	20	0	20	0	14	20
	0	20	0	20	0	20	0	20	0	20	1	15	20
	1	19	0	20	0	20	0	20	1	17	0	2	20
	0	20	0	20	0	17	3	14	6	7	–	–	20
	0	20	0	20	0	20	1	19	6	10	–	–	20
	2	18	0	18	0	15	3	10	2	5	–	–	20
	0	20	0	20	0	20	0	20	0	18	–	–	20
	3	14	1	13	1	12	0	11	1	9	–	–	19
	1	18	1	18	0	15	0	15	2	13	–	–	19
unconditioned	0	18	0	18	1	15	1	12	–	–	–	–	18
	2	18	0	19	1	12	2	7	2	1	–	–	20
	1	19	0	18	0	18	1	16	0	15	–	–	20
	0	20	0	20	0	20	0	20	1	18	0	3	20
	1	19	1	19	0	18	0	15	1	5	–	–	20
	0	20	0	20	1	13	0	15	0	7	–	–	20
	0	19	0	19	2	17	0	19	0	17	–	–	20
	3	16	0	17	4	13	0	12	0	5	–	–	19
	0	20	3	17	4	9	0	7	–	–	–	–	20
unstarved:													
conditioned	0	20	0	20	1	19	0	20	0	20	0	20	20
	5	13	2	10	0	1	0	1	–	–	–	–	19
	5	12	3	5	–	–	–	–	–	–	–	–	17
	1	19	0	20	0	20	0	17	0	19	1	18	20
	0	17	0	16	1	15	0	16	1	14	0	11	17
	1	19	1	19	0	20	0	20	2	18	0	18	19
	1	18	0	19	0	19	0	19	0	17	0	19	19
	1	19	1	19	1	19	2	18	0	20	0	20	20
	0	20	0	19	0	19	0	19	2	17	0	15	20
unconditioned	0	20	0	20	0	20	0	20	0	20	2	17	20
	0	20	0	20	1	19	0	20	0	20	0	7	20
	0	20	0	20	0	20	0	20	5	15	0	20	20
	0	20	0	20	0	20	0	20	0	20	1	15	20
	1	19	1	19	1	19	1	19	2	18	0	5	20
	0	20	0	20	0	18	0	18	2	15	–	–	20
	1	19	0	20	0	20	0	20	5	14	0	6	20
	1	19	0	20	0	20	0	20	1	19	3	7	20
	4	14	5	13	6	12	6	12	5	13	1	11	18

starved: held for 24 h without food + water following removal from culture tin.

unstarved: held for 24 h with food + water following removal from culture tin.

unconditioned: allowed immediate access to treated half of chamber following addition to choice box.

conditioned: held for 2 h in untreated half of choice box prior to being given access to treated half.

APPENDIX 5.5

Number of dead male adults of German cockroaches in choice boxes half-treated with Gasil HP37 at 1.15 mg/m²

relative humidity	exposure period 1 h	2 h	4 h	6 h	12 h	24 h	total
58% r.h.	2 0	7 0	18 14	20 18	20 20	20 20	20 20
	0 0	0 0	0 0	0 0	0 0	10 15	20 20
control	0 0	0 0	0 0	0 0	0 0	3 0	20 20
	0 0	0 0	0 0	0 0	0 0	0 0	20 20
85% r.h.	0 0	1 1	6 6	12 13	20 19	20 19	20 19
	0 0	0 0	0 0	0 0	2 0	7 6	14 20
control	0 0	0 0	0 0	0 0	0 0	0 0	20 20
	0 0	0 0	0 0	0 0	0 0	0 0	19 20
95-100%	0 0	0 1	0 1	0 1	0 1	0 13	20 20
	0 0	0 0	0 0	0 0	0 0	1 1	18 20
control	0 0	0 0	0 0	0 0	0 0	2 2	18 19
	0 0	0 0	0 0	0 0	0 0	1 1	18 17

APPENDIX 5.6

Number of live male adult German cockroaches (out of 20) in treated (T) and untreated (UT) side of choice boxes half-treated with Gasil HP37 at 1.15 mg/m² (n=4)

relative humidity	exposure period 1 h a[1] T	1 h b UT	2 h a T	2 h b UT	4 h a T	4 h b UT	6 h a T	6 h b UT	12 h a T	12 h b UT	24 h a T	24 h b UT	total in box
58% r.h.	6	12	5	8	0	2	-	-	-	-	-	-	20
	0	20	2	18	1	4	0	2	-	-	-	-	20
	0	18	0	18	0	18	0	18	0	18	0	8	18
	0	20	0	20	0	20	1	19	0	20	0	5	20
control	18	2	19	1	20	0	18	2	4	16	14	3	20
	0	20	0	20	0	20	0	20	2	18	1	19	20
	9	11	10	10	14	6	3	17	11	9	18	2	20
	19	0	19	0	18	0	18	1	17	2	17	2	19
85% r.h.	1	19	4	15	0	14	1	7	-	-	-	-	20
	1	18	0	18	0	13	0	6	-	-	-	-	19
	1	13	0	14	0	14	1	13	1	11	1	6	14
	0	20	0	20	0	20	0	20	0	20	2	12	20
control	15	5	12	8	19	1	20	0	20	0	10	10	20
	0	20	0	20	0	20	0	20	3	17	0	20	20
	14	5	17	2	14	5	18	1	17	2	10	9	19
	0	20	1	19	1	19	1	19	6	14	0	20	20
95-100% r.h.	0	20	0	20	0	20	0	20	1	19	0	19	20
	5	15	2	17	2	17	1	18	2	17	0	7	20
	0	18	0	18	0	18	1	17	1	17	0	17	18
	0	20	0	20	0	20	0	20	4	16	0	19	20
control	2	16	0	18	8	10	8	10	8	10	7	9	18
	0	19	0	19	0	19	0	19	0	19	1	16	18
	2	16	2	16	0	18	1	17	0	18	0	17	18
	6	11	13	4	7	10	3	14	2	15	8	7	17

[1] (a) cockroaches placed initially in chamber (a)
(b) cockroaches placed initially in chamber (b)

APPENDIX 5.7

Effect of the presence of water dispensers on toxicity of Gasil HP37 dusted on one side of choice boxes at 1.15 mg/m² at 75% r.h. and 30° C (n=4)

	number of cockroaches killed						
	1 h	2 h	4 h	6 h	12 h	24 h	total
no water	0	4	9	10	19	20	20
dispenser	0	1	6	10	20	20	20
	1	1	7	14	15	17	17
	1	8	15	18	20	20	20
with water	0	0	1	1	2	5	20
dispenser	0	0	0	0	0	1	20
	0	1	2	4	4	6	20
	0	0	1	1	1	5	20

APPENDIX 5.8

Number of live male adults[1] of German cockroaches in treated (T) and untreated (UT) sides of choice boxes half-treated with Gasil HP37 at 1.15 mg/m² (n=4)

	exposure period												
	1 h		2 h		4 h		6 h		12 h		24 h		total
	T	UT	T	UT	T	UT	T	UT	T	UT	T	UT	teated
no water	6	14	5	11	7	4	5	5	1	0	–	–	20
dispenser	7	13	7	12	6	8	5	5	–	–	–	–	20
	2	14	3	13	4	6	0	3	0	2	–	–	17
	3	16	2	10	2	3	0	2	–	–	–	–	20
with water	7	13	6	14	3	15	4	15	4	14	0	15	20
dispensers	1	19	1	19	3	17	1	19	6	14	0	19	20
	5	15	8	11	5	13	3	13	4	12	4	10	20
	2	18	1	19	0	19	3	16	2	17	1	14	20

[1] cockroaches deprived of food and water for 24 h prior to introduction into choice boxes and allowed immediate access to the treated side.

APPENDIX 5.9

Number of dead cockroaches (out of 10) at 24 h following 3 min exposure to combinations of pyrethroids and Gasil HP25 or talc at 1 g/m²

combination	concentration (w/w)														
	0.01%					0.05%					0.1%				
deltamethrin plus															
talc	0	0	0	0	0	10	10	7	6	7	-				
silica	10	0	8	2	0	10	10	10	10	10	-				
alphamethrin plus															
talc	0	0	0	0	0	2	0	1	0	1	-				
silica	3	5	6	9	9	10	9	10	10	10	-				
cyfluthrin plus															
talc	0	0	0	0	0	0	2	0	0	1	4	4	2	5	2
silica	0	0	5	3	0	9	5	6	4	5	10	10	10	10	10
cypermethrin plus															
talc	0	0	0	0	0	0	0	0	0	0	5	3	5	2	0
silica	5	0	0	2	0	7	2	10	4	4	10	9	7	10	10
permethrin plus															
talc	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
silica	0	6	0	0	0	0	0	7	0	2	6	0	2	4	4
control															
talc	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
silica	1	0	0	0	0	1	4	0	1	1	0	0	2	3	0

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