APPLICATION OF INSECTICIDES TO CONTROL THE GERMAN COCKROACH, *Blattella germanica* (L.)

by

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(ABSTRACT)

Control strategies for the German cockroach, Blattella germanica (L.), are usually based on the application of liquid insecticides in or near infested harborages. Cockroach mortality occurs when they are exposed to insecticide residue by walking on insecticide-treated surfaces. Cockroach walking depends on the three pairs of legs. Only tarsal pads and arolium on each leg are involved in travelling and picking up insecticide residue. The total contact area at each step was 0.1879 mm^2 and 0.1771 mm^2 for laboratory susceptible (VPI) and field-collected (RHA) cockroach strains, respectively. There were no significant differences ($P > 0.05$) in the walking distance in two steps among three legs, or between the two strains. However, walking movement rate greatly influences the amount of insecticide accumulated on tarsal pads in a distinct time period. RHA-strain cockroach walked a significantly ($P < 0.05$) greater distance than VPI-strain on untreated and 10 ug/cm^2 of cypermethrin treated glass plates. RHA-strain cockroaches picked up significantly ($P < 0.05$) more cypermethrin than VPI-strain in 5 min, but there was no difference when exposed for 30 movement units. Knockdown time was significantly correlated

($P < 0.05$) to estimated dose transfer in 0.025 ug/cm^2 and 0.049 ug/cm^2 treatments for the VPI-strain, and in 0.245 ug/cm^2 and 0.392 ug/cm^2 for the RHA-strain. Cockroach knockdown time and mortality were determined by cockroach walking on different numbers of cypermethrin droplets on treated glass plates. Insecticide dose could be reduced from 10 ug/cm^2 to 0.025 ug/cm^2, but resulted in increasing the KT_{50} from 5.4 min to 15.9 min in VPI-strain cockroaches. To achieve 50% cockroach knockdown, a VPI-strain cockroach must walk on at least 33 droplets (130 um in diameter) of 0.1% cypermethrin; a RHA-strain cockroach must walk on at least 3174 droplets. High insecticide dosage increased the amount of antennal grooming in VPI-strain cockroaches, and stimulated leg grooming in RHA-strain. Walking movement activities decreased in response to increased antennal and leg grooming.

For my parents

who guide me to be a scientist with great patience and diligence

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My thoughts go back to a small street in Shanghai, where my parents, relatives, and friends are living. My parents always do the best for their daughter, and send their only child across Pacific to another country. Their encouragement, understanding, and love give me strength to go through trouble and being home sick. Special thanks also go to my relatives for their taking care of my parents. To my lovely cousin, Yinyin, thank you for making my parents life happier.

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Chapter I

INTRODUCTION, LITERATURE REVIEW AND OBJECTIVES

The German cockroach, Blattella germanica (L.), is one of the most important pests in urban environments around the world. This species can occur in large numbers in commercial and residential buildings, and is often considered a serious pest by urban residents (Wood et al. 1981, Zungoli & Robinson 1984, Robinson & Bao 1988). Zungoli & Robinson (1984) surveyed urban apartment residents in three Eastern cities, and reported that two German cockroaches sighted in a dwelling was considered a serious problem. This cockroach species is usually associated with damp and cluttered household conditions, and has been implicated as a direct or indirect disease carrier (Jettmar 1935, Janssen & Wedberg 1952, Bernton & Brown 1970a, b, Schulaner 1970, Fernandez & Zaror 1971, Alcamo & Frishman 1980). Recently, household cockroaches have been implicated in causing serious allergic reactions (Brenner et al. 1990, Kang 1990).

The German cockroach is a household pest throughout the year, with a preference for infesting kitchens and bathrooms in urban apartments (Akers & Robinson 1981). Ebeling et al. (1967), Ono & Tsuji (1972), and Mueller (1978) reported the importance of proximity of food and water to German cockroach survival. Cornwell (1968) and Zhai (1990) reported this species preferred humid environments, and a temperature range of 24-33^{0} C. Kunkel (1966), Cochran (1983), Durbin & Cochran (1985) reported the effect of water and food deprivation on female German cockroach reproduction.

Control strategies for the German cockroach are based on the application of liquid insecticides to infested harborages, or placing toxic bait stations close to harborages (Tsuji & Ono 1969, Robinson & Zungoli 1985, Robinson 1988). Modern neurotoxic insecticides, such as the organophosphates, carbamates, and pyrethroids can function as both contact and stomach poisons. Cockroach mortality occurs when adults and nymphs are exposed to insecticide residues by walking on treated surfaces and/or ingesting toxic quantities of insecticide while grooming legs and antennae. Reingold & Camhi (1977) reported cockroach grooming behavior occurred in response to increased chemical irritation. German cockroach tarsal grooming peaked at 24-30 min after exposure to a propoxur formulation (Bret & Ross 1986). The most important routes-of-entry for cockroach control chemicals are the cuticle and digestive system. Movement of cockroaches over a surface depends on the legs. The tarsi on each leg are always touch the substrate; they are the primary appendage through which cockroaches contact insecticides. The tarsal pads and arolium are the part of the leg
that actually come in contact with the insecticide residue on treated surfaces.

Traditional application methods for liquid insecticides for cockroach control include the use of a compressed-air sprayer. The typical nozzle delivers a spray of liquid in droplets in the range of 50-400 um. In urban building environments, pest control operators usually use one of four nozzles: coarse-fan (50015), fine-fan (800067), pin-stream (0001), or a crack-and-crevice tip. The mass median diameter of the spray droplets from a fine-fan (800067) nozzle ranges 120-150 um (Byron 1987).

Insecticide resistance in German cockroach field populations has been a problem (Cochran 1984, 1987, 1989, 1990, Zhai & Robinson 1991). This cockroach has developed some level of resistance to most insecticides used for control. Zhai & & Robinson (1991) reported that exclusive use of cypermethrin resulted in high level (LD_{50} ratio 180) of resistance in a field population of German cockroaches. Resistance management programs can help delay the development of resistance in the field population, and effective and efficient insecticide application will be an most important part of such programs.

LITERATURE REVIEW

Morphology. Cockroach locomotion depends on the three pairs of legs. Snodgrass (1965) described the basic structure of an American cockroach leg, and Cornwell (1968) illustrated the structure of a typical cockroach leg. Zhai & Robinson (1990) presented photomicrographs showing the basic structure and ventral view of German cockroach legs. There are six segments on each leg: coxa, trochanter, femur, tibia, tarsus and pretarsus. Each tarsus is subdivided into five tarsomeres which are connected by a flexible membrane. Each of the first four tarsomeres bears a single, pad-like structure called an euplantula (Roth & Willis 1952). The ventral view of pretarsus of Periplaneta sp., illustrated by Chapman (1969), consists of a large membranous or partly sclerotized arolium, a pair of claws with the unguifer, and a pair of small auxilia between the claws and unguitractor. Brousse-Gaury (1981) prepared a topographical map of sensilla on the tarsi of the American cockroach. She showed that the largest number of four different sensilla (basiconic, campaniform, trichodea, mechanorecepters and contact chemorecepters) in ventral view of tarsus are mechanoreceptors and contact chemoreceptors. Frings

& Frings (1949) reported that the legs of American and German cockroaches lack contact chemoreceptors. Sarkaria & Patton (1949) reported that the position of the arolium on susceptible German cockroaches during walking is an important factor to their exposure to dry films of the insecticide DDT.

Walking behavior. Chapman (1969) reported that the tarsi of the midlegs and hindlegs in Periplaneta sp. are always placed on the substrate when the insect walks. The tarsi of five cockroach species, P. americana (L), P. australasiae (F.), Blatta orientalis (L), B. germanica (L), and Supella longipalpa (F.), were studied by Roth & Willis (1952). They reported that the parts of tarsi touching the substrate are euplantulae and pretarsus. Usually the tips of the claws and euplantulae of the 3rd and 4th tarsomeres contact the surface. The functions of these structures vary on different surfaces. Bowerman (1977) summarized the arthropod walking movements analyzed by a large number of researchers. Much of the work was concerned with neural mechanisms of movements of single legs in cockroaches, locusts, and grasshoppers (Nijenhuis & Dresden 1952, 1955a, b, Dresden & Nijenhuis 1958, Hoyle 1964, Runion & Usherwood 1968, Usherwood et al. 1968, Young 1969, Usherwood & Runion 1970, Delcomyn & Usherwood 1973, Fourtner 1976, Delcomyn 1985, 1987). The behavior and neurophysiology of walking in Arthropoda has been analyzed by Manton (1953) and Bowerman (1977). Burns (1973) reported on coordination mechanisms of normal walking in some Orthoptera.

Cornwell (1968), and Guthrie & Tindall (1968) reported the different functions of the American cockroach legs involved in walking. Reingold & Camhi (1977) reported only one hindleg of the American cockroach is used for grooming.

Research on the walking movements of the oriental cockroach, B. orientalis indicated that the normal walking rhythm is R1, L2, R3, L1, R2, L3, R1, etc (Hughes 1952). This conclusion was the same as that reported by Full & Tu (1990) for Blaberus discoidalis (Serville). During locomotion, foreleg and midleg resist the forward motion of the body (Graham 1983), and the hindleg must be in a supporting position before the foreleg or midleg is protracted (Hughes 1952). Delcomyn (1971a, b) studied the locomotion of P. americana, and reported that this cockroach never walks in a straight line for long distances. He reported the midlegs remained on the substrate a little longer than any of the other four legs. Full & Tu (1990) measured ground reaction forces and mechanical energy changes when B. discoidalis travelled. They reported alternating tripod locomotion speeds in this species ranging from 0.08 to 0.66 m/sec. Zhai & Robinson (1990) described the normal walking behavior of a German cockroach. Akers & Robinson (1983) reported that there was significant difference in the movement between a field- and laboratory-reared strain of German cockroaches. The field strain was more active than the VPI-Normal strain.

Sprayer equipment. Matthews (1979) described the function of different types of nozzle. Traditional application in the urban environment for cockroach control includes use of compressed-air sprayer and a fan nozzle to deliver insecticide to infested harborages. The B&G compressed-air sprayer nozzle and spray pattern of nozzles on Multee-jet tip were described by Byron & Robinson (1986, 1987). They demonstrated the different height and pressure that influences the pattern of droplet distribution, and size of droplets (Byron, unpublished data).

Droplet size and density. Matthews (1979) pointed out four important factors for insect control: 1) the control strategy, 2) the type of pesticide, 3) the habitat of the pest, and 4) behavior of the pest. When applying insecticides in the urban environments, the minimum insecticide usage to get the maximum pest control effects must be considered (Himel 1969, Benzon 1987, Powell & Robinson 1989). Effective insecticide application depends, in part, on the droplet size and the amount of droplets that reach substrate (Bals 1969, 1982). Development of resistance to insecticides can be increased through poor application technology or inappropriate placement of insecticides on target substrates.

Consideration of the insecticide droplet size, pattern, and formulation as it relates to biological activity is an important research area (Hall 1987). The optimum spray droplet size, droplet densities, and insecticide concentration for controlling of Aedes taeniorhynchus (Wied.), Trialeurodes vaporariorum (West.), Plutella xylostella (L.) larvae, Heliothis virescens (F.) larvae, Tetranychus urticae (Koch), and Phyllocoptrura oleivora (Ashmead) has been reported by Weidhaas et al. (1970), Hall (1986), Adams et al. (1987), Omar & Matthews (1987), Wofford et al. (1987), Hall & Adams (1988), and Salyani & McCoy (1989), respectively. Yendol et al. (1990) reported that the droplet size and density deposit on a individual leaf of oak canopy is an important factor in determining microbial pesticide effectiveness. However, there is little information on the effect of spray droplet size, and droplet density for cockroach control.

Adams et al. (1987) reported the relationship between mortality of insects and distance from insecticide droplets. Droplet size was considered an influential factor in control. Density and distribution of droplets on the substrate was also important for controlling insects (Munthali 1984, Omar & Matthews 1987, Adams &

Hall 1989, Hall et al. 1990). Adams et al. (1990) used a video-tracer technique to determine the mortality of Aphis gossypii (Glover) exposed to different densities of insecticide droplets.

Equipment for applying insecticides. Various types of equipment have been used for studying the effects of droplet size, droplet distribution, and insecticide dosage in pest control (Ennis & Williamson 1963, Buehring et al. 1972, Fisher et al. 1974, McKinlay et al. 1974, Young 1986, Hall & Reichard 1978, Reichard et al. 1987). Reichard et al. (1987) reported on a droplet generator for studying spray droplets pest control. This system can produce uniform-size drops within a range of 50-500 um to determine the optimal drop sizes and minimum insecticides to control insects. Young et al. (1987) used a single droplet generator to study the impaction of droplets with a leaf surface. They recommended a two layer silicone medium for measuring droplet size.

Droplet transfer and performance. Tadros (1987) reviewed the interactions of insecticide solution in air and droplets on a surface. He described the variety of droplet performance on different surfaces, and the concept of runoff. Hart & Young (1987) showed scanning electron microscopy pictures of wet and drying droplets on a leaf surface.

The penetration and permanence of insecticides in structural wood was reported by several authors (Dodson & Robinson 1988, Powell & Robinson 1989, Powell et al. 1989, Powell & Robinson 1990). They investigated the amount of insecticide residue maintained at different depths in structural wood after different times of application. Powell & Robinson (1989) reported the impact of using runoff and less than runoff volumes to treat pine sapwood with chlorpyrifos.

Insecticide control of field population. Rust & Reierson (1978) reported repellency and resistance to several insecticide in a field population of German cockroaches. They reported different levels of effectiveness in field and laboratory strains. Sanitation, resistance, repellency, and dispersal of cockroaches (Bennett & Wright 1971, Burden 1975, Cornwell 1968, Ebeling et al. 1968, Grayson 1965, Gupta et al. 1973, Nelson & Wood 1982, Rust & Reierson 1978, Wright 1979, Robinson & Zungoli 1985, Ross & Bret 1986, Schal 1988, Zhai & Robinson 1991, Zungoli & Robinson 1982) can influence insecticide efficacy and effectiveness. If an insecticide is repellent, cockroaches can move to an untreated harborage and avoid insecticide residue on surfaces (Ebeling et al. 1968, Ebeling & Reierson 1969, Bret & Ross 1985). Barcay et al. (1990), and Owens & Bennett (1982) reported movement decreasing within apartments in a field population of German cockroaches after insecticide treatment. The cockroaches left after insecticide treatment, and most returned to original harborages within 1 to 2 weeks, indicating a thorough insecticide application to all possible harborages is necessary (Barcay et al. 1990, Owens & Bennett 1982, Ross & Bret 1986, Runstrom & Bennett 1984).

Grooming behavior. Insecticide residue may be transferred to the mouthparts during grooming (Scott 1990). Grooming behavior is an immediate response to an irritant compound (Dethier 1972, Reingold & Camhi 1977, 1978, Bret & Ross 1986). Chemoreceptors on the antennae and tarsi can detect these compounds (Brousse-Gaury 1981, Dethier 1972). Bret & Ross (1986) reported that propoxur vapors increased German cockroach movement and grooming behavior; antennal grooming increased earlier than tarsal grooming. They also reported that as soon as tarsal grooming increased the antennal grooming decreased.

OBJECTIVES

The overall objectives of this research were to study the relationship of morphology of tarsal pads and arolium, cockroach walking behavior, and effectiveness of insecticide residues. Insecticide application research is based on studies of German cockroach adults leg morphology and walking behavior, so that the optimum insecticide droplet size and density can be determined. Specific objectives included:

Morphology and behavior

1) To describe basic external morphology of the tarsal segments and pretarsus of German cockroach adults. The fine structure of the tarsal pads and arolium was examined and illustrated.
2) To study the role of individual legs of male German cockroach during normal walking. Specific attention was given to the distance each leg (tarsal segments) travels during normal walking.

Insecticide application technology

1) To determine the size (um) and density (dosage/cm^2) of cypermethrin on a nonporous substrate that gives 90% mortality of male German cockroach.
2) To determine the influence of cypermethrin droplet density on male German cockroach grooming and walking behavior.

Chapter II

LEG MORPHOLOGY AND WALKING MOVEMENT OF THE GERMAN COCKROACH, BLATTELLA GERMANICA (L.)

Cockroach walking depends on the three pairs of legs. Snodgrass (1965) described the basic structure of an American cockroach leg, and Cornwell (1968) illustrated the structure of a typical cockroach leg. Zhai & Robinson (1990) presented photomicrographs showing the basic structure and ventral view of German cockroach legs. The ventral view of the pretarsus of Periplaneta sp. was illustrated by Chapman (1969). Brousse-Gaury (1981) showed mechanoreceptors and contact chemoreceptors on the ventral tarsomeres and pretarsus of the American cockroach. Frings & Frings (1949) reported that the legs of American and German cockroaches lack contact chemoreceptors.

Chapman (1969) reported that the tarsi of the midlegs and hindlegs in Periplaneta sp. are always placed on the substrate when the insect walks. Roth & Willis (1952) reported that the parts of leg touching the substrate are claws, euplantulae, and arolia in five cockroach species. Usually the tips of the claws and euplantulae of the 3rd and 4th tarsomeres contact the surface. The functions of these structures vary on different surfaces. Research on the walking movements of the oriental cockroach, B. orientalis indicated that the normal walking rhythm is R1, L2, R3, L1, R2, L3, R1, etc (Hughes 1952). This rhythm was also reported by Full & Tu (1990) for Blaberus discoidalis (Serville). During locomotion, the foreleg and midleg resist the forward motion of the body (Graham 1983), and the hindleg must be in a supporting position before the foreleg or midleg is protracted (Hughes 1952). Delcomyn (1971a, b) reported the midlegs of P. americana remained on the substrate a little longer than any of the other legs. Full & Tu (1990) measured ground reaction forces and mechanical energy changes when B. discoidalis travelled. They reported alternating tripod locomotion speeds in this species ranging from 0.08 to 0.66 m/sec. Akers & Robinson (1983) reported that there was significant difference on the movement between field and laboratory reared German cockroaches. Field strains were more active than the VPI-Normal strain. Zhai & Robinson (1990) described the normal walking behavior of a German cockroach.

The objectives of the research reported here were to 1) examine the basic external morphology of the tarsal pads and arolium of adult German cockroaches from laboratory and field strains, and 2) determine the role individual legs of male German cockroach in each strain have during normal walking, including the distance each leg travels during walking, and walking movement rates on different doses of cypermethrin-treated glass surfaces.

MATERIALS AND METHODS

Cockroaches. Two strains of the German cockroach were used in this research: VPI, a susceptible strain; and RHA, a resistant strain. VPI-strain has been reared at VPI&SU Entomology Department for about 40 years, and has no history of insecticide exposure. The RHA-strain is from Roanoke Redevelopment and Housing Authority, Roanoke, Virginia, and was collected in July, 1990.

Chemical and equipment. A pyrethroid insecticide, cypermethrin (Demon EC, 25.3% AI, ICI Americas Inc.), (±)a-cyano-(3-phenoxyphenyl)methyl (±)-cis, trans-3-(2, 2-dichloroethenyl)-2, 2-dimethylcyclopropanecarboxylate, was used in the evaluation. Two droplet generators, 'Drop On Demand' System 100, (Aerometrics, Inc.) and Droplet Generator (Ohio State Univ.) were used to produce individual droplets. The Drop On Demand System includes a computer with the software installed on the hard disk. A 130 um diameter droplet of insecticide was produced by the droplet generators. The droplet volume was 1 nl, and there was 1 ng (0.1%) of cypermethrin in each droplet. There were 5 ng and 8 ng of cypermethrin in the 0.5% and 0.8% concentrations.

Leg morphology. Tarsi and pretarsus of male German cockroaches from VPI and RHA strains were examined with scanning electron microscopy (SEM). Five replicates of the hind-, middle-, and forelegs of males, and females of each strain were treated in alcohol concentrations (25, 50, 75, 85, 95, and 100%) to prepare them for mounting and examination. Electron microscope photographs of the tarsi and pretarsus were analyzed, and data on euplantulae and arolium size were recorded.

Areas of tarsal pads and arolium were analyzed by integral calculus. Each pad was divided into a number of areas. Each area was modified to form two rectangles which were 1 mm in width, one in and the other out of the arc lines. The length of the rectangles was measured, and then the areas of two rectangles were calculated. The mean area of two rectangles equaled the area of this portion of the pad. The means of all portions were then added to provide the surface area of the entire pad. Surface contact area of each leg was total areas of 2nd, 3rd, and 4th tarsal pads, and arolium.

Walking behavior. Walking behavior of adult male cockroaches from the VPI and RHA strains was examined using video tape images. Cockroaches were confined to and filmed on a 3 x 3 mm^2 square grid to record normal walking behavior. The film was slowed to 4 sec/frame, and the movement of individual legs were analyzed from a television monitor. Data were obtained on distance between steps which each individual leg travels during normal walking. Differences between two strains were analyzed.

Walking movement rate on untreated glass surface. Walking movement units of male cockroaches from a field and laboratory strain were observed on 9 x 9 cm^2 of clean glass in day-light condition. A square plastic cover (9 x 9 cm^2), with a thin film of petroleum jelly on the inside, was used to confine the cockroaches to the glass plate. The plastic cover was marked into squares, each square was 2.8 x 2.8 cm^2, for a total 9 squares. Each square was assigned a value of 1 unit. Each time a cockroach entered a different square it earned one movement unit. Individual cockroach was confined on clean glass plate at least 20 min before it was moved into test arena. Cockroach movement units were accumulated at each minute in 20 minutes. Movement units on RHA- and VPI-strain cockroaches were analyzed.

Walking movement rate on cypermethrin treated glass surface. Thirty males from the VPI-strain were placed individually on less than runoff (0.1% cypermethrin) treated glass surface (9 x 9 cm^2) in the day-light condition. There were 2,025, 2,916 and 3,969 droplets (130 um diameter) on each glass. The distances between two droplets were measured with a microscope ocular micrometer. The amount of cypermethrin (AI) on each plate was 0.025, 0.036, and 0.049 ug/cm^2. There were four clean glass plates adjacent to the treated glass plate. Each cockroach was confined on clean glass plate at least 20 min before it was moved into treated surface. The movement units were counted at each minute until knockdown observed. Movement rate was calculated by the formula: movement units/time of knockdown. The movement units were divided by 20 minutes, if knockdown time was not observed in 20 minutes. Thirty males from the RHA-strain were placed on 0.1%, 0.5%, and 0.8% cypermethrin with 3,969 droplets on each glass plate. Dosage density on these treatments were 0.049, 0.245, and 0.392 ug/cm^2, respectively. The observation procedure was the same as above.

Thirty of each RHA- and VPI-strain cockroaches were placed individually on runoff (10 ug/cm^2 of cypermethrin) treated 9 x 9 cm^2 glass plates. Movement units were observed in 5 min, and knockdown time was recorded. Movement units data were obtained from cockroaches that were not knockdown down at 5 min.

Data analysis. Data were analyzed using a computer-based statistics program (SAS). Analysis of variance (ANOVA) and Tukey multiple comparison (SAS 1985) were performed to quantify various aspects of cockroach tarsal pads and arolium and walking behavior. Differences between movement units by VPI-strain and RHA-strain male German cockroaches were subjected to log transformations and determined with a general linear model (GLM). All tests were considered significant at the critical level of 5% (SAS 1985).

RESULTS

Leg morphology. There are six distinct segments to each German cockroachleg: coxa, trochanter, femur, tibia, tarsus, and pretarsus (Fig. 1). The tarsus is subdivided into five tarsomeres (Fig. 2, 3, 4). On the underside of the first four tarsomers are pad-like euplantula. At the end of tarsus is a pretarsus which is composed of a pair of claws, and a pad-like arolium between the claws. These small pads and claws contact the surface a cockroach walks upon, and they probably provide the traction necessary to walk and climb on a variety of surfaces.

The surface area of each pad on the fore-, mid-, and hindleg of the VPI-strain cockroach ranged from a mean of 0.0092 mm^2 to 0.0208 mm^2 (Table 1). The 4th tarsal pad and arolium are significantly (Tukey, $P < 0.05$) larger than those on other segments. The total area of the tarsal pads of segments T2, T3, T4, and arolium which contact the surface is presented in Table 2. There is a significant difference (Tukey, $P < 0.05$) in the surface areas of three legs in males; surface contact area of foreleg is smaller than contact area of hindleg, and contact area of midleg is intermediate.

Male RHA-strain cockroaches have significant differences (Tukey, $P < 0.05$) in the surface areas of tarsal pads and arolium in each leg. The range of these pad areas is 0.0094 mm^2 to 0.0219 mm^2. The 4th tarsal pad and arolium have the largest areas (Table 3). There are no differences in the areas of 1st, 2nd, and 3rd tarsal pads, and no differences on the total surface contact areas of the three pairs of legs in this strain.

In both strains the pads of female German cockroaches are larger than those of males (Table 4). The mean weight of females without an eggcase was 0.071 ±

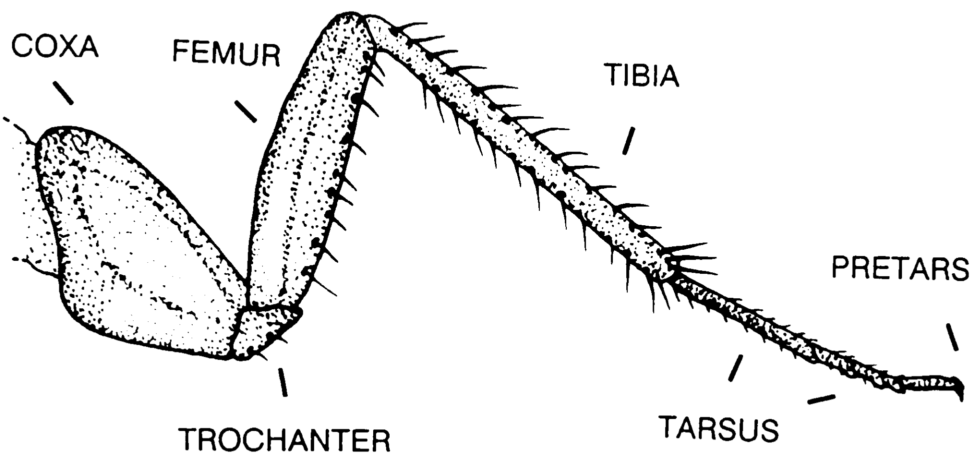

Figure 1. External leg structure of the German cockroach, Blattella germanica (L.).

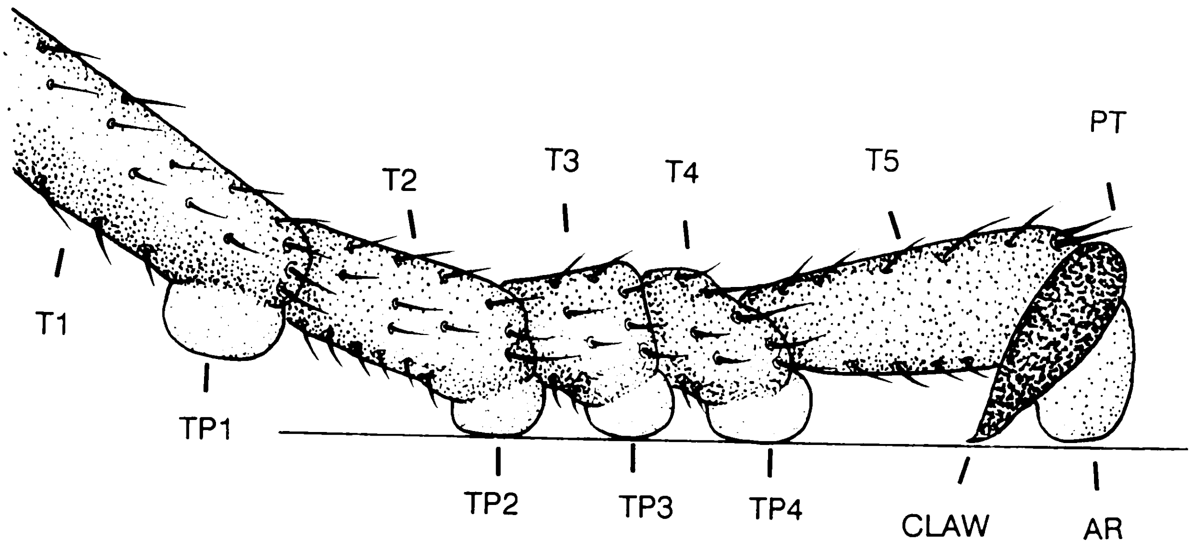

Figure 2. External structure of tarsus and pretarsus of a German cockroach, Blattella germanica (L.) leg. T1, 1st tarsomere; TP1, first tarsal pad; T2, 2nd tarsomere; TP2, 2nd tarsal pad; T3, 3rd tarsomere; TP3, 3rd tarsal pad; T4, 4th tarsomere; TP4, 4th tarsal pad; T5, 5th tarsomere; PT, pretarsus; AR, arolium.

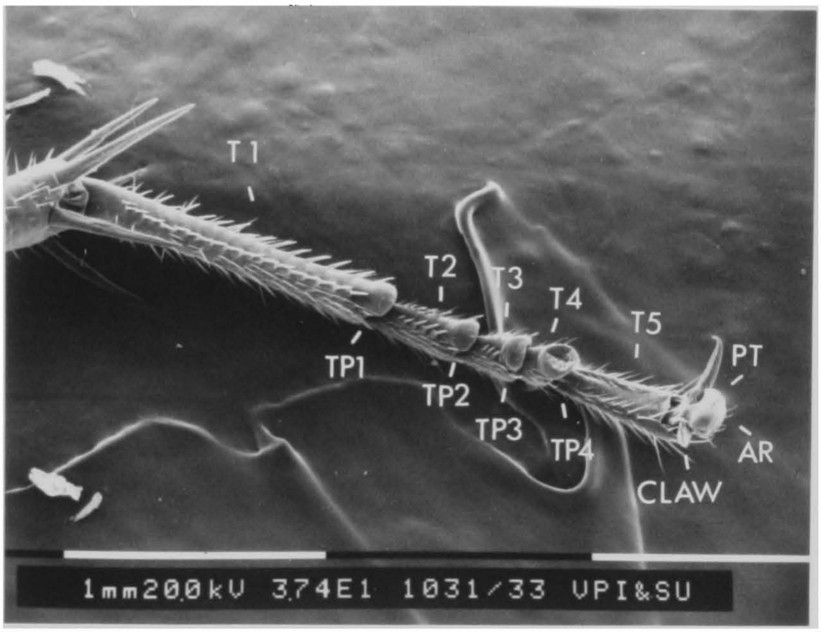

Figure 3. Ventral view of midleg tarsus and pretarsus of male German cockroach, Blattella germanica (L.). T1, 1st tarsomere; TP1, first tarsal pad; T2, 2nd tarsomere; TP2, 2nd tarsal pad; T3, 3rd tarsomere; TP3, 3rd tarsal pad; T4, 4th tarsomere; TP4, 4th tarsal pad; T5, 5th tarsomere; PT, pretarsus; AR, arolium.

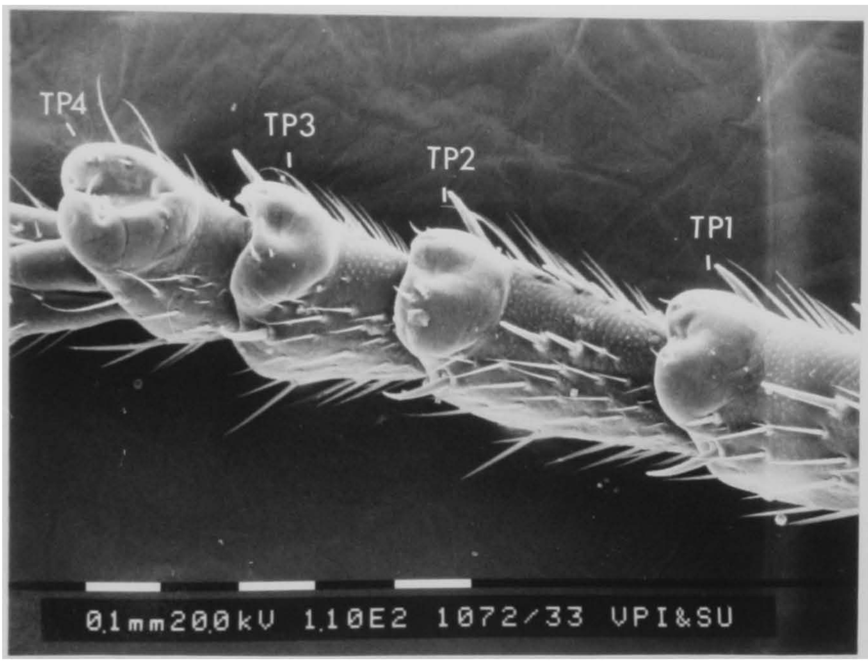

Figure 4. Tarsal pads on foreleg of female German cockroach, Blattella germanica (L.). TP1, first tarsal pad; TP2, 2nd tarsal pad; TP3, 3rd tarsal pad; TP4, 4th tarsal pad.

Table 1. Surface areas of tarsal pads and arolium in VPI-strain male German cockroach.

Segment	$\bar{x}$ area of tarsal pad in mm^2 [a] (± SD)		
	Foreleg	Midleg	Hindleg
TP1	0.0130b (0.0009)	0.0141b (0.0010)	0.0135b (0.0021)
TP2	0.0130b (0.0005)	0.0118bc (0.0012)	0.0137b (0.0027)
TP3	0.0095c (0.0011)	0.0092c (0.0011)	0.0115b (0.0026)
TP4	0.0170a (0.0009)	0.0184a (0.0041)	0.0208a (0.0011)
AR	0.0190a (0.0032)	0.0206a (0.0018)	0.0206a (0.0017)

[a] means followed by same letter in column are not significantly different (Tukey, $P < 0.05$)

Table 2. Surface contact areas of each leg in VPI-strain and RHA-strain male German cockroach.

Leg	$\bar{x}$ contact area in mm^2 [a] (± SD)	
	VPI	RHA
Foreleg	0.0584b (0.0029)	0.0597 (0.0052)
Midleg	0.0629ab (0.0051)	0.0568 (0.0058)
Hindleg	0.0666a (0.0055)	0.0606 (0.0050)
Total [b]	0.1879c (0.0071)	0.1771c (0.0146)

[a] means followed by same letter in column are not significantly different (Tukey, $P < 0.05$)

[b] total contact area of one step; means followed in this row are not significantly different (Tukey, $P < 0.05$)

Table 3. Surface areas of tarsal pads and arolium in RHA-strain male German cockroach.

Segment	$\bar{x}$ area of tarsal pad in mm^2 [a] ($\pm$ SD)		
	Foreleg	Midleg	Hindleg
TP1	0.0123b (0.0016)	0.0104b (0.0009)	0.0121b (0.0006)
TP2	0.0112b (0.0014)	0.0110b (0.0016)	0.0115b (0.0010)
TP3	0.0102b (0.0025)	0.0099b (0.0009)	0.0094b (0.0021)
TP4	0.0169a (0.0028)	0.0167a (0.0019)	0.0178a (0.0039)
AR	0.0189a (0.0025)	0.0206a (0.0049)	0.0219a (0.0030)

[a] means followed by same letter in column are not significantly different (Tukey, $P < 0.05$)

Table 4. Total surface contact area and body weight of adult German cockroaches in VPI-strain and RHA-strain.

Strain	Contact area in mm^2 ($\pm$ SD) [a]		Body weight in g ($\pm$ SD) [b]		
	M	F	M	F1	F2
VPI	0.188 (0.007)	0.202 (0.018)	0.052 (0.001)	0.071 (0.007)	0.108 (0.008)
RHA	0.177 (0.015)	0.264 (0.019)	0.054 (0.003)	0.092 (0.013)	0.111 (0.009)

[a] total contact area of three legs

[b] F1: female without eggcase; F2: female with eggcase

0.007 g in VPI-strain and 0.092 $\pm$ 0.013 g in RHA-strain cockroaches. The mean weight of females with an eggcase was 0.108 $\pm$ 0.008 g and 0.111 $\pm$ 0.009 g in the VPI and RHA strains, respectively. The mean male weight was 0.052 $\pm$ 0.001 g in VPI, and 0.054 $\pm$ 0.003 g in RHA, almost half the mean weight of females with an eggcase. The surface contact area of the tarsal pads of segments T2, T3, T4, and arolium on fore-, mid-, and hindlegs was 0.202 mm^2 (VPI) and 0.264 mm^2 (RHA) in the female, and 0.188 mm^2 (VPI) and 0.177 mm^2 (RHA) in the male. There is no significant difference in the total contact areas of the three pairs of legs between VPI- and RHA-strain male German cockroaches (Table 4).

Walking. During walking, the body of a German cockroach is supported by a foreleg and hindleg on one side, and midleg on the other side of body (Fig. 5). While these legs are on the substrate, the other three of legs are moving forward to a new position.

Distances between each walking step in male German cockroach were variable, and depended on the walking position and direction. In both strains the forelegs usually stepped the longest distance (Table 5). There are no significant differences (ANOVA, $P > 0.05$) in the walking distances in two steps between right and left legs, and fore-, mid-, and hindlegs during normal walking. There is no significant difference (ANOVA, $P > 0.05$) in the walking distance between each step of right and left legs in RHA-strain male (Table 5). The walking distance (each step) ranged from 3 to 28 mm (VPI) to 4 to 22 mm (RHA), and there are no significant differences in these distances (ANOVA, $P > 0.05$).

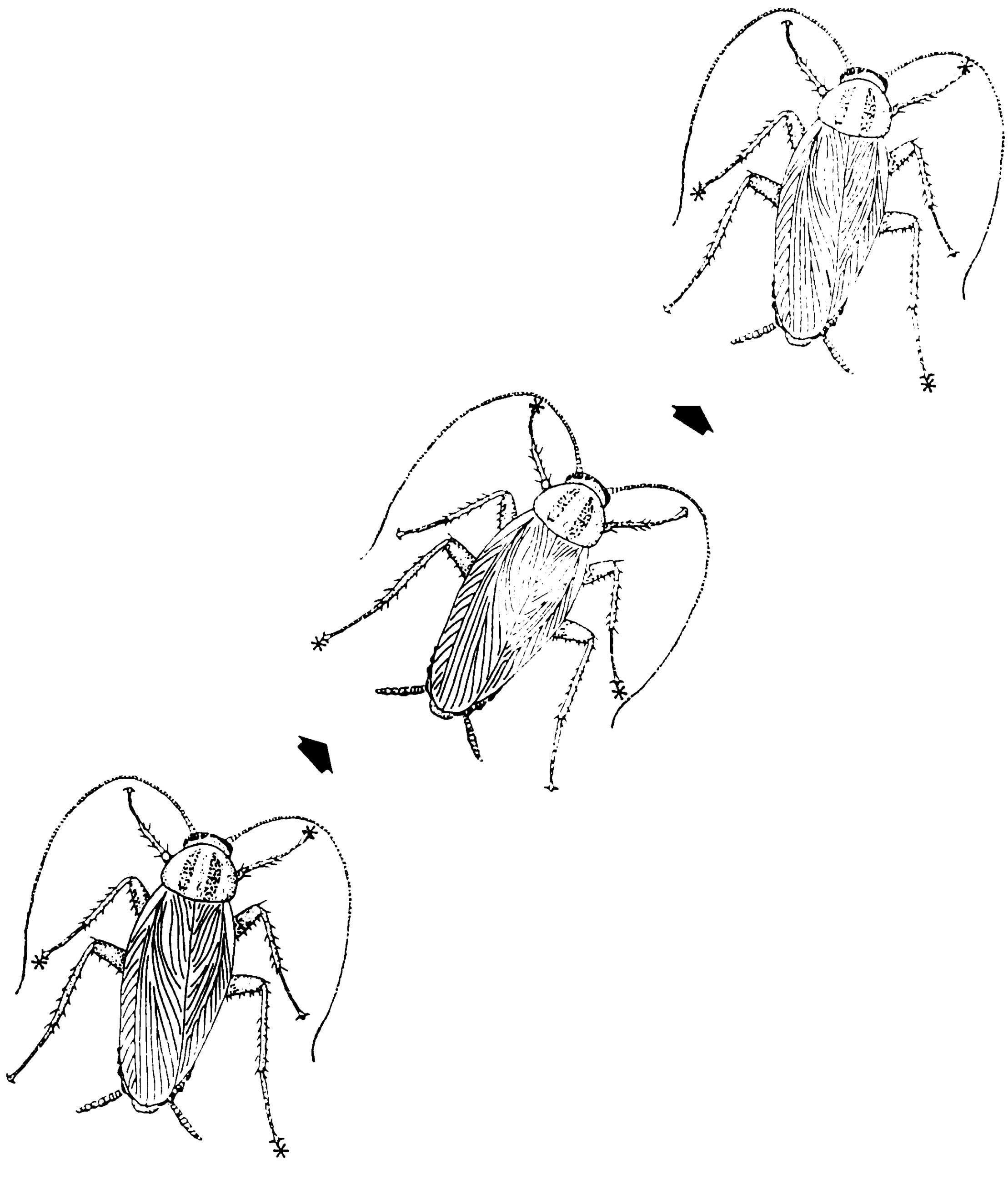

Figure 5. Walking movement of a German cockroach: three legs (shown in *) form a tripod that support the body, the other legs are in motion.

Table 5. Distance between two steps in VPI-strain and RHA-strain German cockroaches during normal walking.

Strain	$\bar{x}$ distance in mmd (± SD)					
	Foreleg		Midleg		Hindleg	
	Right	Left	Right	Left	Right	Left
VPI	11.35 (5.05)	11.88 (3.78)	9.84 (3.93)	10.00 (2.23)	10.64 (3.04)	9.81 (2.48)
RHA	10.65 (3.19)	11.25 (4.87)	9.83 (3.31)	9.42 (3.64)	9.30 (2.16)	8.95 (3.54)

Walking movement rate on untreated and treated glass surfaces. On the untreated glass surface there was no significant difference in the walking distance (bewteen two steps) between VPI-strain and RHA-strain male German cockroaches. The accumulated walking movement units on RHA-strain cockroaches were significantly (GLM, $P < 0.05$) 1.4 times greater than the VPI-strain cockroaches in the first 3 min (Fig. 6), and significantly (GLM, $P < 0.05$) 3.9 times that of the VPI-strain in 20 min (Fig. 7) on the untreated glass surface.

The RHA-strain cockroaches walked 1.1-fold in 2 min, and 1.4-fold in 3 min more than VPI-strain cockroaches on 10 ug/cm^2 cypermethrin treated glass surface, and significantly (GLM, $P < 0.05$) more than VPI-strain cockroaches at 5 minutes. However, these two strains showed decreasing activity on 10 ug/cm^2 cypermethrin treated glass plate compared with movement behavior on an untreated surface (Fig. 6).

On low dosage treatments, the mean distances between two droplets were 1.7 $\pm$ 0.5 mm, 1.3 $\pm$ 0.5 mm, and 1.1 $\pm$ 0.2 mm on 2,025, 2,916, and 3,969 droplets treated glass surface, respectively. VPI-strain cockroaches showed significantly (Tukey, $P < 0.05$) more movement response to the 0.049 ug/cm^2 treatment than to 0.036 ug/cm^2 and 0.025 ug/cm^2 treatments before knockdown (Table 6), but no significant differences in these three treatments, and between treated and untreated glass plates in 5 min (Fig. 8).

RHA-strain cockroaches showed increasing movement in response to a lower dose (0.049 ug/cm^2) of cypermethrin treated glass plate before knockdown (Table 6). This strain had 1.6-fold and 1.3-fold movement rate increase between 0.049 ug/cm^2 and 0.392 ug/cm^2, and between 0.049 ug/cm^2 and 0.245 ug/cm^2, respectively. However, RHA-strain cockroaches showed significantly (Tukey, P <

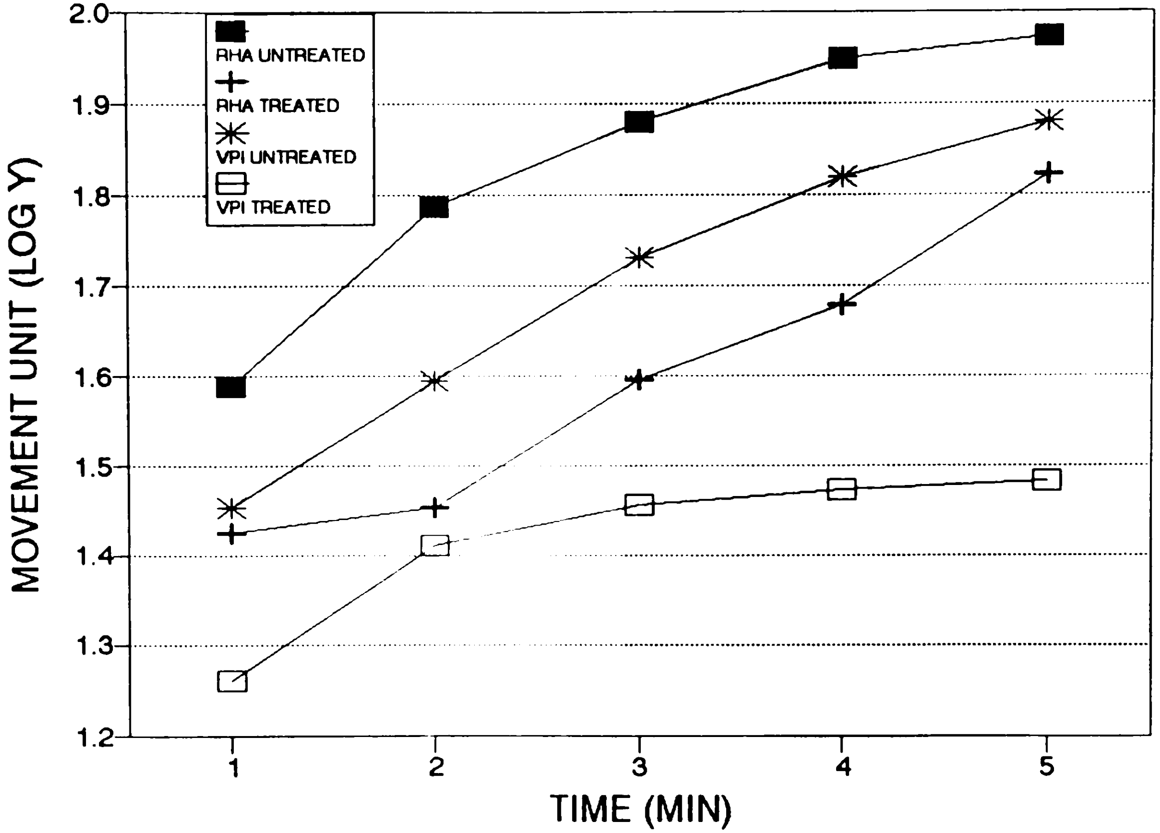

Figure 6. Accumulated walking movement units of VPI- and RHA-strain male German cockroaches on 10 ug/cm^2 cypermethrin treated and untreated glass plates in 5 minutes (log transformated means of movement units).

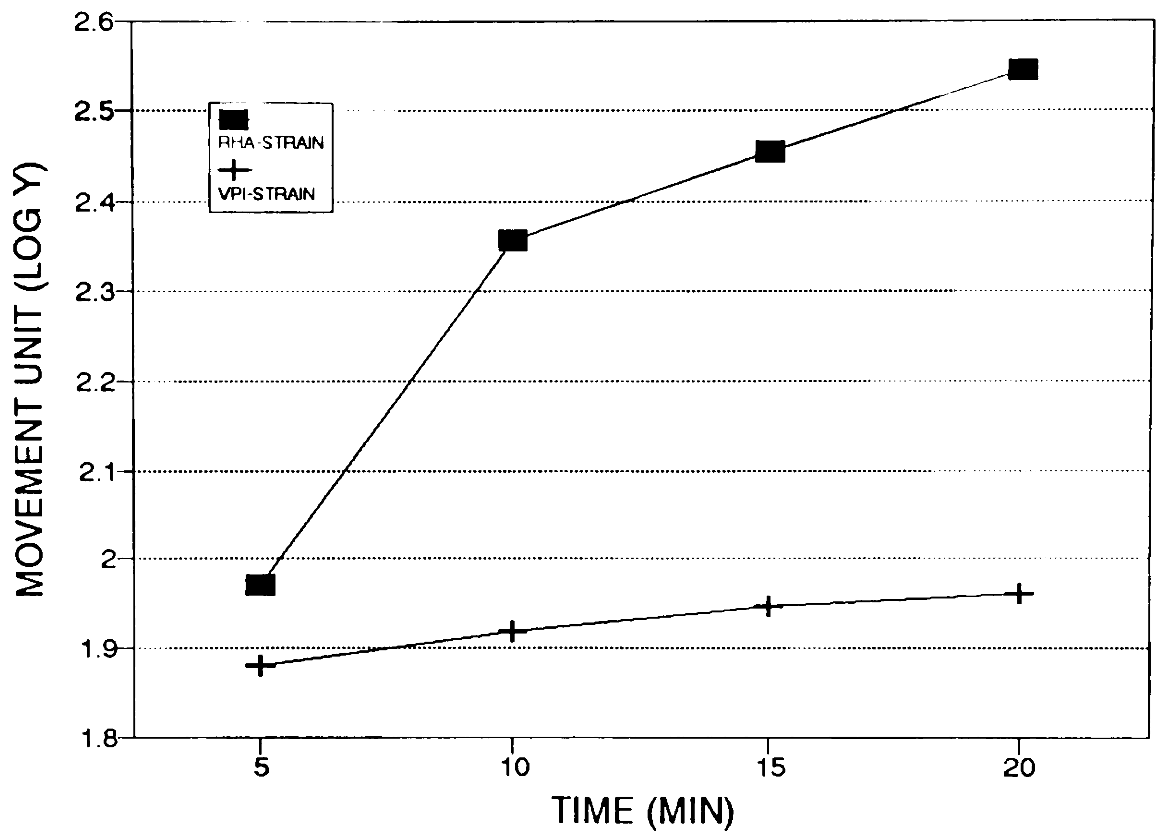

Figure 7. Accumulated walking movement units of VPI- and RHA-strain male German cockroaches on untreated glass plates in 20 minutes (log transformated means of movement units).

Table 6 Walking movement rate before knockdown time in VPI- and RHA-strain male German cockroaches.

Treatment (ug/cm^2)	Movement rate in units/min[a] (± SD)	
	VPI	RHA
0.025	8.1 (0.5)	--
0.036	8.0 (3.0)	--
0.049	12.7[b] (5.6)	4.7 (2.1)
0.245	--	3.8 (3.3)
0.392	--	3.0 (1.4)

[a] movement units/knockdown time, or movement units/20 if knockdown time was not observed in 20 min

[b] it is significantly different from 0.025 ug/cm^2, 0.036 ug/cm^2 treatments in VPI, and 0.049 ug/cm^2 in RHA (Tukey, $P < 0.05$)

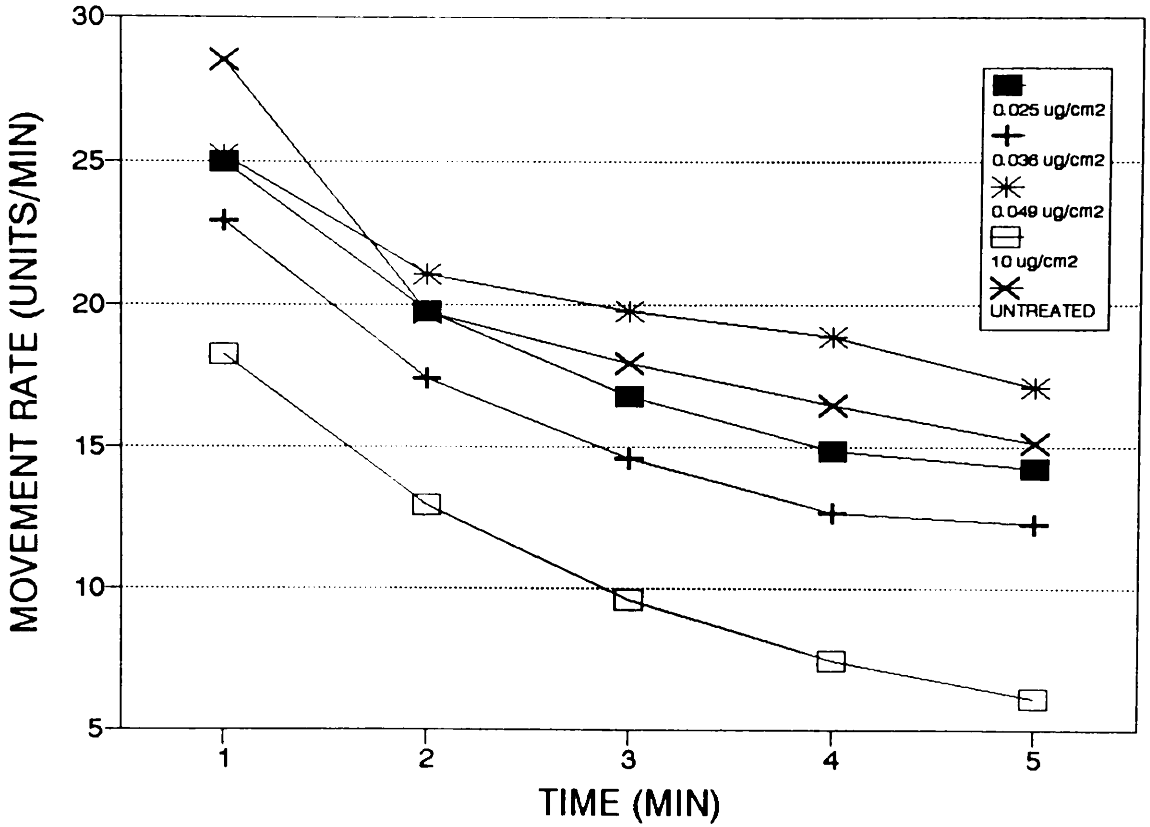

Figure 8. Walking movement rate of VPI-strain male German cockroaches on various cypermethrin treated glass plates in 5 minutes.

0.05) slow activity on these treatments compared to that on untreated surface in 5 min (Fig. 9). In addition, VPI-strain cockroaches walked significantly (Tukey, $P < 0.05$) more than RHA cockroaches on 0.049 ug/cm^2 treated glass plates in 5 min.

DISCUSSION

Modern liquid insecticides are usually applied as a water-emulsion spray to a variety of surfaces in the household and urban environment for cockroach control. Soon after the liquid is applied, the water in the dilution evaporates, to leave a thin residue of insecticide on the surface. When a cockroach walks across this treated surface, insecticide residue will accumulate on the tarsal pads of the six legs, penetrate the cuticle, and produce mortality. Aspects of leg morphology (sizes of tarsal pads and arolium), walking behavior, and resistance influence the impact of insecticides on the cockroach (Bret & Ross 1985, Ross 1991, Roth & Willis 1952, Sarkaria & Patton 1949).

The tarsal pads and arolium are the primary route of exposure for contact insecticides in cockroaches (Roth & Willis 1952). Although grooming and feeding may introduce toxic material into the body (Scott 1990), the tarsal pads of at least three legs are always on the substrate and exposed to insecticide residues (Delcomyn 1971a, b, Hughes 1952). The insecticide exposure in males may be less than females if there are not any other differences in the aspects of morphology and physiology, since their tarsal pads are smaller than those of the female. The ratios of total surface contact area on six legs to body weight were 7.2 (VPI) and 6.6 (RHA) in males; and 5.7 without an eggcase, 3.7 with an eggcase in VPI

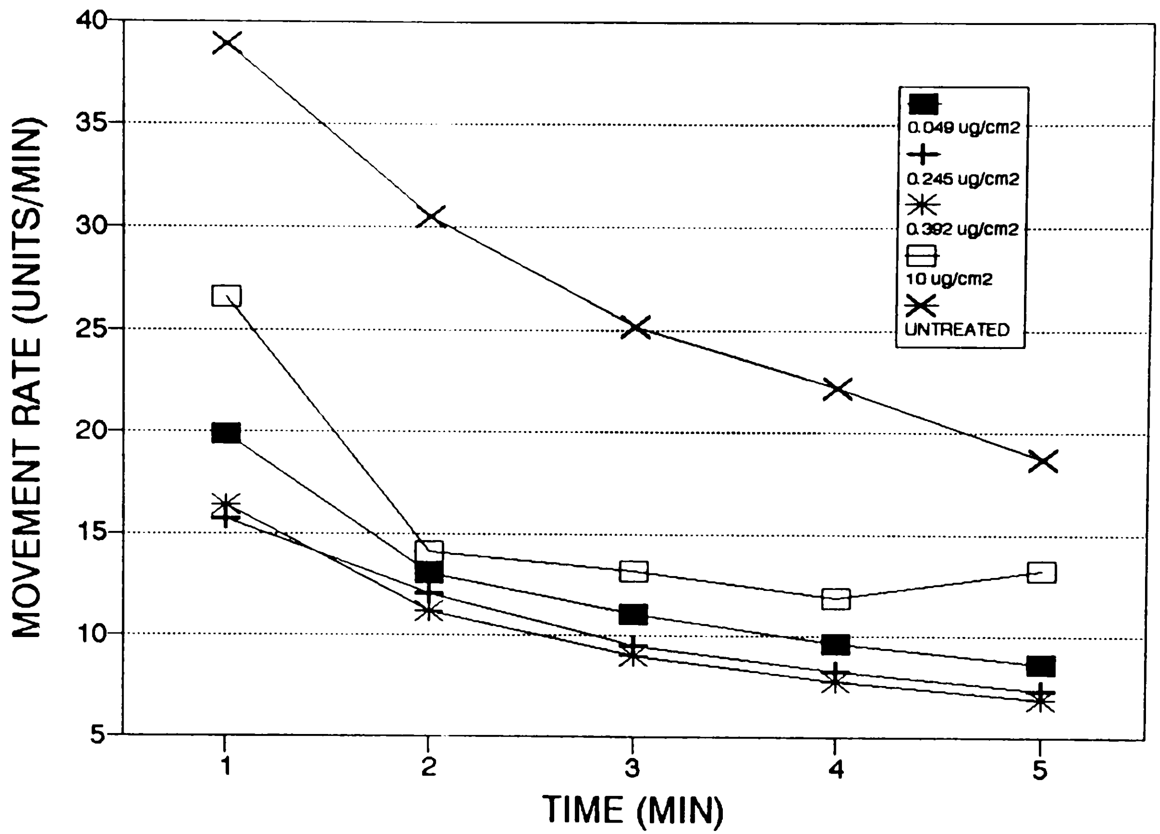

Figure 9. Walking movement rate of RHA-strain male German cockroaches on various cypermethrin treated glass plates in 5 minutes.

cockroaches; and 5.7 without an eggcase, 4.8 with an eggcase in RHA cockroaches. The female German cockroach may be exposed to more insecticide because of the size of her tarsal pads, and the increased weight when carrying an eggcase (Table 4). Although female German cockroaches may have greater exposure to insecticides from tarsal pads and arolium than males, they are generally more resistant than males. The insecticide susceptibility of female German cockroach is often decreased by body weight and certain physiological factors (such as lipid content) (Munson & Gottlieb 1953, Nagasawa 1957).

Considering there is no significant difference in the contact areas of VPI- and RHA-strain cockroaches, the potential insecticide residue exposure would be the same for both strains. However, walking behavior of cockroaches may also influence their exposure to insecticide residues (Bret & Ross 1985, Wooster & Ross 1989). The significant difference in the movement rate between the two strains may influence the amount of insecticide accumulated on tarsal pads and arolium in a distinct time period. On an untreated surface after 20 min exposure, the RHA-strain male walked nearly 4 times more than VPI-strain male. On a surface treated to 10 ug/cm^2 the RHA-strain males walked two times more than VPI-strain male in 5 min. The distinct difference in normal walking movement between RHA- and VPI-strain cockroaches may be the result of selection pressure on the laboratory strain. The VPI-strain has been in colony for about 40 years. There is abundant water and food in the small (about 1 gal.) rearing containers. The cockroaches do not have to move far to find food and a mate. The limited space in the rearing containers may have selected for those individuals with less movement activity, especially if less movement was advantageous to survival/reproduction. In the field, cockroaches are not limited in the movement needed to obtain water and food.

Male cockroaches are active in searching for females for mating (Owens 1980) and harborage sites (Owens & Bennett 1982, Zungoli 1982).

A resistant strain of German cockroach could have different insecticide-induced behavior (Wooster & Ross 1989, Ross 1991), or physiological adaptations that enhances resistance to the insecticide. This was evident in the RHA-strain cockroach response to cypermethrin-treated glass plates. When RHA-strain cockroaches were confined on low dosage treated glass plates, the decreased movement activities in response to the 0.049 ug/cm^2, 0.245 ug/cm^2, and 0.392 ug/cm^2 treatments indicates that this strain may increase avoidance toward cypermethrin. There was a mean of 1.1 $\pm$ 0.2 mm between droplets on the 3,969 droplet-treated surface. This is sufficient space for a cockroach to stand and to avoid cypermethrin residue on the tarsal pads. The RHA-strain may have developed cypermethirn-induced walking behavior changes to deal with lower dosage treatments. In contrast, VPI-strain cockroaches showed increased movement activities in response to 0.025 ug/cm^2, 0.036 ug/cm^2, and 0.049/cm^2 cypermethrin treatments. This susceptible strain has never been exposed cypermethrin, and these cockroaches may not have developed cypermethrin-induced behavior changes to avoid insecticide exposure. Insreased movement may be due to increased insecticide avoidance in this susceptible strain. RHA-strain cockroaches showed decreased movement compared to VPI-strain cockroaches when they walked on 0.049 ug/cm^2 treated glass plates. This walking behavior difference may also indicate cypermehtirn-induced behavior can be different between susceptible and resistant strain. Decreased walking movement was also displayed in VPI-strain cockroaches on 10 ug/cm^2 treatment, it might be due to exposure to the insecticide. Bret & Ross (1985) reported that VPI-strain German cockroaches increased movement in response to propoxur vapors, but a resistant strain showed lower movement activity. Barcay et al. (1990), and Owens & Bennett (1982) reported that pyrethroids decreased movement of a field strain within apartments because of increased mortality. In this study there was decreased movement recorded on treated surfaces for both VPI- and RHA-strain German cockroaches.

Less than runoff insecticide application may be not effective in controlling a highly resistant field population. Complete insecticide coverage may be necessary to control such a population (Barcay et al. 1990, Owens & Bennett 1982, Ross & Bret 1986, Runstrom & Bennett 1984). Knowing the insecticide resistance profile of a field population can be important when designing an integrated control program (Robinson & Zungoli 1985). It will help in selecting the chemical control strategy for the population and provide for using the minimum amount of insecticide to achieve maximum control.

Chapter III

RELATIONSHIP OF LEG MORPHOLOGY, WALKING BEHAVIOR, AND CYPERMETHRIN DOSE TRANSFER IN THE GERMAN COCKROACH, BLATTELLA GERMANICA (L.)

Control strategies for the German cockroach are based on the application of liquid insecticide to infested harborages, or placing toxic bait stations close to harborages (Tsuji & Ono 1969, Robinson & Zungoli 1985, Robinson 1988). The effectiveness of these strategies can be influenced by the level of insecticide resistance in the population. Rust & Reierson (1978) reported repellency and resistance to several insecticides in field populations of German cockroaches. Sanitation, resistance, repellency, and dispersal of cockroaches can influence insecticide efficacy (Bennett & Wright 1971, Burden 1975, Cornwell 1968, Ebeling et al. 1968, Grayson 1965, Gupta et al. 1973, Nelson & Wood 1982, Rust & Reierson 1978, Wright 1979, Robinson & Zungoli 1985, Ross & Bret 1986, Schal 1988, Zhai & Robinson 1991, Zungoli & Robinson 1982). If an insecticide is repellent, cockroaches may move to an untreated harborage and avoid toxic residues on surfaces (Ebeling et al. 1968, Ebeling & Reierson 1969, Bret & Ross 1985). Barcay et al. (1990), and Owens & Bennett (1982) reported the movement of German cockroach decreasing within apartments after insecticide treatment. The dispersal of cockroaches after insecticide treatment and a return to original harborage within 1 to 2 weeks (Ross & Bret 1986) indicates that a thorough insecticide application to all the harborages is necessary for control (Barcay et al. 1990, Owens & Bennett 1982, Ross & Bret 1986, Runstrom & Bennett 1984).

When applying insecticides in urban environments, the minimum amount of insecticide to get the maximum control effects must be considered (Himel 1969, Benzon 1987, Powell & Robinson 1989). Effective insecticide application depends, in part, on the droplet size and the amount of droplets that reach the target substrate (Bals 1969, 1982). Resistance to insecticides and treatment efficacy may be influenced by poor application, or inappropriate placement of insecticides on target substrates.

Consideration of the insecticide droplet size, spray pattern, and formulation, as it relates to biological activity, is an important research area (Hall 1987). Adams et al. (1987) reported the relationship between insect mortality and distance from insecticide droplets. Droplet size was considered an influential factor in control. Density and distribution of droplets on the substrate is also important for controlling insects (Munthali 1984, Omar & Matthews 1987, Adams & Hall 1989, Hall et al. 1990).

The objective of the research reported here was to determine the number of droplets and density of cypermethrin on a nonporous substrate that give 90% cockroach mortality. A direct application and indirect application method were used to treat two strains of German cockroach. The dose transferred to the tarsal pads from different application methods were compared, and the relationship between knockdown time and dose transfer were analyzed.

MATERIALS AND METHODS

Cockroaches. Two strains of German cockroach were used in this research: VPI, a susceptible strain, and RHA, a resistant strain. VPI-strain has been reared at VPI&SU for about 40 years, and has no history of insecticides exposure. The RHA-strain is from Roanoke Redevelopment and Housing Authority (RRHA), Roanoke, Virginia, and was collected in July, 1990. Zhai & Robinson (1991) reported a high level of cypermethrin resistance in the German cockroach population in RRHA apartments.

Chemical and equipment. A pyrethroid insecticide, cypermethrin (Demon EC, 25.3% AI, ICI Americas Inc.), ($\pm$)a-cyano-(3-phenoxyphenyl)methyl ($\pm$)-cis,trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate, was used in application on glass plates. Technical cypermethrin (99.3% AI, FMC Corporation) was used in topical applications.

Two droplet generators, 'Drop On Demand' System 100, (Aerometrics, Inc.) and Droplet Generator (Ohio State Univ.) were used to produce individual insecticide droplets. The Drop On Demand System includes a computer with the software installed on the hard disk. An automatic microapplicator (Burkard Mfg. Co. Ltd., England) was used to produce 1 ul of different concentrations of cypermethrin.

An insecticide droplet of 130 um diameter was produced by the droplet generators. This size of droplet contained 1 nl of insecticide. Three insecticide concentrations were used: 0.1% (1 ng cypermethrin), 0.5% (5 ng cypermethrin), and 0.8% (8 ng cypermethrin).

Direct and indirect application. Direct application was performed by topical application of a known-dose cypermethrin on the cockroach tarsal pads (LDT). A 1 ul cypermethrin-acetone solution was applied on the tarsal pads of anesthetized male cockroaches. There were five concentrations used for each strain. Acetone was applied on the tarsal pads as control. Ten cockroaches per strain were used for each concentration, with three replicates. Mortality was recorded at 24 h.

For the indirect application, individual cockroaches contacted a known-dose of cypermethrin droplets on a glass surface, and knockdown time (KT) was recorded. Thirty male cockroaches per strain were placed individually on cypermethrin treated glass plates (9 x 9 cm^2). The treatment and evaluation methods on the glass surface was the same as the movement evaluation (Chapter II). Knockdown time and 24 h mortality were recorded.

Correlation of dose transfer and knockdown time. The insecticide dose transferred to the cockroach tarsal pads was calculated by the formula:

$$\text{Dose transferred} = \frac{R \times T \times A \times D}{S} \times 2.8$$

Where, R = movement rate (units/min); T = knockdown time (min); A = total surface contact area of six legs (cm^2); D = density of insecticide dose (ug/cm^2); S = distance in two steps (cm); and 2.8 (cm) is the size of 1 unit. This formula is based on the assumption that the 2nd, 3rd, and 4th tarsal pads, and arolium of a cockroach leg pick up all the insecticide in one droplet when stepped upon.

Data analysis. Data were analyzed using a computer-based statistics program (SAS). Insecticide residue data were analyzed with analysis of variance (ANOVA). The relationship between dose transfer and knockdown time were analyzed by using correlation test. All test were considered significant at the critical level of 5% (SAS 1985). Data obtained from direct and indirect application were subjected to probit analysis (SAS Institute 1985) for the lethal dose on tarsal pads (LDT_{50} and LDT_{90}), and the knockdown time (KT_{50} and KT_{90}).

RESULTS

Dose transfer and mortality: direct application. Direct application of cypermethrin to the tarsal pads of VPI-strain cockroaches resulted in a LDT_{50} of 0.03 ug/cockroach (95% FL = ± 0.02 ug), and LDT_{90} of 0.33 ug/cockroach (95% FL = ± 0.84 ug) (Table 7). To achieve 50% mortality, a VPI-strain cockroach must be exposed to at least 33 droplets of 130 um in diameter of 0.1% cypermethrin; and must walk on 330 droplets to achieve 90% mortality.

There was a high cypermethrin LDT_{50} and LDT_{90} recorded in RHA-strain German cockroach, probably because of the high level of resistance to cypermethrin in this population (Zhai & Robinson 1991). The LDT_{50} was 3.17 (± 2.09 of 95% FL) ug/cockroach (Table 7). These data indicated that RHA-strain cockroaches must walk on at least 3,174 droplets (130 um diameter) of 0.1% cypermethrin to achieve 50% mortality. The LDT_{90} of 29.88 ug/cockroach with ± 208.51 ug/cockroach (95% FL) indicated that there was a high level of cypermethrin resistance in this population.

Table 7. Relationship between lethal dose and number of droplets of cypermethrin applied on the tarsal pads of VPI- and RHA-strain male German cockroaches.

Strain	LDT_{50} (ug) ($\pm$ 95% FL)	No. droplets [a] ($\pm$ 95% FL)	LDT_{90} (ug) ($\pm$ 95% FL)
VPI	0.03 (0.02)	33 (15)	0.33 (0.84)
RHA	3.17 (2.09)	3174 (2086)	29.88 (208.51)

[a] number of 0.1% cypermethrin droplets with 130 um in diameter related to LDT_{50}

Relationship between number of droplets and mortality: indirect application. Cockroach knockdown time decreased as the insecticide droplet density on the glass plates increased. When VPI-strain males walked on 10 ug/cm^2 of cypermethrin on the glass surface, a KT_{50} of 5.4 min and KT_{90} of 7.2 min were recorded (Table 8). However, KT_{50} increased to only 8.4 min and a KT_{90} to 14.1 min when they walked on 0.049 ug/cm^2 cypermethrin-treated glass surface. This treatment had 204 times less cypermethrin compared to the 10 ug/cm^2 treatment. A KT_{50} of 12.9 min, 15.8 min, and KT_{90} of 22.9 min, and 23.5 min were recorded when they walked on 0.036 ug/cm^2 and 0.025 ug/cm^2 treatment, respectively. All tests achieved 100% mortality after 24 h. When the amount of insecticide was reduced 400 times (0.025 ug/cm^2), the time to achieve 90% knockdown increased from 6.9 min to 16.3 min.

The RHA-strain has high level resistance to cypermethrin, an LD_{50} resistance ratio of 180 was recorded in 1990 (Zhai & Robinson 1991). The KT_{50} of 15.6 min and KT_{90} of 27.5 min (93% mortality after 24 h) recorded in this study indicate a level of cypermethrin resistance in this strain, compared to the susceptible strain when they walked on a 10 ug/cm^2 cypermethrin-treated glass surface (Table 8, 9). A KT_{50} greater than 24 h (27% mortality in 24 h) was recorded when they walked on 0.049 ug/cm^2 of cypermethrin treatment, on which there were 3,969 droplets on the glass surface. These data are consistent with the data from direct application, which indicated that to achieve mortality in the RHA-strain a cockroach must walk on at least 3,174 $\pm$ 2,086 droplets of cypermethrin. Unless they walked on the entire surface of the glass plate with the 0.049 ug/cm^2 treatment, individual cockroaches could not walk on enough droplets to achieve knockdown. When the amount of insecticide on the plates was reduced from 0.392 ug/cm^2 to 0.245 ug/cm^2, the KT_{90}

Table 8. Knockdown time of VPI-strain male German cockroaches to various cypermethrin dose treatments.

Treatment (ug/cm^2)	KT_{50} (min) ($\pm$ 95% FL)	KT_{90} (min) [a] ($\pm$ 95% FL)
10	5.4 (0.5)	7.2 (1.3)
0.049	8.4 (0.5)	14.1 (1.7)
0.036	12.9 (0.6)	22.9 (3.2)
0.025	15.9 (0.6)	23.5 (2.7)

[a] all treatments achieved 100% mortality in 24 h

Table 9. Knockdown time of RHA-strain male German cockroaches to various cypermethrin dose treatments.

Treatment (ug/cm^2)	KT_{50} (min) ($\pm$ 95% FL)	KT_{90} (min) ($\pm$ 95% FL)	24 h (%) mortality
10	15.6 (0.6)	27.5 (6.0)	93
0.392	301.0 (33.9)	1073.0 (406.0)	90
0.245	220.0 (46.5)	2558.0 (3134.0)	86
0.049	> 24 h	--	27

values increased from 1073.0 min (17.9 h) to 2558.0 min (42.6 h), and 24 h mortality decreased from 90% to 86%. However, a KT_{50} of 301.0 min (5 h) in 0.392 ug/cm^2 treatment was greater than the KT_{50} of 220.0 min (3.6 h) for 0.245 ug/cm^2.

Correlation of dose transfer and knockdown time. How soon a lethal dose of insecticide is accumulated on cockroach tarsal pads depends on how much it walks on a treated substrate. Dose transfer is directly related to the distance travelled, to the contact area of the tarsal pads, to the density of insecticide droplets on the substrate, and to the area covered by the droplets. For the 0.025 ug/cm^2 cypermethrin treatment (VPI-strain, KT_{50} of 15.9 min), the estimated mean amount of cypermethrin transferred at knockdown time was 0.03 ug ($\pm$ 0.01 ug) (Table 10). For the 0.049 ug/cm^2 treatment (KT_{50} of 8.4 min), the mean amount of cypermethrin transferred was 0.05 ug ($\pm$ 0.02 ug). There were significantly positive (CORR, $r = 0.67, 0.92, P < 0.05$) correlations between knockdown time and estimated dose transfer when VPI-strain male cockroaches were exposed to these two treatments. The dose transfer data indicated that VPI-strain cockroaches picked up significantly (Tukey, $P < 0.05$) more residue on the 0.049 ug/cm^2 treatment than on the 0.036 ug/cm^2 and 0.025 ug/cm^2 treatments at knockdown time. The susceptibility of this strain to cypermethrin may have been enhanced by a significantly (Tukey, $P < 0.05$) higher movement rate (12.7 units/min) on the 0.049 ug/cm^2 treated surface than on 0.036 ug/cm^2 and 0.025 ug/cm^2 treatments.

RHA-strain German cockroaches showed significantly (CORR, $r = 0.75, 0.64, P < 0.005$) negative correlation between knockdown time and estimated dose transfer for the 0.245 ug/cm^2 and 0.392 ug/cm^2 treatments (Table 11). For the

Table 10. Correlation of the estimated cypermethrin transferred to tarsal pads by contact surface with cypermethrin treated surface and knockdown time in VPI-strain male German cockroaches.

Treatment (ug/cm^2)	KT_{50} (min) ($\pm$ 95% FL)	Movement rate (units/min)[c] ($\pm$ SD)	Dose transfer (ug)[d] ($\pm$ SD)
0.049 [a]	8.4 (0.5)	12.7a (5.6)	0.05a (0.02)
0.036	12.9 (0.6)	8.0b (3.0)	0.04b (0.01)
0.025 [b]	15.9 (0.6)	8.1b (3.2)	0.03b (0.01)

[a] there is a significant correlation between knockdown time and dose transfer (CORR, $r = 0.67$, $P < 0.05$)

[b] there is a significant correlation between knockdown time and dose transfer (CORR, $r = 0.92$, $P < 0.05$)

[c], [d] movement rates and doses within the same column followed by the same letter are not significantly different (Tukey, $P < 0.05$)

Table 11 Correlation of the estimated cypermethrin transferred to tarsal pads by contact surface with cypermethrin treated surface and knockdown time in RHA-strain male German cockroach

Treatment (ug/cm^2)	KT_{50} (min) (± 95% FL)	Movement rate (units/min) (± SD)	Dose transfer (ug) [c] (± SD)
0.392 [a]	301.0 (33.9)	3.0 (1.4)	3.93 (4.16)
0.245 [b]	220.0 (46.5)	3.8 (3.3)	1.52 (1.77)
0.049	> 24 h	4.7 (2.1)	--

[a] there is a significant correlation between knockdown time and dose transfer (CORR, $r = 0.64$, $P < 0.05$)

[b] there is a significant correlation between knockdown time and dose transfer (CORR, $r = 0.75$, $P < 0.05$)

[c] there is a significantly different dose transfer (ANOVA, $P < 0.05$)

0.245 ug/cm^2 treatment, the estimated mean amount of cypermethrin the cockroach tarsi accumulated at knockdown time was 1.52 ug ($\pm$ 1.77 ug) with a movement rate of 3.8 units/min ($\pm$ 3.3 units/min). There was 3.93 ug ($\pm$ 4.16 ug) of cypermethrin transferred to the tarsi from 0.392 ug/cm^2 treatment surfaces, with a movement rate 3.0 units/min ($\pm$ 1.4 units/min).

DISCUSSION

The dose directly applied to the tarsal pads (LDT_{50}, Table 7) was similar to the theoretical dose transferred to the pads by walking on a treated substrate (Table 10, 11) before knockdown time for both the susceptible and resistant strains. These data indicate that the number of droplets that a cockroach must walk on to achieve mortality can be predicted by the theoretical equation. Total contact areas and distance covered in two walking steps should be relatively constant, since there were no significant differences in walking steps between the VPI- and RHA-strain male German cockroaches. The major differences would be movement rates of German cockroach strains before knockdown in response to different insecticide dosages on the substrate. If a standard "less than runoff" treatment rate is used, it is possible to predict the number of droplets that must be applied or contacted, when the susceptibility of the strain to the insecticide has been established. Further work with several German cockroach field strains would confirm the accuracy of the theoretical equation. Direct application is a more accurate measurement of dose exposure since there is movement involved in indirect application, and it is not known exactly how much insecticide is picked up by a cockroach in one step, or how much more is picked up in later steps.

For less than runoff treatments, the knockdown time decreased as the insecticide droplet density (ug/cm^2) increased in VPI-strain cockroaches (Table 8). RHA-strain cockroaches took longer time to achieve 50% knockdown on 0.392 ug/cm^2 (301.0 min) than on 0.245 ug/cm^2 (220.0 min) cyperemthrin treated glass plates. This unusual response may be due to decreased movement or the heterogenity of the RHA population. Although the movement rates of RHA-strain cockroaches on 0.245 ug/cm^2 (3.8 units/min) and 0.392 ug/cm^2 (3.0 units/min) treatments were not significantly different, this difference might be large enough to influence the KT_{50}. The RHA-strain cockroaches were kept in colony for less than two generations, and this may have retained heterogenity in the population. The phenomena seen at KT_{50} was not observed at KT_{90} for the 0.392 ug/cm^2 and 0.245 ug/cm^2 treatments. Higher dosages of insecticide, such as runoff, on the substrate will achieve quick knockdown, however, it may not be an efficient or economical application rate. Effective cockroach control can be achieved with application rates that are less than "runoff".

Different susceptibility of German cockroach strains to a chemical can result in significant differences in effectiveness (Burden 1975, Rust & Reierson 1978, Zhai & Robinson 1991). This research showed that insecticide application can be decreased by 400 times less than runoff (10 ug/cm^2) and still achieve 100% mortality in 24 h of a susceptible strain of German cockroaches in laboratory tests. For a German cockroach strain with high level of cypermethrin resistance, it was also possible to decrease the application rate to 40 times less than runoff and achieve effective control. In urban environments it may not be necessary to completely cover surfaces with insecticide to achieve cockroach control. A known-dose or known-droplet density application could be used to give satisfactory results.

Chapter IV

INFLUENCE OF WALKING ON DETECTING CYPERMETHRIN RESISTANCE IN THE GERMAN COCKROACH, BLATTELLA GERMANICA (L.)

Cockroach mortality occurs when adults and nymphs are exposed to insecticide residues by walking on treated surfaces and/or ingesting toxic quantities of insecticide while grooming legs and antennae. The level of infestation reduction following a typical cockroach control treatment may be linked to the level of insecticide susceptibility in the pest population. Measuring resistance in German cockroaches includes using a surface-contact method (Keller et al. 1956) to determine KT_{50} or LT_{50} values, and topical-application methods to determine LD_{50} or LC_{50} values. These two methods may provide conflicting information on the level of resistance to insecticides in field populations. Milio et al. (1987) and Wadleigh et al. (1989) reported that surface-contact methods did not accurately assess chlorpyrifos susceptibility in field populations of German cockroaches. He et al. (1986) reported topical application to be more sensitive than surface-contact tests for measuring resistance to permethrin.

The excitatory response of cockroaches to pyrethroid-treated surfaces (Miall & le Patourel 1989), the different sites of toxity between surface-contact and topical-application methods (Scott et al. 1986), the different mode of action of Type I (e.g. permethrin) and Type II (e.g. cypermethrin) pyrethroids (Scott 1990), behavior differences in field strains, and the decrease in movement behavior of laboratory-strain German cockroaches (Akers & Robinson 1983) may influence results of surface-contact methods for measuring resistance to cypermethrin in the German cockroach.

The objectives of the research reported here were to determine the influence of walking activity on dose transfer in VPI and RHA strain cockroaches, and the impact of walking behavior on measuring cypermethrin resistance in these two strains.

MATERIALS AND METHODS

Cockroaches. Two strains of German cockroach were used: VPI, a susceptible strain, and RHA, a resistant strain. VPI-strain has been reared at VPI&SU for about 40 years, and has no history of insecticides exposure. The RHA-strain is from Roanoke Redevelopment and Housing Authority, Roanoke, Virginia, and was collected as adults and nymphs in July, 1990. The RHA-strain has high level (LD_{50} RR = 180) of resistance to cypermethrin (Zhai & Robinson 1991).

Resistance ratio. Methods for determining KT_{50}, KT_{90}, LDT_{50} (lethal dose to tarsal pads), and LDT_{90} are provided in Chapter III. The resistance ratio (RR) was calculated by formula:

$$RR = \frac{\text{KT or LDT (RHA)}}{\text{KT or LDT (VPI)}}$$

Dose transfer. A pyrethroid insecticide, cypermethrin (Demon EC, 25.3% AI, ICI Americas Inc.), (±)a-cyano-(3-phenoxyphenyl)methyl (±)-cis,trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate, was used in dose transfer evaluations.

Five males from each strain were individually placed on 10 ug/cm^2 cypermethrin-treated glass plates (9 x 9 cm^2). Each cockroach was confined on the plates for 5 min, then all six legs were removed and placed in 1 ml hexane. Another five males from each strain were individually confined on 10 ug/cm^2 cypermethrin-treated glass plates, and the insecticide exposure time limited to 30 movement units (see Chapter III). The limit of 30 movement-units was based on evidence that VPI-strain cockroaches accumulate this mean amount of movement units within 3 min (Capter II), and that a lethal dose or more of cypermethrin is transferred to the tarsal pads within this time. After 30 movement units were recorded, the legs of all cockroaches were removed and saved in hexane.

Analysis of cypermethrin residue on the legs was performed by gas chromatography. A Tracor 540 gas chromatograph, equipped with electron capture detectors employing Ni^{63} as a ionization source and nitrogen as the carrier gas, was used for analysis. Operating conditions for the gas chromatograph were: oven temperature 240^0 C, injector temperature 245^0 C, and detector temperature 350^0 C. Samples were injected into a 2-mm ID, glass column packed with 1.5% OV-17, 1.95% OV-210 on 100/120 Chromosorb WHP. Peak areas were determined with a Hewlett-Packard integrator. The recovery rate of 78 ± 2.0% was determined by placing 5 ug of cypermethrin on the tarsal pads.

Data analysis. Data were analyzed using a computer-based statistics program (SAS). Analysis of variance (ANOVA) was performed to quantify dose transferred by cockroach tarsal pads in different strains. All test were considered significant at the critical level of 5% (SAS 1985).

RESULTS

The surface-contact method, 10 ug cypermethrin/cm^2, provided a KT_{50} resistance ratio of 2.9, and a KT_{90} RR of 3.8 for RHA cockroaches. The surface-contact on 0.049 ug cypermethrin/cm^2 resulted a KT_{50} RR > 170, and the LDT_{50} RR and LDT_{90} RR of 106 and 91, respectively, were recorded in topical application of cypermethrin to tarsal pads. The surface-contact method on runoff treatment (10 ug cypermethrin/cm^2) indicated there was only low-level cypermethrin resistance in the RHA population. However, the topical application to tarsal pads, and surface contact on less than runoff treatemt (0.049 ug/cm^2) clearly indicated high level resistance to cyperemthrin, which further confirmed the control failure and resistance reported by Zhai & Robinson (1991). These results indicate that the topical application methods or surface-contact on less than runoff treatment are more sensitive indicators of cypermethrin resistance in German cockroaches than surface-contact on runoff treatments. However, because of the high dosage (10 ug/cm^2) of cypermethrin the comparison with VPI-strain to develop the RR may be inappropriate.

In previous studies (Chapter II) it was determined that the RHA-strain cockroaches moved more per unit time than did the VPI-strain on untreated and 10 ug/cm^2 cypermethrin treated glass plates, but not on 0.049 ug/cm^2 cypermethrin treated glass plates. Analysis of the cypermethrin residue on the tarsal pads showed that RHA-strain males accumulated significantly (ANOVA, $P < 0.05$) more cypermethrin ($\bar{x} = 10.47 \pm 1.30$ ug), than the less active on 10 ug/cm^2 cypermethrin treatment, VPI-strain ($\bar{x} = 7.02 \pm 1.23$ ug) at 5 min (Table 12). The increased amount of movement by RHA-strain cockroaches probably resulted in significantly more insecticide accumulating on the tarsal pads, as indicated by the residue analysis.

The differences in movement exhibited by field- and laboratory-strain and between strains of field-collected cockroaches can influence surface-contact methods (LT_{50} or KT_{50}) for measuring insecticide resistance. Increased movement on treated surface may increase exposure to insecticide and decrease knockdown time, thus influencing the RR. Further evidence of the influence of walking movement on RR, derived from surface-contact method, is shown by data from the restricted movement study. This evaluation provided cockroaches from both strains the opportunity to receive a nearly equal dose of insecticide before knockdown. There was no significant difference in the mean amount of cypermethrin detected on the tarsal pads of the VPI ($\bar{x} = 6.03 \pm 0.84$ ug) and RHA-strain ($\bar{x} = 4.38 \pm 0.24$ ug) cockroaches exposed for 30 movement units to cypermethrin-treated plates (Table 12).

Table 12. Mean amount (ug) of cypermethrin residue on tarsal pads of VPI-strain and RHA-strian German cockroaches after walking on 10 ug/cm^2 cypermethrin-treated glass plates.

Strain	$\bar{x}$ amount (ug) cypermethrin / tarsal pads / cockroach [a] ($\pm$ SD)	
	30 movement units	5 minutes
VPI	6.03a (0.84)	7.02a (1.23)
RHA	4.38a (0.24)	10.47b (1.30)

[a] means within the same column and row followed by the same letter are not significantly different (ANOVA, $P < 0.05$)

DISCUSSION

Insecticide resistance in a pest population can be expressed as a ratio in which knockdown time (KT or LT) or lethal dose (LD or LC) for susceptible (S) and resistant (R) strains are compared (S/R). The KT or LT ratio can best depict the level of resistance when the two strains are exposed to the same dose of insecticide. Data presented here indicated that KT methods for measuring cypermethrin resistance may not deliver the same dose to both susceptible and resistant (field strain) cockroaches. Although some insecticide may be transferred to the mouthparts during grooming (Scott 1990), the lethal dose is probably delivered through the tarsal pads. Insecticide exposure on the treated plates therefore is related to the amount of walking a cockroach does on the treated surface. As data presented in Chapter III, if dosage does play a major role when it presents lethal or sublethal level to locomotion in different strains of cockroach, an increase in the amount of walking on the runoff-treated surface results in increased exposure, because of the increase in the amount of insecticide accumulating on the tarsal pads of each leg. The strain differences in walking movement rate and subsequent accumulation of insecticide on the tarsal pads in surface-contact method assays may result in overestimating or underestimating resistance. Data reported here (Chapter II) indicated that walking movement on runoff treatment in a laboratory strain may be significantly less than field strains of German cockroach, and an underestimation of resistance is likely. Scott et al. (1986) reported different results on DDT-resistant strains of German cockroaches against cypermethrin by using surface- and topical-application methods.

The success of integrated pest management (IPM) and resistance management programs for German cockroaches may depend on detection of low to moderate levels of resistance to different classes of insecticides (Robinson & Zungoli 1985). Resistance management programs include alternating application of organophosphates, carbamates, and pyrethroids (Cochran 1990, Georghiou 1990), and may require monitoring resistance to these chemicals. The surface-contact on high dose treatment may not detect evolving resistance or low-level resistance in a German cockroach population, and this degree of sensitivity may be necessary in some resistance management programs. A topical application method (LD), or surface contact on low dose treatment (KT) may be more sensitive to evaluate susceptibility to insecticides in a field population. However, it may be difficult to standardize a level of less than runoff treatment for surface-contact method which will accurately detect the evolving resistance or low-level resistance. On the other hand, behavior resistance via avoidance of residue will not be observed in topical application bioassays.

Chapter V

INFLUENCE OF CYPERMETHRIN DOSE ON GROOMING BEHAVIOR OF THE GERMAN COCKROACH, BLATTELLA GERMANICA (L.)

Grooming behavior in insects is an immediate response to an irritant (Dethier 1972, Reingold & Camhi 1977, 1978, Bret & Ross 1986). The chemoreceptors on the antennae and tarsus can detect a variety of chemical irritants, including insecticides (Brousse-Gaury 1981, Dethier 1972). Reingold & Camhi (1977) reported cockroach grooming behavior occurred in response to increased chemical irritation, and Scott (1990) reported insecticide residue may be transferred to the mouthparts during grooming. Bret & Ross (1986) reported that propoxur vapors increased German cockroach movement and grooming behavior, and antennal grooming increased before tarsal grooming. They also reported that when tarsal grooming increased, antennal grooming decreased. Armstrong & Eonner (1985) found that permethrin induced a antifeedant effect on Drosophila melanogaster when they were topically treated with a sublethal dose. They thought that increased preening was due to the reduction in walking movement which ultimately resulted in decreased feeding. Pyrethroid-induced repellency is observed at sublethal levels (Matsunaga 1991, Su & Scheffrakn 1990), and usually shows a positive correlation between physiological and behavior resistance (Quisneberry et al. 1984).

The objectives of the research reported here were to determine the influence of discriminating dose of cypermethrin to the tarsal pads on cockroach grooming behavior in VPI- and RHA-strain German cockroaches.

MATERIALS AND METHODS

Cockroaches. Two strains of German cockroach were used: VPI, a susceptible strain, and RHA, a resistant strain. VPI-strain has been reared at VPI&SU for about 40 years, and has no history of insecticide exposure. The RHA-strain is from Roanoke Redevelopment and Housing Authority, Roanoke, Virginia, and was collected as adults and nymphs in July, 1990.

Insecticide. A pyrethroid insecticide, cypermethrin (Demon EC, 25.3% AI, ICI Americas Inc.), (±)a-cyano-(3-phenoxyphenyl)methyl (±)-cis,trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate, was used in grooming behavior evaluation.

Grooming behavior. Thirty males from VPI-strain were individually placed on a 9 x 9 cm^2 glass plate which was treated with cypermethrin at the rate of 0.025 ug/cm^2, 0.036 ug/cm^2, 0.049 ug/cm^2, and a runoff rate of 10 ug/cm^2. Thirty RHA-strain males were individually placed on glass plates which were treated with 3,969 droplets of 0.1%, 0.5% and 0.8% of cypermethrin, which resulted in 0.049, 0.245, and 0.392 ug cypermethrin per cm^2 on these plates. As a control, thirty VPI- and RHA-strain males were placed on untreated glass plates and grooming behavior recorded. Tarsal and antennal grooming behaviors were recorded each minute for 20 minutes. Differences in grooming behavior in response to different doses were recorded and analyzed.

Data analysis. Data were analyzed using a computer-based statistics program (SAS). Influences of different dose on grooming behavior in each strain were analyzed with Tukey multiple comparison (SAS 1985). All test were considered significant at the critical level of 5% (SAS 1985).

RESULTS

Antennal and tarsal grooming activity was observed in both strains at all concentrations of cypermethrin. Antennal grooming started at the base of the antennae, then continued forward to the tip. Tarsal grooming was most common on the forelegs, although mid- and hindleg grooming was also observed. Both cockroach strains groomed the tarsomeres and pretarsus more often than other segments of a leg.

A high percentage of VPI-strain cockroaches groomed legs (75%) and antennae (80%) on untreated surfaces. On untreated surfaces, VPI-strain antennal grooming peaked at 3 min (Fig 10). However, this strain started with a high percentage (53%) of antennal grooming, and it remained high for 10 min, with peaks at 7 min (73%) and 10 min (80%). The percentage of the total cockroaches involved in antennal grooming increased 1.2-fold, 1.2-fold, and 1.3-fold respectively on 0.025 ug/cm^2, 0.036 ug/cm^2, and 0.049 ug/cm^2 treated surfaces. The mean time

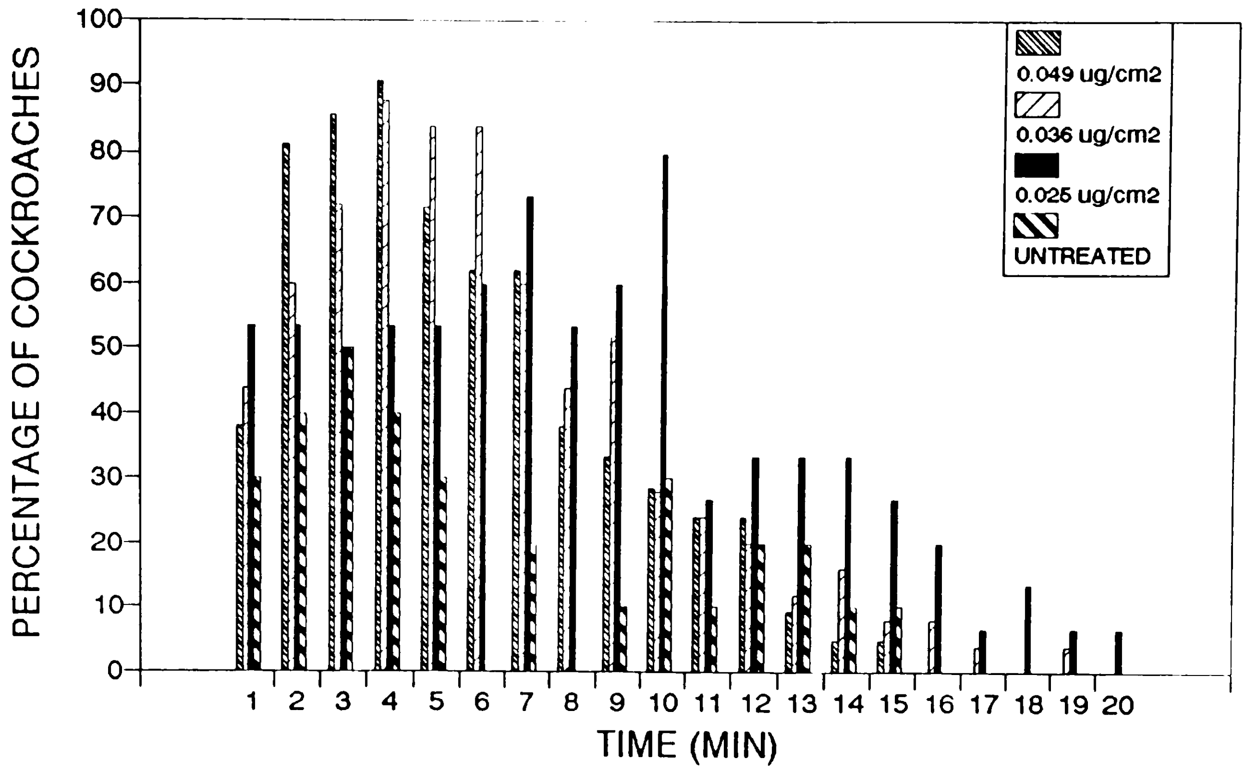

Figure 10. Percentage of VPI-strain male German cockroaches involved in antennal grooming when they walked on various cypermethrin treated glass plates in 20 minutes.

spent grooming antennae when exposed to these three treated surfaces were significantly (ANOVA, $P < 0.05$) greater than for the control. On 0.036 ug/cm^2 and 0.049 ug/cm^2 treated surfaces, grooming peaked sharply in the first 4 min. The 0.036 ug/cm^2 treatment had higher peaks at 5 min (88%) to 7 min (84%). Comparatively, the 0.049 ug/cm^2 treatment had peaks at 2 min (81%) to 4 min (91%). Higher insecticide dosage increased the number of cockroaches grooming their antennae, and also caused the peak of antennal grooming to occur early.

The percentages of total of cockroaches involved in leg grooming on treated surfaces were significantly (ANOVA, $P < 0.05$) less than on untreated surface. The mean amount of leg grooming was also significantly (ANOVA, $P < 0.05$) reduced (Table 13). There was a peak of leg grooming at 2 min (29%) on the 0.049 ug/cm^2 treatment, and this was lower than the control (Fig. 11).

RHA-strain cockroaches had a low percentage (30%) of total cockroach leg grooming and high percentage (100%) of antennal grooming in the control (Table 14). There were no differences in the percentage of total cockroaches involved in antennal grooming on various treatments. This species showed a high percentage on antennal grooming during the first 7 min of exposure (Fig. 12) to insecticide. On 0.049 ug/cm^2 and 0.392 ug/cm^2 cypermethrin-treated surfaces, there was a large number of RHA-strain cockroaches grooming antennae at the 20 min observation. Peaks were more stable on 0.392 ug/cm^2 treatment than 0.049 ug/cm^2 treatment. Cockroaches had a peak at 2 min (75%) on 0.245 ug/cm^2 treatment, then grooming slowly decreased. High dosage (0.392 ug/cm^2) increased leg grooming significantly (Tukey, $P < 0.05$) (Fig. 13). There were grooming leg responses on various treatments in first 2 min, but no such responses on control. This data indicates that cypermethrin stimulated the leg grooming on RHA-strain German cockroach.

Table 13. Mean amount of leg and antennal grooming behavior activity of VPI-strain male German cockroach on cypermethrin-treated glass surfaces in 20 min.

Treatment (ug/cm^2)	Leg grooming event [a]		Antennae grooming event	
	% of cockroach grooming	$\bar{x}$ no. [b] grooming ($\pm$ SD)	% of cockroach grooming	$\bar{x}$ no. grooming ($\pm$ SD)
Untreated	75a	10.9a (8.5)	80	4.6b (2.1)
0.025	13c	3.0b (1.4)	93	15.1a (6.2)
0.036	16c	3.8b (2.1)	96	17.6a (8.5)
0.049	40b	3.8b (3.2)	100	16.9a (9.8)

[a] percentages and means within the same column followed by the same letter are not significantly different (Tukey, $P < 0.05$)

[b] mean times of grooming in 20 minutes

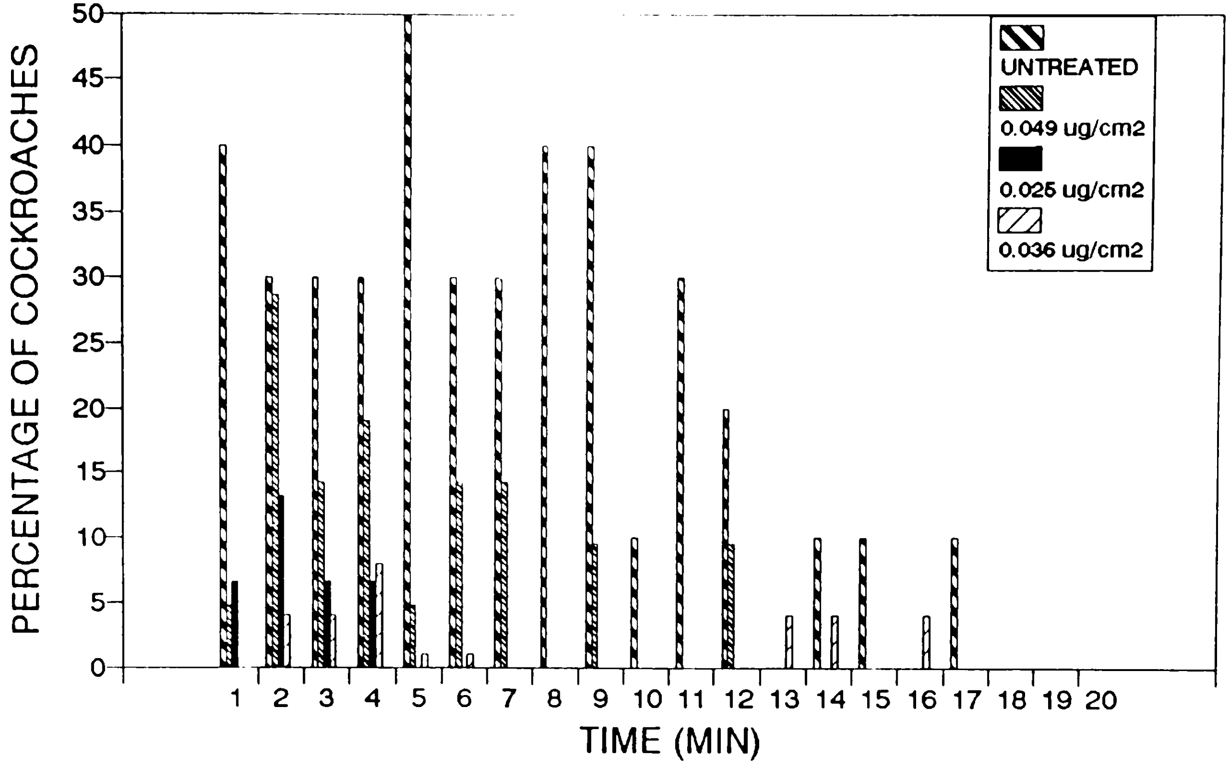

Figure 11. Percentage of VPI-strain male German cockroaches involved in leg grooming when they walked on various cypermethrin treated glass plates in 20 minutes.

Table 14. Mean amount of leg and antennal grooming behavior activity of RHA-strain male German cockroach on cypermethrin-treated glass surfaces in 20 min.

Treatment (ug/cm^2)	Leg grooming event [a]		Antennae grooming event	
	% of cockroach grooming	x̄ no. [b] grooming (± SD)	% of cockroach grooming	x̄ no. grooming (± SD)
Untreated	30b	5.0 (3.6)	100	19.0 (8.1)
0.049	43b	7.9 (12.7)	100	25.5 (13.1)
0.245	39b	5.5 (6.6)	96	13.6 (8.8)
0.392	87a	11.2 (7.3)	100	28.6 (12.6)

[a] percentages and means within the same column followed by the same letter are not significantly different (Tukey, $P < 0.05$)

[b] mean times of grooming in 20 minutes

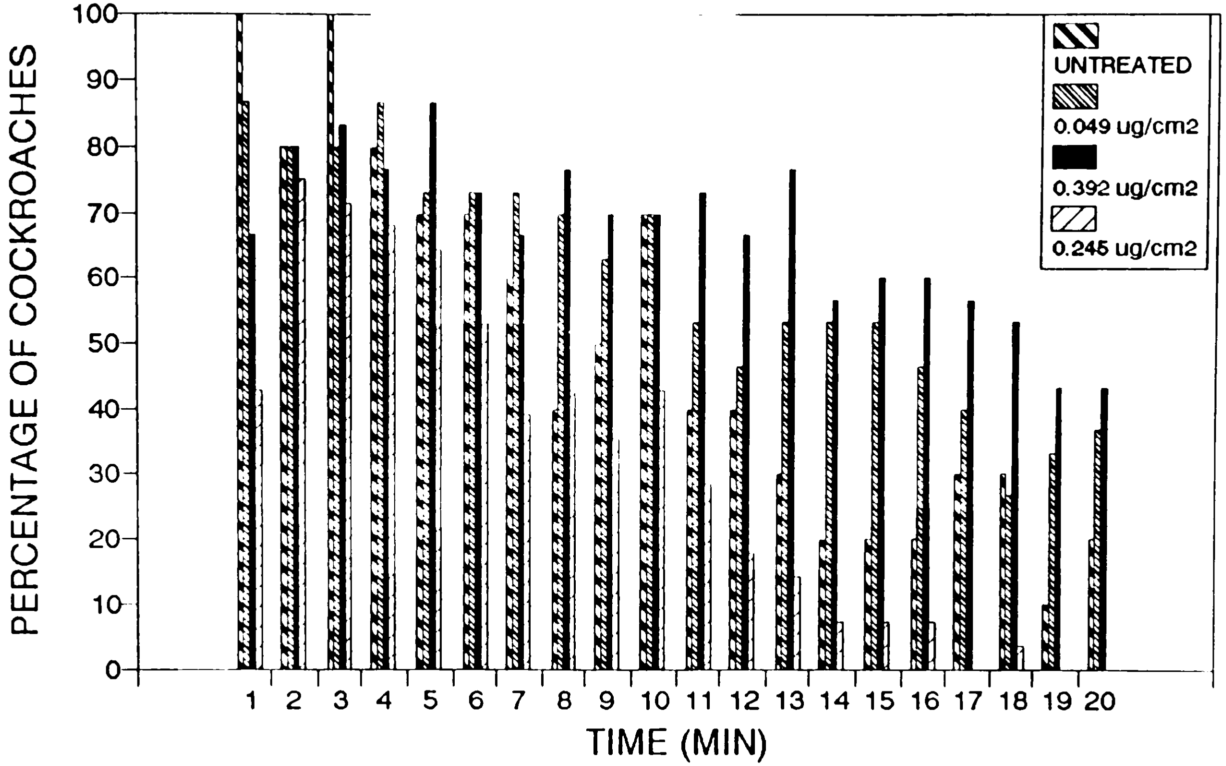

Figure 12. Percentage of RHA-strain male German cockroaches involved in antennal grooming when they walked on various cypermethrin treated glass plates in 20 minutes.

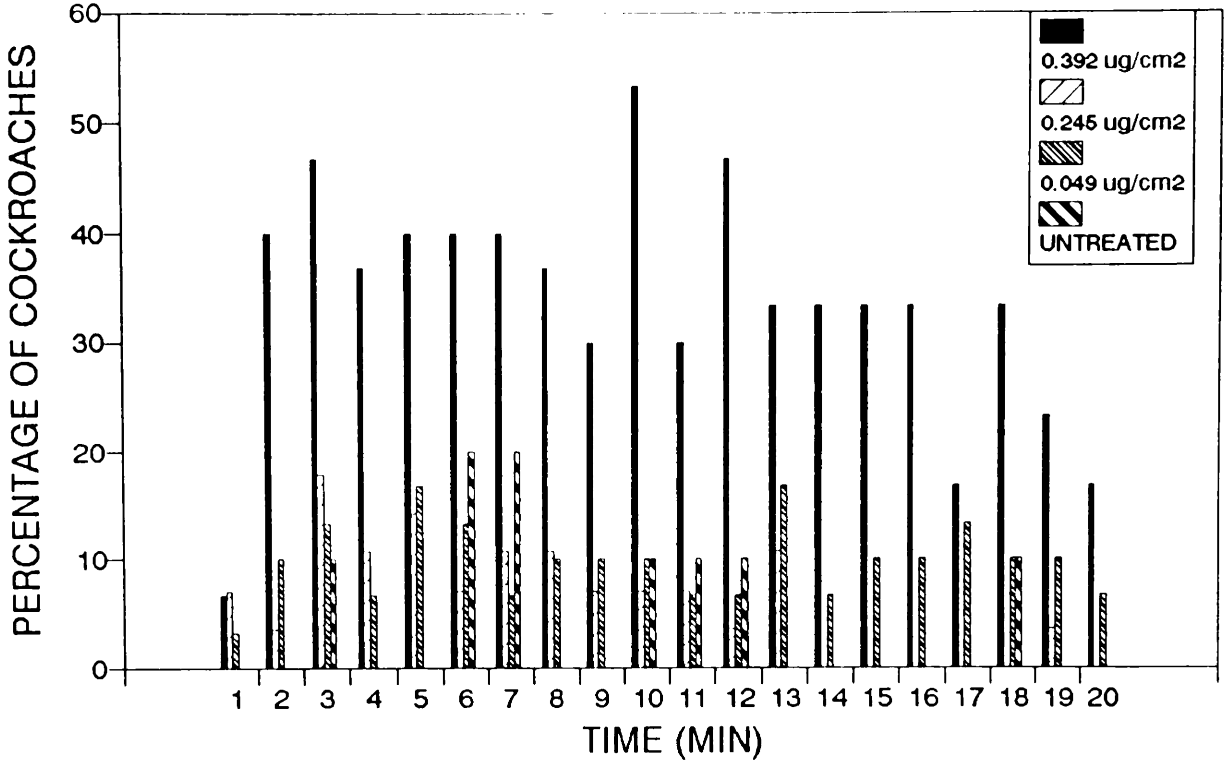

Figure 13. Percentage of RHA-strain male German cockroaches involved in leg grooming when they walked on various cypermethrin treated glass plates in 20 minutes.

DISCUSSION

Modern insecticides have both contact and stomach poison action. Scott (1990) reported that insecticide may be transferred internally through mouthpart grooming of legs and antennae. There was increased grooming behavior in VPI- and RHA-strain cockroaches in response to exposure insecticide. Grooming behavior is probably necessary to keep sensory surfaces of cockroach free from contamination (Matsunaga 1991). High dosage of insecticide increased the amount of antennal grooming in VPI-strain cockroaches. Antennae are the primary receptor sites for volatile odors (Reddy 1970a, b), and in response to increased antennal grooming, leg grooming decreased. This phenomenon was also reported by Bret & Ross (1986). High insecticide dosage stimulates leg grooming greatly in the RHA-strain. Leg irritation in response to high dosage may due to reduction of the walking movement rate.

Matsunaga (1991) reported that foreleg tarsus has the most chemoreceptors in A. aegypti, and most of the sensilla were on the 4th and 5th tarsus of each leg. Although the distribution of chemoreceptors on tarsi of a cockroach leg may be different from the mousquito, Brousse-Gaury (1981) reported the largest number of sensilla on the venter of the tarsus are mechanoreceptors and chemoreceptors in the American cockroach. Tarsal grooming in German cockroaches may result from an abundance of chemoreceptors on the tarsal segments. The phenomenon of a large amount of foreleg tarsal grooming may be explained by attempts at cleaning a large portion of the chemoreceptors, to which a large amount of insecticide residue is attached. The differences in the leg grooming and walking movement (Chapter II) response to various treatments in VPI- and RHA-strain of German cockroaches may have resulted from the amount of chemoreceptors on the tarsal segments or the sensitivity of these chemoreceptors (Mclver 1978).

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