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Potential of glucose-aversion development in field-collected populations of the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae) from Malaysia

Lee C.Y. & Soo J.A.C.
Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia.

Abstract: The potential of glucose-aversion development in Malaysian field populations of the German cockroach was investigated in the laboratory. A total of 41 strains of field-collected populations were starved and screened for their response to glucose in agar. Only 12% of the total strains screened contained individuals that showed negative response towards glucose agar. They were removed and further tested with boric-acid-based glucose agar. The survivors (Group I) were isolated, mixed and reared for progenies (Group II). Results indicated that Group I individuals showed a higher level of tolerance to a 2% hydramethylnon-based gel bait (containing glucose) when compared to those of the unselected individuals. However, there appeared to be a reduction in tolerance when Group II was tested. As glucose-aversion is an autosomal incompletely dominant trait, this possibly suggested an increase in heterozygous or wild type individuals after a generation turnover. This present study suggested the potential of glucose-aversion development in field populations tested, as a novel behavioural resistance mechanism in the German cockroach from Malaysia.

INTRODUCTION

Being synanthropic in nature, the German cockroach, *Blattella germanica* (L.) is an important urban insect pests in many parts of Asia, especially in the food preparative industry (Lee *et al.*, 1999a; Lee & Robinson, 2001). Its obnoxious behaviour that move from one place to another when foraging for food makes it a potential mechanical vectors of various pathogenic organisms (Lee, 1997a). Current control of this species relies heavily on the use of residual insecticides. However with the increase in incidents of insecticide resistance in this species (Lee, 1997b; Lee & Lee, 1998; Lee *et al.*, 1996; Lee *et al.*, 1999b; Lee *et al.*, 2000), the use of baits as a resistance management tool is gaining popularity among the pest control operators in this region (Lee, 1998). Baiting is an effective method and has many advantages against this insect species because bait can be precisely placed, fast action, child-proof (especially those in containerised form) (Lee, 1998; Lee & Lee, 2000) and demonstrated secondary poisoning effects (Mook, 1999) through necrophagy (Gahlhoff *et al.*, 1999) and coprophagy (Silverman *et al.*, 1991).

However long-term usage of bait products may select for glucose-aversion individuals in field populations of the German cockroach. Glucose-aversion is defined as an inherited behaviour that avoid glucose-based food or materials. It has a serious implication on bait usage, because many bait products contain glucose as one of the major food attractants. Silverman & Bieman (1993) first reported a strain of the German cockroach in Florida which was aversed to glucose. Subsequently, more strains were found in the U.S.A. and South Korea (Silverman & Bieman, 1993; Silverman & Ross, 1994) in locations where baits have been used for a few years against German cockroaches. Broad geographical range of spread of this phenomenon could be possibly due to independent evolvement in isolated locations, spread through commerce, and evolvement prior to cockroach/human association, particularly due to selective allelochemicals such as glucosinolates or cynaogenic glycosides (Silverman & Ross, 1994).

Gel baits against German cockroaches in Malaysia has registered several years of usage. In our routine collection of German cockroaches for insecticide resistance studies, we investigated the potential of glucose-aversion in various field-collected populations which had been subjected to bait treatments.

MATERIALS AND METHODS

A total of 41 field populations of the German cockroach were collected with bread-baited glass jars from various premises in several cities in Peninsular Malaysia, at different dates from 1997 – 2000 (Table 1). They were brought back to the laboratory and reared under environmental conditions of 27 ± 2°C, 50 ± 5% relative humidity and 12-hour photoperiod, for 1 – 3 generations before being used for this study. A standard susceptible strain, ICI, which was obtained from Zeneca Agrochemicals, UK earlier, was used for comparison.

Preliminary screening for glucose-aversed individuals was conducted by introducing uncounted numbers of starved German cockroaches (without food for 2 days period) of multiple stages and sex into a test arena which contained two petri dishes placed side by side. One contained 1 M D(+)-glucose anhydrous (Merck, Germany) in 4% agar, while another one contained only blank 4% agar. The individuals that were seen to feed on blank agar (instead of glucose-based agar) were isolated and reared in a separate container.

About a week after the preliminary screening, the isolated individuals were starved again for 24 hours. After this period, they were introduced into a test arena containing two agar-filled petri-dishes, one containing 30% boric acid in 1 M D(+)-glucose anhydrous in agar, while the other one contained blank agar only. The cockroaches were allowed to feed for 24 hours, after which the survivors were isolated into another rearing container. This process was repeated for three cycles every five days on the surviving individuals of the previous selection. These survivors were considered as Group I. Group I were further reared and the gravid females were isolated to harvest newly emerged nymphs which were considered as Group II.

Both Group I and II, along with unselected strain were subjected to bait susceptibility tests to determine the presence of glucose aversion characteristic in these individuals. A total of 20 each of adult males, females and mid-instar nymphs were introduced into a test arena (35 x 30 x 10 cm) and provided with food and water. After 24 hours, 0.15 g of a 2% hydramethylnon-based gel bait (Siege®, BASF Malaysia) was introduced into the test arena. Mortality of the cockroaches were scored daily up to 14 days post-treatment and three replicates were done.

Data were pooled and subjected to probit analysis (Finney 1971) using POLO-PC software. Resistance ratio was calculated using RR-PC97 program (C.Y. Lee, unpublished program) according to procedures described by Robertson & Preisler (1992).

Table 1: Information on strains of field populations of the German cockroach, *Blattella germanica* used in this study

Strain collection	Collection site	City	Date of
BBR	Hotel kitchen	Penang	11 Jan 1999
CBO	Hotel kitchen	Penang	22 Dec 1998
Cigo	Restaurant's kitchen	Kuala Lumpur	28 Dec 1998
CK	Hotel kitchen	Kuantan	11 Aug 1999
CP	Hotel kitchen	Penang	2 Nov 1998
CT	Hotel kitchen	Kuantan	18 Aug 1999
EMP	Cruise ship	–	2 Dec 1999
FBR	Hotel kitchen	Penang	20 Jan 2000
GL	Hotel kitchen	Kuantan	18 Aug 1999
GCJB	Hotel kitchen	Johor Bahru	29 Dec 1998
GCPG	Hotel kitchen	Penang	29 Jun 1999
GT	Hotel kitchen	Kuantan	3 Aug 1999
HT	Food court	Kuala Lumpur	16 Dec 1998
HUSM	University cafetaria	Penang	20 Nov 1997
IE	Express bus	–	24 May 1998
IHKL	Hotel kitchen	Kuala Lumpur	15 Oct 1999
Inai	Food Court	Kuala Lumpur	16 Dec 1998
Ita	Hotel kitchen	Penang	13 Dec 1999
KTM	Train	–	10 Mar 2000
LHFA	Restaurant's kitchen	Kuala Lumpur	25 Jun 1998
LHFB	Restaurant's kitchen	Kuala Lumpur	27 Jun 1998
Mand	Hotel kitchen	Kuala Lumpur	14 Aug 1999
Mal	Hotel kitchen	Kuala Lumpur	16 Dec 1998
May	Restaurant's kitchen	Penang	6 Jan 1999
Mids	Restaurant's kitchen	Penang	30 Jun 1999
ML	Hotel kitchen	Kuala Lumpur	13 Feb 1997
MT	Hotel kitchen	Penang	25 Jan 1999
MV	Hotel kitchen	Penang	25 Jun 1998
PEAK	Hotel kitchen	Kuantan	9 Aug 1999
PGC	Club house	Penang	20 Sep 1999
PK	Hotel kitchen	Kuantan	17 Aug 1999
PRKT	Hotel kitchen	Kuala Trengganu	23 Apr 1998
PRPG	Hotel kitchen	Penang	6 Jan 1999
Pudu	Hotel kitchen	Kuala Lumpur	28 Dis 1998
Raja	Hotel kitchen	Kuantan	18 Aug 1998
Sedap A	Food court	Johor Bahru	5 Jan 1999
Selesa	Restaurant's kitchen	Johor Bahru	5 Jan 1999
SG	Restaurant's kitchen	Penang	23 Nov 1998
SW	Hotel kitchen	Penang	6 Feb 1998
TS	Sundry shop	Kuala Lumpur	17 Aug 1999
ZT	Boutique	Kuala Lumpur	28 Nov 1999

RESULTS AND DISCUSSION

Sugar, especially those of mono- and disaccharide groups are often regarded as phagostimulants to many insects (Bernays, 1985; Silverman & Bieman, 1993). Many sugars stimulate feeding in cockroaches (Tsuji, 1965) and thus, they are often incorporated into toxic cockroach baits. Glucose, a universal metabolic fuel, is by far the most abundant monosaccharide in nature (Silverman & Ross, 1994). Cockroaches required diets of >50% carbohydrate to promote optimal development (Cohen *et al.*, 1987; Forgash, 1958), particularly high levels of glucose as carbohydrate source (House, 1949; Gordon, 1959; 1968).

Based on the 41 strains of field populations of the German cockroach tested in preliminary screening, only 12% (5 strains) showed potential of glucose aversion development. They were [Emp, GCPG, LHFA, LHFB and ML] strains. Results obtained indicated that there is a potential for development of glucose aversion in the five strains tested. After being subjected to selection using boric acid-based glucose agar, most insects in Group I showed a higher tolerance to the hydramethynon gel bait (Table 2). Adult females of Group I LHFB showed 3.8x more tolerant to the bait, when compared to that of the unselected ones. Similar observation was recorded in the same strain for mid-instar where it demonstrated a 2x tolerance to the hydramethylnon gel bait, than that of the unselected individuals. This suggested that the glucose-averse (*glu/glu*)

Table 2. Susceptibility of potential glucose-aversed selected strains of the Malaysian field populations of the German cockroach to 2.0% hydramethylnon gel bait (LT_{50} in days)

Strain	Stage/sex					
	Male		Female		Mid-instar	
	LT_{50} (95% F.L.) (days)	RR_{50} (95% F.L.)	LT_{50} (95% F.L.) (days)	RR_{50} (95% F.L.)	LT_{50} (95% F.L.) (days)	RR_{50} (95% F.L.)
ICI	1.8 (1.4 – 2.0)	-	2.0 (1.8 – 2.2)	-	3.3 (2.9 – 3.8)	-
LHFA						
Unselected	1.7 (1.1 – 2.3)	1.0 (0.8 – 1.2)	1.9 (1.5 – 2.2)	0.9 (0.8 – 1.1)	3.9 (3.5 – 4.2)	1.2 (1.0 – 1.3)
Group I	2.1 (1.7 – 2.4)	1.2 (1.0 – 1.4)	2.7 (2.4 – 2.9)	1.3 (1.1 – 1.5)	4.2 (3.8 – 4.6)	1.3 (1.1 – 1.4)
Group II	1.8 (1.7 – 2.0)	1.0 (0.9 – 1.3)	2.1 (1.9 – 2.4)	1.1 (0.9 – 1.2)	3.4 (3.2 – 3.6)	1.0 (0.9 – 1.1)
LHFB						
Unselected	1.6 (1.1 – 1.9)	0.9 (0.5 – 1.7)	2.2 (1.9 – 2.5)	1.1 (0.9 – 1.3)	3.0 (2.8 – 3.2)	0.9 (0.8 – 1.0)
Group I	3.4 (3.0 – 3.7)	1.9 (1.6 – 2.3)	7.6 (7.1 – 8.2)	3.8 (3.3 – 4.3)	6.8 (6.4 – 7.2)	2.0 (1.8 – 2.3)
Group II	2.3 (2.0 – 2.4)	1.3 (1.0 – 1.6)	3.6 (3.3 – 3.9)	1.8 (1.5 – 2.0)	5.2 (4.9 – 5.4)	1.6 (1.4 – 1.7)
ML						
Unselected	1.8 (1.3 – 2.3)	1.0 (0.8 – 1.3)	1.9 (1.6 – 2.3)	1.0 (0.8 – 1.1)	3.5 (3.1 – 3.9)	1.1 (0.9 – 1.2)
Group I	3.3 (2.2 – 3.6)	1.8 (1.5 – 2.3)	5.1 (4.5 – 5.7)	2.5 (2.3 – 2.9)	5.7 (5.2 – 6.2)	1.7 (1.6 – 1.9)
Group II	2.3 (1.9 – 2.5)	1.3 (1.0 – 1.6)	1.9 (1.4 – 2.3)	0.9 (0.7 – 1.2)	3.1 (2.9 – 3.2)	0.9 (0.8 – 1.0)
GCPG						
Unselected	2.2 (1.8 – 2.6)	1.3 (1.0 – 1.6)	3.5 (3.1 – 3.9)	1.7 (1.5 – 2.0)	3.6 (3.3 – 3.8)	1.1 (1.0 – 1.2)
Group I	2.5 (2.2 – 2.7)	1.4 (1.1 – 1.7)	3.1 (2.8 – 3.4)	1.5 (1.3 – 1.8)	4.7 (4.3 – 5.1)	1.4 (1.3 – 1.6)
Group II	2.4 (1.9 – 2.8)	1.4 (1.1 – 1.7)	3.2 (2.6 – 3.8)	1.6 (1.3 – 1.9)	3.4 (3.2 – 3.6)	1.0 (0.9 – 1.1)
EMP						
Unselected	1.9 (1.4 – 2.2)	1.1 (0.8 – 1.4)	3.8 (3.4 – 4.1)	1.9 (1.6 – 2.2)	2.8 (2.5 – 3.1)	0.8 (0.7 – 0.9)
Group I	2.0 (1.7 – 2.4)	1.2 (1.0 – 1.4)	3.5 (3.2 – 3.9)	1.7 (1.5 – 2.0)	3.7 (3.3 – 4.0)	1.1 (1.0 – 1.2)

genotypes are likely existing in these strains. Earlier, Silverman & Bieman (1993) reported that glucose aversion is inherited as an autosomal incompletely dominant trait, which is controlled by a single major gene.

Despite recording a reduction in susceptiblity to glucose-based bait in Group I, an increase in susceptibility was seen in Group II. Most of the insects from Group II tested showed a shorter LT_{50} period when compared to those of Group I (Table 2). This could possibly due to a reduction in the number of cockroaches carrying *glu/glu* genotypes in Group II. It was reported earlier that individuals with *glu/glu* genotypes have a disadvantage in its biological parameters, namely consuming less glucose-supplemented diet, gaining less weight, showing longer developmental period and had a lower survival rate, when compared to the wild type (*glu+/glu+*) or heterozygous individuals (*glu/glu+*) (Silverman, 1995). It is possible that the Group II individuals (progenies of Group I) tested in this study were mainly consisted of heterozygous individuals (*glu/glu+*) or wild-type (*glu+/glu+*) due to the fact that the presence of normal individuals (*glu+/glu+*) will dilute the proportion of glucose-aversed cockroaches within a generation turnover. This speculation is very likely, as similar observation had been registered for insecticide resistant German cockroaches (Ross, 1991; Lee *et al.*, 1996). Laboratory-rearing of German cockroaches often selects for individuals that develop most rapidly and that are most fecund (Koehler *et al.*, 1994; Strong *et al.*, 1997).

This present study suggested the potential of glucose-aversion development in Malaysian field populations of the German cockroach, as a novel behavioural resistance mechanism. There is a serious implication of this finding because increased prevalence of glucose-aversed German cockroach populations would render many bait products with glucose as main food attractant to be useless. A continuous selection process for glucose-aversed individuals in the five strains reported above were currently undertaken. More screening of field populations for this behavioural trait should be conducted to further substantiate current findings.

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