

## **BIOLOGY AND MANAGEMENT OF BED BUGS: REVIEW OF RECENT RESEARCH**

**ALVARO ROMERO**

Department of Entomology, Campus Box 7613, North Carolina State University,  
Raleigh, NC USA 27695-7613

**Abstract** The near absence of bed bugs from human dwellings for fifty or more years has left us with limited knowledge of its biology and few answers to eliminate populations. Research findings with relevance to global bed bug management are presented. Monitoring of spontaneous activity of bed bugs demonstrated that this activity is periodical, endogenously generated by circadian clocks and can be entrained by light conditions. Short-term starved adults moved more frequently than long-starved adults. Extremely high levels of resistance to deltamethrin was detected in more than 20 populations collected across the United States indicating that resistance to this insecticide is already widespread. Synergism studies with piperonyl butoxide (PBO) suggested that cytochrome P450 monooxygenases (P450) were involved in deltamethrin resistance. Identification and high frequency of two mutations in the voltage-gated sodium channel  $\alpha$ -subunit gene in pyrethroid resistant populations suggested that target site mechanisms may be responsible for pyrethroid resistance in bed bugs. Behavioral studies of bed bugs to insecticides showed that responses vary depending on several factors including the class of insecticide, and the presence of harborages or a heat source. Chlorfenapyr was effective against pyrethroid resistant strains but it had a slow killing action.

**Key Words** *Cimex lectularius*, locomotor activity, circadian rhythm, insecticide resistance, behavioral responses, chlorfenapyr

### **INTRODUCTION**

Prior to the mid-twentieth century bed bugs, *Cimex lectularius* L., were fairly common in the USA and other countries (Usinger, 1966). However, after the advent of powerful synthetic insecticides such as DDT in the 1940's, their prevalence greatly decreased, particularly in developed countries, and only sporadic infestations were detected, primarily in dwellings with high occupant turnover (Krueger, 2000). Over the last ten years however, a general global resurgence of bed bugs has been reported in Asia, Europe, North America, and Australia (Krueger, 2000; Boase, 2001; Doggett et al., 2004). The current resurgence of bed bugs has been called a "perfect storm" (Potter, 2006).

Bed bug infestations can have adverse effects on health and quality of life. Bed bug bites produce irritating and itchy skin reactions that can be severe in some cases, but emotional stress is often the most severe consequence of infestations (Hwang et al., 2005). Vigorous scratching and concomitant erosions predispose the skin to secondary bacterial infection (Millikan, 1993). Chronic blood loss and iron-deficiency anemia have been reported in people who have been continuously exposed to severe bed bug infestations (Pritchard and Hwang, 2009). Bed bug infestations often require expensive ongoing inspections and treatments, disposal and replacement of infested beds and other furnishings, and quarantine of infested areas (Potter, 2005). Bed bug infestations are expected to increase in the coming years, presenting many challenges to the pest control industry. Basic and applied knowledge about this urban pest is extremely limited compared to other major pest insects. What follows is a summary of findings of research designed to provide not only practical information for the pest control industry and its stakeholders, but also to begin to resolve the knowledge vacuum that surrounds this pest.

### **LOCOMOTOR ACTIVITY**

Much locomotor activity of bed bugs concerns searching for a host, acquiring a blood meal, and returning to harborages, where they remain while digestion takes place. Few studies have addressed in detail locomotion of bed bugs particularly about the effect of internal and external on this activity. The aim of this study was to reveal characteristics of the periodicity of spontaneous locomotor activity, and to determine if the periodicity is influenced

by endogenous factors or an exogenous light cycle. In addition, the influence of feeding status on locomotor activity was evaluated.

#### **Activity Under 14 hr of Light and 10 hr of Dark Photoperiod**

Locomotor activity of bed bugs monitored with infrared sensitive digital camera for six days was periodical and showed a clear nocturnal pattern (Romero et al., 2010a). Some bed bugs began to move shortly after lights-off and remained relatively active for several hours before declining toward the end of scotophase. In the transition from dark to light, most of the insects showed a sudden increase in activity, but the frequency of movements rapidly decreased, persisting at low levels throughout the photophase (Romero et al., 2010a).

#### **Activity in Conditions of Light or Dark**

Spontaneous locomotor activity of bed bugs under continuous light (LL) or dark (DD) conditions persisted for at least three cycles. Free running of locomotor activity in the absence of other obvious time cues (such as temperature or humidity) suggested that the activity has an endogenous circadian nature (Romero et al., 2010a).

#### **Entrainment**

Entrainment of locomotor activity was demonstrated when bed bugs, having transferred to a reverse cycle of light and dark (i.e. from L:D 12:12 to D:L 12:12), adopted the new pattern within four cycles, as expected for an endogenously controlled system (Romero et al., 2010a). Entrainment of circadian rhythm of locomotor activity by environmental light cycles may be crucial for bed bug survival (less conspicuous activity) considering the frequent changes in light conditions to which bed bugs are exposed in human environments.

#### **Effect of Feeding History on Locomotor Activity**

Bed bugs that were subjected to a short period without food (one week after emergence) were more active than those starved longer. Recently fed adults showed activity but their activity was less intense than unfed groups. This relationship between length of time of food deprivation and activity level suggests that the intensity of activity is a measure of the nutritional state of bed bugs, and that metabolic reserves play a role in modulating activity level (Romero et al., 2010a).

## **INSECTICIDE RESISTANCE**

#### **Detection of Insecticide Resistance in Bed Bug Populations**

Dose-response analysis showed extremely high levels of resistance to deltamethrin and lambda cyhalothrin in four populations collected from human dwellings (Romero et al., 2007a). The resistance ratios (RR) of these populations relative to a susceptible colony was  $>12,765$ . Further evaluations of samples collected across the United States indicated that resistance to pyrethroid insecticides is already widespread (Romero et al., 2007a, b; Zhu et al., 2010). Resistant bed bugs to pyrethroids has also been detected in the United Kingdom, Denmark and Australia indicating that pyrethroid resistance is becoming a global phenomenon (Boase et al., 2006; Kilpinen et al., 2008; Lilly et al., 2009).

#### **Mechanisms of Resistance to Pyrethroids**

The cytochrome P450 monooxygenase (P450) inhibitor piperonyl butoxide (PBO) was used to assess the role of P450s in deltamethrin resistance in three field-collected bed bug strains, LA-1, CIN-1 and WOR-1. Piperonyl butoxide synergized deltamethrin in all three strains, but its impact was variable (Romero et al., 2009a). Because the resistance ratio in the highly pyrethroid resistant strains CIN-1 and WOR-1 was 174 and 39, respectively after piperonyl butoxide treatment, it is suggested that P450s have some involvement in deltamethrin resistance (Romero et al., 2009a). Later studies with DNA from  $>100$  bed bug samples collected across the USA identified the V419L and L925I mutations in the voltage-gated sodium channel  $\alpha$ -subunit gene and suggested that target site resistance may be also responsible for pyrethroid resistance (Zhu et al., 2010).

#### **Behavioral Responses to Insecticides**

Behavioral assays evaluated the responses of bed bugs to deltamethrin or chlorfenapyr. Results showed that three of four strains (LA-1, CIN-3, and LEX-1) preferred to settle in acetone-treated rather than in deltamethrin-treated tents (Romero et al., 2009b). Insects from all strains did not avoid resting in tents with dry deposits of chlorfenapyr.

Video recordings of bed bugs showed that insects increased their activity when they contacted sublethal doses of deltamethrin. Harborages treated with a deltamethrin remained attractive. In addition, a nearby heat source overcame avoidance to deltamethrin (Romero et al., 2009b).

### EFFECTIVENESS OF A NON-PYRETHROID INSECTICIDE

Chlorfenapyr is an insecticide that disrupts oxidative phosphorylation in insect mitochondria and has emerged as alternative to pyrethroid insecticides. Results of laboratory assays showed that chlorfenapyr was effective against susceptible and pyrethroid resistant strains (Romero et al., 2010b). In addition, dry residues of chlorfenapyr aged for 1, 2 or 4 months remained as toxic as fresh deposits. However, this insecticide had slower effect on bed bugs, compared with conventional neurotoxicants such as pyrethroids on susceptible insects, with mortality of >50% recorded after 3 days of continuous exposure. A faster insecticidal effect is obtained with an aerosol formulation, suggesting greater bioavailability of the toxicant (Romero et al., 2010b).

### CONCLUSIONS AND IMPLICATIONS

-The locomotor activity of bed bugs is periodical, endogenously generated by circadian clocks and can be entrained by light conditions. Short-starved insects are more active than long starved insects. Based on these findings, it can be suggest that during prolonged periods of starvation (no host available) bed bugs make a transition from exploratory behavior to host-stimulus dependent searching. During this latter period, long-starved insects might not only reduce their spontaneous locomotor activity but the threshold of host-seeking related responses that would allow them to detect host stimuli when they are present. This suggestion remains speculative but it produces the testable prediction that long-starved bed bugs under the presence of host cues (e.g., temperature and carbon dioxide) increase activity.

Pyrethroid resistance in bed bugs was documented for the first time. Extremely high levels of resistance to deltamethrin and  $\lambda$ -cyhalothrin, was detected in populations collected across the USA. P450-mediated resistance and target site resistance were identified as resistance mechanisms in pyrethroid resistant populations. Failure of pyrethroids to quickly control infestations of resistant populations increases the opportunity for their spread. In addition, high incidence of *kdr*-like insensitivity in bed bug populations in the USA limits the effectiveness of pyrethroids and requires the selection of an insecticide with a different mode of action.

Behavioral responses of bed bugs to insecticides and their implications for control may vary depending on several factors including insecticide susceptibility of populations, class of insecticide, and the presence of other stimuli in the environment. The presence of aggregating factors in harborages and attraction to a heat source might reduce the avoidance behavior of bed bugs to deltamethrin. Survival of bed bugs after their contact with deltamethrin residues, with the subsequent increase in locomotor activity, represents a potential problem for the spread of bed bugs to adjoining areas. This is a behavioral effect that should be considered with all insecticides used for bed bug management.

The non-repellent insecticide chlorfenapyr emerges as an alternative to pyrethroid insecticides to control bed bugs. Its slow active action, however, limits the immediate effect on infestations. Dry residues of chlorfenapyr remain effective over an extended period of time which is encouraging because bed bugs that are not sprayed directly may still succumb after residing on treated surfaces.

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