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# Circadian Dynamics of Locomotor Activity and Deltamethrin Susceptibility in the Pine Weevil, *Hylobius abietis*

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Rhythmic locomotor activity and daily susceptibility to deltamethrin were tested in the pine weevil, *Hylobius abietis* (L.) (Coleoptera, Curculionidae), a pest of young conifer plants. In constant darkness, beetles revealed a free-running circadian pattern of locomotor activity (average period 22h : 20min). Under long photoperiod, L:D 18:6, entrainment of motor activity was observed. In the entrained population of the weevils, a peak of locomotor activity occurred at about the beginning of the dark phase, and the minimum occurred in the middle of the light phase. Fluctuations of susceptibility to a standard dose of deltamethrin (0.5 $\mu$g/g body weight) varied across the day, and were inversely correlated to the changes in locomotor activity. The importance of considering the daily organization of insect biology in studying insect resistance to pesticides is re-emphasized.

KEY WORDS: *Hylobius abietis*; circadian rhythmicity; chronotoxicology; pyrethroids; video-digitizing system OXALIS.

## INTRODUCTION

Numerous physical, chemical or pathophysiological factors are known to influence insecticide toxicity, and are often controlled during toxicological studies. However, the time of administration of the toxicant is usually not controlled systematically. As early as 1963, the time of treatment was shown by Beck (1) to influence the susceptibility of insects to lethal agents This researcher found that German cockroaches, *Blattella germanica* (L.), exhibit a 24-h susceptibility rhythm when administered a standard dose of potassium cyanide at different times of the day. Surprisingly, since then not many insects have been shown to exhibit daily or circadian patterns of susceptibility to insecticides. At the end of 1997, inspection of 12 representative databases connected to Internet Database System Cambridge,[3] yielded 17 papers on chronotoxicity of pesticides, and only ten referred to chronotoxicity in insects.

Studies published on daily or circadian rhythms of insect susceptibility to pesticides stem mostly from the 1960s and 1970s. Cole and Adkisson (2) reported that boll

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[3] www.csa.com. The following databases were screened: Animal Behaviour Abstracts, AGRICOLA, Aquatic Science and Fishery Abstracts, Biological Sciences, Ecology Abstracts, Entomology Abstracts, Environmental Science and Pollution Abstracts, Health and Safety Science Abstracts, Pollution Abstracts, Water Resource Abstracts, Toxicology Abstracts, TOXLINE.

weevils, *Anthonomus grandis* (Boheman), showed a circadian rhythm in the susceptibility to methyl parathion. Both house flies (Fales normal strain) and Madeira cockroaches, *Leucophaea maderae* (F.), exhibited a circadian rhythm of susceptibility to a pyrethrum aerosol (12). Fondacaro and Butz (4) presented daily changes in susceptibility to methyl parathion in adults of *Tenebrio molitor* (L.). Ware and McComb (14) described circadian susceptibility of pink bollworm moths, *Pectinophora gossypiella* (Saunders), to azinphosmethyl. Circadian rhythms of sensitivity of the house fly to DDT, dieldrin and malathion were examined by Shipp and Otton (10). Daily changes in sensitivity to methyl parathion were also found in *Drosophila melanogaster* (Mg.) (6,8).

Among the species tested, timing of peaks in susceptibility or tolerance to insecticides varied considerably, and variation in techniques used to evaluate these changes contributed to a rather confused overall picture. As a universal rule it was suggested (10) that "the time of greatest susceptibility occurs at about the time of the onset of increased activity," but in no case was sensitivity of the insects tested in relation to the most obvious parameter of biological rhythms, namely, quantitative daily changes of motor activity. We examined this point in regard to the pine weevil, *Hylobius abietis*, an important pest of young conifer plants (7). Previously, several authors (3,11) had shown that in early summer, during gradation, adult pine weevils exhibit daily changes of locomotor activity in the field. More recently, diurnal rhythm of abundance on foliage was reported in males of the sweetpotato weevil, *Cylas formicarius* (Fab). In this species increased activity was observed during late afternoon and the night, and corresponded with increased flight activity (13).

In this paper we present an extension of the studies of pine weevil, and report that *H. abietis* shows circadian rhythmicity under laboratory conditions, by entraining its rhythm of locomotor activity to a long photoperiod (L:D 18:6). We also report daily changes in susceptibility of an entrained laboratory population of *H. abietis* to a standard dose of deltamethrin, and refer these fluctuations to both the time of day and daily changes of locomotor activity.

## MATERIALS AND METHODS

### *Insects and chemicals*

The stock colony of *Hylobius abietis* (L.) (Coleoptera, Curculionidae), originating in central Poland, was established in glass containers, at 22±1°C, 75% r.h., on defoliated twigs of Scots pine, *Pinus silvestris* (L.). The insects were provided with water and maintained at L:D 18:6 regime. The lamps (fluorescent tubes) were jacketed with cold water in order to avoid overheating. During photophase the light was maintained at 100 lux.

Deltamethrin [(1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate of (S)-alfa-cyano-3-phenoxybenzyl] of 98% purity, produced by Roussel-Uclaf, was employed. Analytical grade acetone was used as solvent.

### *Monitoring of locomotor activity*

Locomotor activity of the beetles, detected by infrared beam actographs, was monitored by means of a computerized recording unit, based on the video-digitizing system OXALIS (9). During recording, weevils were maintained at 22±1°C. Entrainment was achieved by using the same photoperiodic regime throughout all experiments: first beetles were kept in constant darkness (DD) for 10 days, and then exposed to a photoperiod of L:D 18:6 for the following 8 days. In some experiments the insects were finally maintained in a subsequent DD regime of 7–8 days' duration again.

Period lengths of the locomotor rhythm during DD and entrainment were analyzed by using OXALIS software based on fast Fourier analysis of time series. Distribution of locomotor activity during day 7 of the entraining period, was determined by OXALIS software-based analysis of individual recordings, in each individual insect separately, and expressed as number of impulses (corresponding to the number of passages of an insect through the infrared beam) produced by one insect during a 1-min interval. Changes in locomotor activity of the entrained laboratory population of 28 males and 28 females were pooled in 1-h intervals, expressed as means ±SEM, and referred to Arbitrary Zeitgeber Time. Onset of the photoperiodic dark phase was adopted as Arbitrary Zeitgeber Time point zero (AZT 0).

### *Determination of the standard dose of the pesticide*

$LD_{50}$ was routinely determined on day 7 of the entrainment, between AZT 13 and AZT 14 (*i.e.,* 12:00 and 13:00 hours local time). Doses of deltamethrin, varying between 2.6 and 0.04 $\mu$g/g of insect fresh weight, were topically applied by using a precise Sigma Sequencing Micropipette (range 0.05–3 $\mu$l, at 0.05 $\mu$l increments) and disposable precision tapered microtips. Ten doses were tested (2.6, 1.5, 1.3, 0.9, 0.6, 0.5, 0.33, 0.2, 0.08 and 0.04 $\mu$g/g), each on 20–30 insects of both sexes (ratio 1:1). $LD_{50}$ (95% confidence interval), calculated by means of POLO PC (5), was equal to 0.502 $\mu$g/g (0.425–0.587). For further tests a dose of 0.5 $\mu$g/g of insect fresh weight was used.

*Determination of daily dynamics of the pesticide toxic effect.* The daily pattern of deltamethrin toxic effect was examined as follows. The doses of 0.5 $\mu$g/g dissolved in 1 $\mu$l of acetone were applied to the ventral part of the body of the beetles at various AZT's. Control animals were treated with 1 $\mu$l solvent only. Each data point refers to a group of 20 females and 20 males. After treatment, both control and treated beetles were placed in stock culture conditions (with water and food *ad libitum*), and observed for 10 days following the application. During the photoperiodic dark phase all manipulations lasted for no longer than 5 min, and were performed in the dim red light of luminance $<1$ lux (Ilford filter No. 207, wavelength $>720$ nm). The weevils were not sensitive to short exposure to such light conditions; in a separate series of experiments we did not observe phase shift response in locomotor activity of the beetles maintained in DD, and exposed to dim red light pulses lasting for 30 min. Percent mortality (dead animals only) was calculated for every sample.

### *Statistics*

On the basis of visual inspection, two sets of data were chosen for statistical analysis: set 1 referring to hours 13–17 AZT, and set 2 referring to hours 1–12 AZT plus 18–24 AZT. Null hypothesis that there is no difference between the two sets of data was tested by Mann-Whitney test.

## RESULTS

The periods of the rhythms of the pine weevils maintained in DD and under a photoperiod of L:D 18:6 are shown in Table 1. All beetles placed in constant darkness (DD)

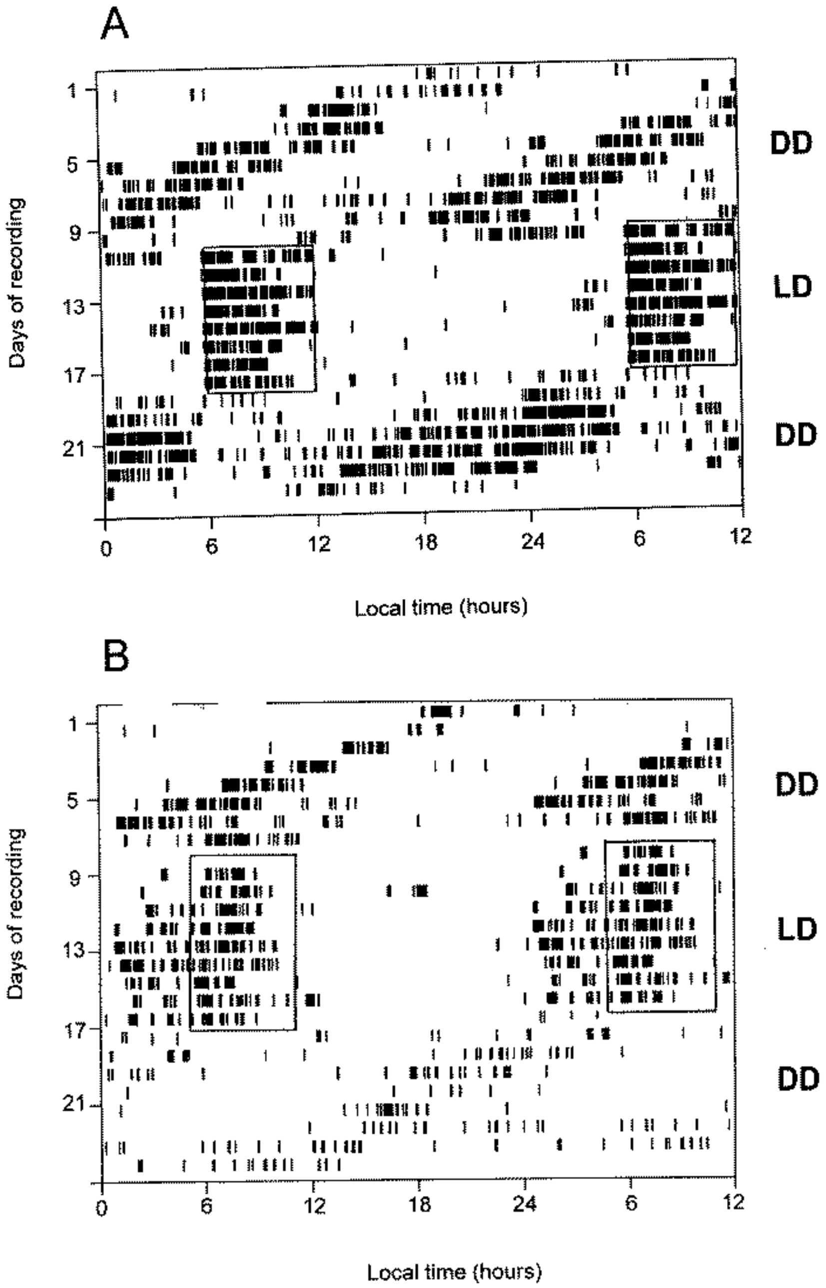

Fig. 1. Two representative event recordings of locomotor activity in adult females of *Hylobius abietis*. Data for successive days are plotted one below the other in chronological order. The records have been partially duplicated in order to provide better visual inspection of the data. The records begin with the insects maintained in constant darkness (DD), after which the weevils were entrained to L:D 18:6 (LD), and eventually exposed to DD again. Panels A and B show two different types of entrainment (see Results section for details). Scotophases are indicated by frames.

revealed a clear, free-running circadian pattern of locomotor activity. All insects entrained to L:D 18:6 revealed a clear, daily pattern of locomotor rhythmicity, in accordance with the photoperiod; however, two types of response were observed. About 60% of the insects started to walk immediately after lights-off (Fig. 1A). The remaining insects increased their locomotor activity in advance, before the end of the photophase (Fig. 1B). These two basic patterns of entrainment appear to be independent of sex, with no statistically significant difference between males and females. In DD following entrainment, the beetles again exhibited their endogenous rhythm of locomotor activity.

Fluctuations of locomotor activity in a population of 56 pine weevils, during day 7 of the entrainment period are shown in Figure 2. Beginning from AZT 16, a gradual increase in locomotor activity was observed. The peak of activity occurred at the beginning of the photoperiodic dark phase (AZT 0), followed by continuous decrease to a minimum between AZT 12 and AZT 15. Daily pattern of susceptibility to topically applied deltamethrin, in the experimental population entrained to L:D 18:6, is shown in Figure 2 as well. The period of the highest susceptibility (50%) was observed at AZT 14 and AZT 17. Then the mortality dropped, reaching 17% at AZT 23. Between AZT 23 and AZT 13 the mortality rate increased gradually. Solvent-treated insects did not show any mortality. Both the changes in locomotor activity and susceptibility to deltamethrin are statistically significant. Toxicity of deltamethrin is significantly higher between AZT 13 and AZT 17 than in the remaining part of the day (Mann-Whitney test, $P<0.0005$). Mean locomotor activity of the beetles is significantly lower between AZT 13 and AZT 17 than in the remaining part of the day (Mann-Whitney test, $P<0.001$).

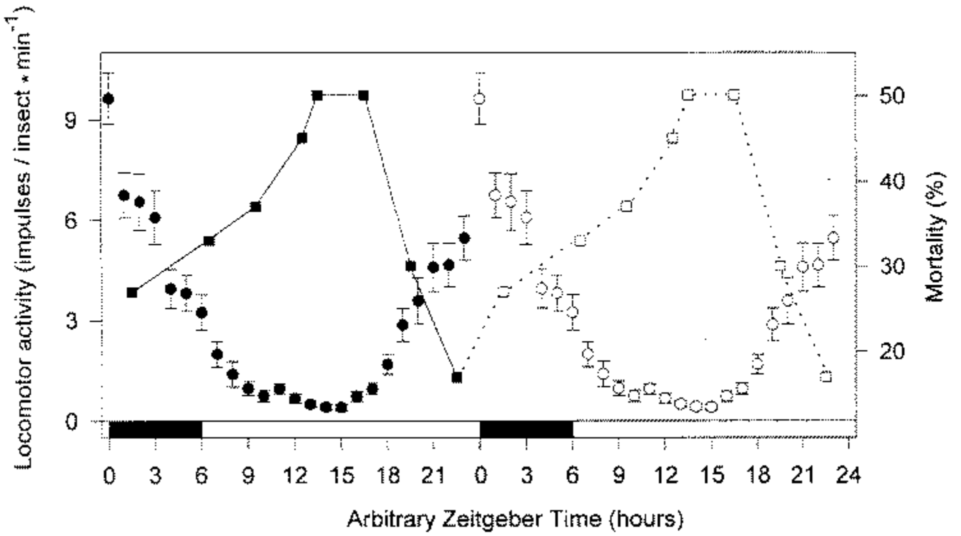

Fig. 2. Daily fluctuation of susceptibility to deltamethrin (0.5 $\mu$g/g of insect fresh weight) *vs* changes of locomotor activity in *Hylobius abietis*. In order to visualize better the changes at the end of the photophase, data are double-plotted against Arbitrary Zeitgeber Time. Percent of mortality (squares) refers to 40 adults, tested in three independent replications. Change in locomotor activity (circles) is based on 56 different individual measurements, and expressed as mean ± SEM. For each data point the sex ratio was 1:1.

TABLE 1. Periods of locomotor activity rhythm in *Hylobius abietis* maintained under constant darkness DD and L:D 18:6 photoperiod.

| Light conditions | N | Period of the rhythm (hours:minutes ±SD) |
|---|---|---|
| DD | 40 | 22:20±00:30 |
| L:D 18:6 | 56 | 23:59±00:07 |
| DD after entrainment | 40 | 22:42±00:25 |

n = number of animals tested.

## DISCUSSION

Adult pine weevils exhibit free-running locomotor rhythmicity in constant darkness and entrainment of locomotor activity to long photoperiod. The activity of a laboratory population, entrained to LD 18:6, is neither clearly nocturnal nor diurnal. Its daily active period starts around late photophase and persists throughout the entire scotophase. Our data are in accordance with previous suggestions of Eidmann (3) and Solbreck and Gyldberg (11), who reported that in the field, at the beginning of gradation, and if the weather is fine, the beetles reveal a distinct daily pattern of flight activity, with a peak in the late afternoon or early night. Changes in locomotor activity of the entrained laboratory population reflect changes of locomotor activity in *H. abietis* in the field. The time of increased activity in laboratory populations of *H. abietis* corresponds also with that in field populations of sweet potato weevil (13). Further experiments are under way in order to explain why *H. abietis* reveals two distinct patterns of entrainment.

However, our results indicate that susceptibility of pine weevils to deltamethrin varies in the course of the day, and the weevils are most sensitive at times of decreased locomotor activity. Fluctuations in mortality ratio in *H. abietis* resemble the daily pattern of susceptibility to methyl parathion in *T. molitor*, published by Fondacaro and Butz (4). The extent of changes in daily susceptibility of *H. abietis* to deltamethrin (33%) is almost equal to that reported by Sullivan *et al.* (12) for *Musca domestica* treated with pyrethrum (34%). However, these authors did not consider any functional relationship between rhythms in motor activity and susceptibility to toxic agents in flies.

In our experiments deltamethrin was administered topically; thus results were not affected by uptake of various doses of the lethal agent relative to changes in locomotor activity. Probably the differential sensitivity of *H. abietis* to deltamethrin at different times reflects daily oscillations in detoxification, translocation to the site of action, the rate of penetration or absorption of deltamethrin and/or changing responsiveness of target tissues to the pesticide. Further experiments are planned to elucidate the mechanism of the differential sensitivity of pine weevils to deltamethrin. However, at present, our finding points to the experimental relevance of circadian organization of insect biochemistry, physiology and behavior with respect to toxicological experiments with insecticides, particularly establishment of the $LD_{50}$, and studying resistance to pesticides. Moreover, we believe that in the field, susceptibility of the pine weevil to pesticides may also fluctuate in the course of the day, and a study of the appropriate timing of control measures concerning *H. abietis* remains desirable, particularly if the pesticide’s operative potential does not persist for longer than several hours.

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