

RESPIRATORY FAILURES IN ADULT COLORADO POTATO BEETLES EVOKED BY NEEM PREPARATION

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*The influence of neem preparation (NeemAzal TS) on the breathing mode in the adults of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), was studied by means of a respirometer-actograph and an infra-red gas analyzer. Both respirometry were combined with an infra-red optical system allowing to record external gas exchange cycles, standard metabolic rate (SMR), and respiratory movements simultaneously. The beetles treated with nonlethal doses of neem lost their ordinary and regular discontinuous gas exchange cycles (DGCs) – the release of carbon dioxide by discrete bursts and abrupt air intakes into tracheae. The DGCs in treated beetles did not restore after the recovering of the beetles from the mild poisoning. Nevertheless, at low temperatures between 5 and 8 °C the treated beetles displayed the typical irregular microcycles of gas exchange. Authors suggest that the respiratory failures were not due to the paralyzing of the spiracles by neem, but affected were rather the neuromuscular, humoral and neuro-hormonal centers involved in the regulation of the external cyclic gas exchange.*

Key words: neem, respiration, cyclic gas exchange, Colorado potato beetle.

Introduction

At present time it has been well documented that neem preparations reveal several distinct modes of action: feeding deterrence, Insect Growth Regulation, oviposition deterrence, repellency, adult sterilization, the disruption of mating behaviour, the inhibition of chitin biosynthesis, and, usually mentioned in the last place, the direct toxicity or poisoning of larvae and adults due to contact immediate action on some insect species. The delayed action on insects is typical of neem.

By our knowledge the effects evoked by neem on the respiratory system of insects have not yet been studied. Insect respiration is an established index of stress imposed by insecticides. There exists an enormous literature concerning the effects of various chemicals compounds on oxygen consumption rates of insects. The respiration may be inhibited or, in other cases accelerated (Kuusik et al., 1983). Commonly the toxicants cause in insects indirect action on respiration via the excitation of the neuromuscular system resulting in enhanced respiration rates. In rare cases the toxicants induce the cytochrome system causing direct stimulatory effects on respiration.

Together with the the direct and indirect influences on oxygen consumption rated the toxic substances may also cause respiratory failures in insects abolishing the discontinuous gas exchange cycles or DGCs. The DGCs mean that insects consume oxygen and release carbon dioxide not continuously but by characteristic cycles – by abrupt intakes of oxygen followed by carbon dioxide release as discrete burst. The cyclic gas exchange has been regarded as the main water conserving mechanism in many insects (Kestler, 1980, 1985; Miller, 1974; Lighton, 1994; for reviews). The DGCs and gas exchange microcycles are the characteristic breathing mode in

quiescent adults of Colorado potato beetle (Kuusik, 1976; Kuusik, Kogerman, 1983; Metspalu et al., 2001).

By some earlier data certain synthetic Insect Growth Regulators, the analogues of juvenile hormones of juvenoids, cause irreversible changes of external gas exchange: the insects treated with nontoxic and even very weak doses of juvenoids lost their normal cyclic gas exchange resulting in increased transpiration rate (Kuusik, Kogerman, 1978; Kuusik et al., 1980). In these insects no other symptoms of direct toxicosis were found. The typical DGCs were also lost in some insect species treated with extracts from certain concentrations resulted in fully abolishment of cyclic gas exchange or the so called discontinuous gas exchange cycles. During the first step of poisoning the loss of DGC was due to ventilating effect of muscular irregular contractions. Later, after the passing of the muscular hyperactivity the breathing of the beetles occurred by regular muscular ventilating due to abdominal regular pumping movements. After some hours the active ventilating movements ended and now the fully restrained beetles showed the continuous gas exchange through the continuously opened spiracles. The normal DGCs were entirely lost and did not restore at all.

It was remarkable that at low temperatures, between 5 and 8 °C, when the moving activity of beetles was suppressed, the treated beetles displayed the typical irregular microcycles of carbon dioxide release and oxygen uptakes (Fig. 2). Thus the neem application affected only the regular and larger cyclic emission of carbon dioxide, while the microbursts of this gas were preserved at low standard metabolic rates.

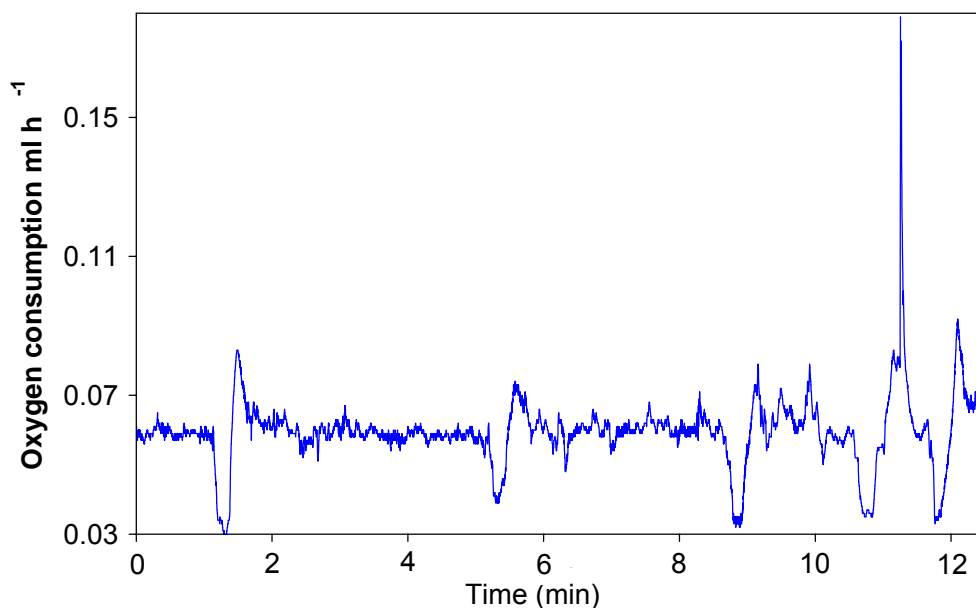


Figure 2. The gas exchange microcycles of an adult Colorado potato beetle registered by electrolytic respirometer-actograph

The DGCs in Colorado potato beetles are coupled with the opening-closing rhythms of the subelytral cavity – an airfilled space between elytra and abdominal tergites (Fig. 3). The abdominal spiracles open into the humid subelytral cavity, and thus this cavity helps lower the respiratory transpiration (our unpublished data). Normally the DGCs are synchronized with the opening-closing rhythms of subelytral cavity. In the beetles treated with neem the subelytral cavity was continuously left semi-open while no regular closing rhythm was to be seen.

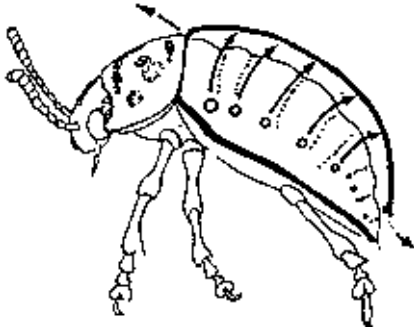


Figure 3. A scheme of the subelytral cavity of adult Colorado potato beetle

Discussion

The discontinuous gas exchange cycles are functioning in insects as a water-conserving mechanism (Kestler, 1980). This was the most probable reason why the neem-treated beetles rapidly lost their body mass. It was evident from these experiments that one of the immediate and externally invisible side effects of the neem were the failures in the gas exchange resulting in increase transpiration and desiccation of insects. The abolishing of the cyclic gas exchange may be regarded as the earliest symptom of poisoning by toxicant in insects (see Kestler, 1991).

It may be suggested that disturbances of DGCs are due to the paralyzed opening-closing mechanisms of the spiracles. However, there also exist other factors abolishing the normal DGCs. Desiccation and very intensive handling stress lead to the same pathological carbon dioxide release pattern as the insecticide (see Kestler, 1991; Kuusik et al., 1999; Harak et al., 1998). By our results the closing-opening mechanism of the spiracles was most susceptible target to the neem in insect body. By our results the the gas exchange microcycles were preserved in neem-treated beetles indicating that the opening-closing mechanism of spiracles were not paralyzed, and thus other affecting factors were involved.

The mechanism by which insects lose their DGCs may either be autointoxication or/and release of neurohormones, or by an influence on the O_2/CO_2 thresholds for the control or on the CO_2 capacitance due to changes in the acid-base status (Kestler, 1991). Thus we conclude that the loss of the DGCs was not the result of a toxicosis evoked by neem (using here the term toxicosis in a strict sense). By our results the mechanism of spiracular regulation is the most susceptible target of the nontoxic doses of neem.. It seems most likely that the primary cause of the respiratory failures described in present work is the indirect effect of neem modulated via the insect endocrine, neurohormonal and neuromuscular centersd.

There are well documented the evidence supporting azadirachtin's ability to to interference with neuroendocrine control of metamorphosis in susceptible insects (reviewed by Addor, 1994; Mordue, 1998). The neem classified as Insect Growth

Regulator really acts as a Insect Growth Inhibitor. Azadirachtin inhibits prothoracicotropic hormone (PTTH) in corpora cardiaca reducing or delaying ecdysone release necessary to trigger moulting.

The treatments of the last instar larvae and early pupae of Colorado potato beetles with nonlethal neem preparation doses always results in the defective metamorphosis – the emerged adults possess morphological failures on elytra. Besides, these adults display an unclear and irregular gas exchange pattern. By Pelletier (1994) the adult Colorado potato beetles with elytra deformation show high mortality rate after exposure to dry conditions during pupal stage. The beetles with deformed elytra as a rule show a high mortality rate during hibernation (our unpublished data) evidently due to the abolishment of the water conserving mechanisms – cyclic gas exchange associated with the functions of subelytral cavity (see Cloudsley-Thompson, 1964).

Acknowledgements: This research was supported by grant no. 3535 from the Estonian Science Foundation

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