A DIFFICULTY IN REARING RHODNIUS PROLIXUS Stahl.

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Usually no difficulties are experienced in maintaining cultures of the blood sucking bug **Rhodnius prolixus,** and usually there is a virtual absence of chance or unexplained deaths so prevalent in some other laboratory insects. This is one of the most pleasing attributes of this insect as an experimental animal, and more than compensates for the tedium of having to feed them in relatively small numbers upon a live rabbit.

This advantage seemed to have been lost when, in January 1958, a malady of unknown aetiology appeared, which in two months killed some four-fifths of a culture of perhaps five thousand specimens at this laboratory. The day to day incidence of the malady was unpredictable and afforded no evidence on which to base a rational experimentation to determine the cause of deaths. After two months the malady rapidly diminished in incidence and then disappeared for nine months, to reappear in equally devastating incidence in January 1959.

At each outbreak the malady was characterised by the sudden death of all or a portion of the 20-120 individuals in each rearing jar, often immediately after that culture had been fed. All instars were affected, but adults appeared particularly susceptible. At both outbreaks all adults were lost within two weeks and repeated attempts to re-establish the breeding stock, failed. When during these outbreaks fifth instar larvae were successfully fed and adults reared, some died within a few days of moulting and the rest always succumbed at or shortly after the first feeding. When the malady passed, adults were reared and maintained without incident.

At the second outbreak all breeding adults, all unfed first instar larvae, and considerable numbers of recently fed nymphs were lost in the few days before the outbreak was discovered. No further routine feeding of cultures was allowed and this curtailed further losses although considerable unpredictable losses still occurred amongst material being fed and used to unravel the problem. At this stage, examination of affected cultures revealed a heavy incipient infestation of the mite **Pymotes ventricosus** (Newport). However this infestation was not unexpected in view of the generally accepted role of this mite in infesting insect cultures which are moribund or unhealthy. To limit this infestation specimens were transferred to clean jars standing in moats of paraffin oil,

utensils were heat sterilised immediately before and after use and barrier plates coated with paraffin oil were installed between each deck in the incubator. As a further precaution the working area of the bench was swabbed with alcohol before cultures were handled. These measures have been entirely successful and **Pymotes** infestation has not since been detected in stock cultures.

About the time **Pymotes** infestation was discovered some 20 recently emerged unfed adults were placed in a clean jar in an incubator. All but one were dead within 48 hours. On examination numbers of slightly swollen **Pymotes** were found on each dead **Rhodnius** but none on the survivor. Eleven recently emerged adult **Rhodnius** were added to the jar with the single survivor. The following morning four specimens were dead. Each dead specimen had mites, the live specimens were mite free.

Experiments have established that **Rhodnius** adults readily succumb to this mite. One mite only is required. On many occasions a single non-gravid female mite has been transferred to an individual adult **Rhodnius.** All the results are consistent. In about two thirds of the transfers the insect succumbs. Those that survive the first mite, succumb in about the same proportion when individual mites are again transferred and no evidence has been obtained to suggest that some individuals are resistant to mite attack. Rather it would seem that about one third of the transferred mites are dislodged or suffer similar accidents before a suitable point of attachment is found.

The effect of the mite is surprisingly rapid. The first insects are fully paralysed within 2.5 hours after the mite is transferred, half have succumbed by five hours and about all within a day. This is an entirely different scale of effects to that described by Swan 1934 for the attack of this mite on larvae of the grain moth **Sitotroga cerealella** where "if a number of mites attack a single larvae it may be immobilised in about 24 hours; with few mites the period is longer". Whether **Rhodnius** adults are particularly susceptible, or the strain of mite particularly virulent has not yet been investigated.

There are no signs to indicate the early stages of attack. If undisturbed, the insect becomes progressively quiescent until it falls from its footholds to lie fully immobilised at the bottom of the tube. Characteristically, the legs are then fully outstretched as in the usual resting position. If disturbed before paralysis is complete, the insect is unable to progress or progresses erratically, the righting reflex is ineffective and the insect is unable to regain the vertical position in which it usually rests. If such insects are stimulated by puffs of air or by prodding, leg tremors are seen, but these tremors are minor in both magnitude and duration. Later,

when paralysis is complete not even micro-tremors can be detected in the appendages, although the heart continues to beat, and the crop, gut and rectum continue their peristaltic movements. If the mite is removed the insect may continue in this state for weeks and months, death, when it ultimately occurs, being readily attributable to exhaustion of metabolic reserves or to desiccation, that is, to factors consequent upon the paralysis rather than to the paralysis itself.

The evidence suggests that this paralysis is brought about by a toxin injected by the mite into the insect haemolymph, for similar paralysis occurred when haemolymph from mite paralysed **Rhodnius** was injected into fresh adults. When the dose injected was much above threshold, the insects became fully paralysed and did not recover. With a dose about three times above threshold, full paralysis was reached about a week after injection and persisted for approximately three weeks when the insects began to recover. After a few days of sluggish behaviour these specimens could not be distinguished from normal adults, indicating complete recovery from the toxin.

That this toxin must be extraordinarily potent is immediately apparent from the relative size of mite and insect. The insect weighs about 100 mg. The mite is too small to weigh directly but an approximate weight of about 0.5 micrograms can be computed from the measurements, which in my culture, are approximately 0.22 mm. long by 0.086 mm. wide by, perhaps, 0.025 mm. thick. Even assuming that one tenth of the mite is toxin (and this seems most unlikely) the dose involved is only 0.05 micrograms and the ratio of toxin to **Rhodnius** is as 1:2,000,000. Yet haemolymph taken from mite paralysed **Rhodnius** will effectively paralyse further **Rhodnius** after overall dilution which is certainly more than 1:50. These figures indicate that the toxin is effective at more than 1:100,000,000 which is 0.01 mg. per kilo. No insecticide is known which even approaches this potency, and amongst other poisons, **Botulinus** toxin, usually considered the most toxic material (Lamanna, 1959) is only 1000 times as potent!

The effect of the mite on immature **Rhodnius** has been investigated, but in less detail. All stages succumb, but whether fed, fasting or starved, none are as susceptible as the adult. When single non-gravid female mites were transferred individually to fasting third, fourth or fifth instar nymphs, two and occasionally three in each dozen would succumb within twenty-four hours. Reinfesting with single mites, killed one or occasionally two of the survivors of the first transfer. After five such reinfestations two-fifths of the nymphs were still alive. Essentially the same result was obtained when mites were transferred to recently fed third and fourth instar nymphs.

The earlier instars are also readily killed by a single female mite. However, these small insects appear to have an acute awareness of the presence of the mite. The nymph postures, rubs the fore tarsi together and if the mite is in that vicinity, repeatedly brushes the side of the abdomen with the hind tarsi. Often the mite is dislodged in less than twenty seconds after the transfer is made. The chances of the dislodged mite finding its host again would seem rather remote but apparently this sometimes occurs. When the smaller nymphs are killed the mites do not attach themselves to the prostrate bodies. Hence examination of the prostrated material fails to reveal the presence of mites and affords no clue as to the causal agent of death.

When mites were detected in the **Rhodnius** culture at the second outbreak, it was obvious that although hundreds of mites were in the grossly distended condition reached after several days feeding, none were sufficiently old to be producing young. This indicated that the infestation was an invasion. The source was easily located as a stock culture of **Stegobium paniceum,** which for convenience and a little warmth, was kept on top of the **Rhodnius** incubator. This culture had become very heavily infested, and vast numbers of non-gravid female mites had migrated from this focus, over and through the **Rhodnius** incubator. Hence it is now clear that the mites which affected the **Rhodnius** culture were not breeding in that culture. Nor does it appear likely that such breeding would occur. In the normal course of maintaining the culture any dead or moribund insects are soon discarded and the risk of chronic mite infestation is greatly reduced by the practice of using clean papers and jars at every feeding. However, serious contamination from other insect cultures is an ever likely occurrence and in particular it would appear to be unwise for a **Rhodnius** culture to share an incubator with cultures of insects known to be particularly susceptible to attack, for example **Sitotroga cerealella** (Swan 1934) **Calandra granaria** (Gay and Greaves 1942) and now **Stegobium.**

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