THE PATHOGENICITY OF AN ENTOMOPOXVIRUS FROM
OTHNONIUS BATESI [COL.: SCARABAEIDAE]
AND ITS POSSIBLE USE AS A CONTROL AGENT (1)

R. J. MILNER & G. G. LUTTON
CSIRO, Division of Entomology, Armidale, N.S.W., 2350, Australia

In a natural population of Othnonius batesi Oll., at Glen Innes, N.S.W.,
4.0 % of larvae in 1972 and 2.2 % in 1973 were exhibiting symptoms of virus
infection, whilst 0.8 % of pupae and adults from the same population were infected
in 1972. These figures, and field observations of infected larvae, suggested that the
pathogenicity of the virus was low. In laboratory experiments with O. batesi the
infection had little effect on mortality, and no significant effect on duration of
the first instar, food intake, or larval growth. The vast accumulation of virus in the
fat body probably results in mortality prior to, or during, the pupal period.
Temperature had a marked effect on both % infection (optimum 30°C) and
production of viral spheroids (optimum 25°C). Very little viral development
occurred below 20°C. In a host range study only O. batesi, Rhopaea verreauxii
BLANCH. and R. morbillosa BLAB. were infected per os. The possible use of the
virus as a control agent is discussed.

Viruses are the safest of all microbial agents proposed for insect control (HEIMPEL,
1971), however work on insect poxviruses as microbial control agents has been inhibited
by fears of their safety since morphological and biochemical similarities to vertebrate
poxviruses exist (BERGOIN & DALES, 1971). To date only one insect poxvirus, that
from Choristoneura fumiferana (CLEM.), has been used experimentally in a biological
control programme which included safety testing. In this work (BUCKNER & CUNNING-
HAM, 1972), small mammals and birds were studied, both in the laboratory and the
field, but none showed any ill effects due to virus. The authors concluded that the
poxvirus is a "safe insecticide from the point of view of homoeotherms".

The first insect pox virus to be described was that from Melolontha melolontha L.
(HURPIN & VAGO, 1963) and an extensive literature now exists on the morphology
(BERGOIN et al., 1969, 1971; DEVAUCHELLE et al., 1971), the biochemistry (BERGOIN et al.,
1970; POGO et al., 1971) and epizootiology (HURPIN & ROBERT, 1967, 1969; ROBERT,
1965, 1968, 1969) of this virus. In field experiments the virus was successfully established
in a population of M. melolontha and remained in the soil (HURPIN & ROBERT, 1972).
The authors concluded that the virus was more promising as a control agent than milky
disease, rickettsia or Adelina but was less promising than Beauveria tenella. In recent
years many more insect pox viruses have been described and in a recent review GRANADOS
(1973) lists 22 viruses from 4 insect orders: Coleoptera, Lepidoptera, Diptera and
Orthoptera.

at Armidale, August 26-30, 1974.
Othonius batesi OLL. is a serious pest of wheat and pastures in the black soil region of northern N.S.W., and an insect poxvirus was first described from O. batesi in 1969 (Goodwin & Filshie). This initial study was confined to morphology but later, preliminary studies on pathogenicity were carried out (Goodwin & Roberts, 1975). A dose of $1.25 \times 10^5$ spheroids/larva was force fed and after 112 days at 21-27°C only 2 out of the 70 treated third-instar larvae were infected. As part of a programme, to evaluate candidate microbial control agents for pasture insects in Australia, further studies on the pathogenicity and host range of this virus have been carried out.

MATERIALS AND METHODS

O. batesi larvae infected with entomopoxvirus are easily recognised (Goodwin & Roberts, 1975) and were collected from a site three miles east of Glen Innes, N.S.W. The diseased fat body was removed aseptically and homogenised with physiological saline. Partial purification was obtained by centrifugation and the suspension stored at about 4°C. To estimate the dose, the number of spheroids were counted in a haemocytometer.

All larvae were either collected in the field or were hatched from eggs laid in the laboratory by adults collected in the field. The larvae were reared individually in cubicles of ice-cube trays with peat as a substrate and sliced carrot as food. The food and peat were replaced at 1 or 2 weekly intervals. Whilst peat is ingested by larvae little or no nourishment is obtained from it.

In the injection experiment larvae were given about 5 µl of the freshly prepared virus suspension containing a large but unknown number of both free virus particles and spheroids.

The susceptibility of O. batesi larvae to virus per os was studied in 2 experiments. Experiment 1 was designed to study the effect of temperature on the disease. Five constant temperatures, 15, 20, 25, 30 and 35°C, and five decimal doses ($3 \times 10^3$ to $3 \times 10^7$ spheroids/larva) were used. Second-instar O. batesi larvae were fed known doses of virus on pieces of carrot and to qualify for inclusion in the experiment each larva had to consume the entire piece of treated carrot in 7 days. The average number of larvae per dose was 24, 24, 45 and 21 for 15, 20, 25 and 30°C respectively. At 35°C the experiment was set up only with 24 larvae fed $3 \times 10^7$ spheroids and 24 control larvae. Due to incubator failure the larvae at 15°C were transferred to 17.5°C after 16 weeks. The larvae were examined weekly for 30 weeks after which all survivors were examined for infection.

Experiment 2 was designed to test the hypothesis that first instar larva might be more susceptible. The carrot feeding technique is not suitable for these small larvae, consequently infection was obtained by mixing known numbers of spheroids into peat. Five decimal doses ($7 \times 10^1$ to $7 \times 10^5$/g dry wt. of peat) and an average of 16 larvae/dose were used. The larvae were incubated at 23°C and were examined every 14 days. After 12 weeks the contaminated peat was replaced by uncontaminated peat and the experiment continued for a further 12 weeks, by which time many larvae had developed symptoms of virus infection. Observations indicated that the infection was having little effect on food intake or larval growth so all surviving larvae were assessed for food intake and growth (wet weight) for an 11-day period.

Food intake was measured by giving each larva a standard cylinder of carrot and 3 or 4 days later the amount eaten was estimated by a visual scoring system. Growth was measured by weighing each larva before and after the 11-day period. Finally, after 24 weeks the fat body was removed from each larva, homogenised in a constant volume