The Great Lakes Entomologist

Volume 15
Number 2 - Summer 1982 *Number 2 - Summer 1982*
Article 2

June 1982

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M. A. Mohamed
University of Wisconsin

H. C. Coppel
University of Wisconsin

J. D. Podgwaite
Northeastern Forest Experiment Station

BIOASSAY OF THE NUCLEOPOLYHEDROSIS VIRUS OF *NEODIPRION SERTIFER* (HYMENOPTERA: DIPRIONIDAE)[1]

M. A. Mohamed,[2] H. C. Coppel,[2] and J. D. Podgwaite[3]

ABSTRACT

Linear regression analysis of probit mortality versus several concentrations of nucleopolyhedrosis virus of *Neodiprion sertifer* resulted in the equation Y = 2.170 + 0.872X. An LC_{50} was calculated at 1758 PIB/ml. Also, the incubation time of the virus was dependent on its concentration.

Most insect viruses possess the potential of causing 100% mortality when employed against some pest species populations (Bailey 1973). However, Franz (1964) pointed out that such a result is not always desirable. For instance, if the virus proves persistent and capable of being transmitted by the target species, one would ideally like a small section of the population to survive, serving as a focus for future epizootics. Not only would this serve to reduce the environmental load of virus, if continuous application of virus was planned, but it would also serve to ensure that predator and parasitoid populations would remain intact. Though studies on mortality as a function of virus concentrations are only one of many aspects ensuring proper usage of these pathogens, they give us some predictive basis to achieve some of the aforementioned results. This paper reports on two aspects of the nucleopolyhedrosis virus (NPV) of *Neodiprion sertifer* (Geoffroy): first, the LC_{50}; and second, mortality as a function of time at fixed concentrations.

METHODS AND MATERIALS

Eggs of *N. sertifer* were obtained from red pine plantations which had no previous history of the presence of NPV of this species. Shoots with egg masses were trimmed and clipped under water. They were then transferred to waxed 1-pt ice cream containers containing water. The units were then covered with lantern globes, the bases of which were sealed with tape and the tops covered with cheese cloth to permit adequate ventilation. The eggs were allowed to hatch and the units were monitored until the larvae were in the second instar. Each pine shoot was cut at regular intervals, and immersed in fresh water to prevent fungal growth.

Fresh shoots of red pine were trimmed to a standard size so that they could fit into the lantern globes. These shoots were then transferred to water filled, wax-coated, 1-pt, ice cream containers and were then sprayed with known concentrations of purified polyhedral inclusion bodies (PIB) obtained from diseased larvae of *N. sertifer*. The foliage was sprayed from several angles with a hand-held atomizer using short bursts to ensure that a fine mist covered all the needles. One ml of each of the following concentrations of PIB/ml of distilled

[1]Research supported by the College of Agricultural and Life Science, University of Wisconsin-Madison, in part by the Wisconsin Department of Natural Resources through the School of Natural Resources, and in part through funds provided by the USDA Forest Service, Northeastern Forest Experiment Station, cooperative study 4500-FS-NE-2202.77.

[2]Department of Entomology, University of Wisconsin, Madison, WI 53706.
[3]Northeastern Forest Experiment Station, Hamden, CT 06514.

water were used: 0, 25, 100, 250, 2500, and 25000. These concentratons were prepared by serial dilution from a stock solution of 2.5×10^8 PIB/ml. Each concentration was replicated twice.

The foliage was allowed to dry for an hour, at which time 50 second instar larvae were transferred onto it with sterile forceps. A total of 12 units was thus employed. Larvae were monitored daily for 21 days or until larvae reached the prepupal stage. Larvae that showed symptoms of NPV-induced death or that did not respond to gentle probing were removed daily and stored singly at 0°C. These were later examined for the presence of inclusion bodies. The daily and cumulative mortalities were recorded for further analysis.

RESULTS AND DISCUSSION

The results of the mortality induced at various concentrations are shown in Figure 1. No mortality was observed in the controls (0 PIB/ml) and subsamples of larvae that died from exposure to PIB at the various concentrations were shown to be virally induced through examination of macerates with bright field microscopy of 600X. The percent mortality was transformed to probit values and plotted against log concentrations of PIB. Using Finney's

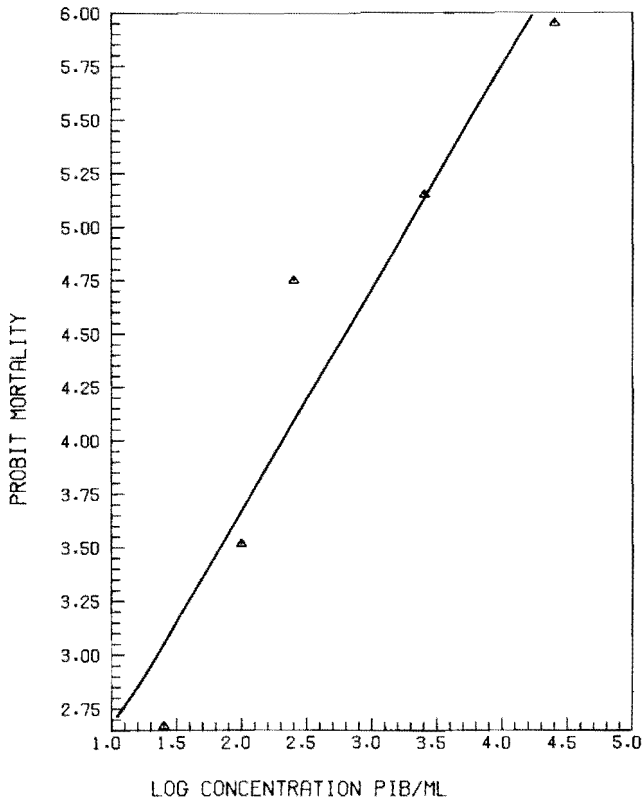

Fig. 1. Regression line fit on a plot of percent mortality versus log concentration of nucleopolyhedrosis inclusion bodies of *Neodiprion sertifer*.

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DOI: 10.22543/0090-0222.1417

(1964) method, the regression equation $Y = 2.170 + 0.872X$ was calculated. The slope of the equation, 0.872, S.E. ± 0.11, was significant at $P < 0.005$, df = 3. The Chi-square test for heterogeneity resulted in $\chi^2 = 9.76$ 3df, $0.02 <$ pval. < 0.05. From this fit an LC_{50} and its 5% fiducial limits were calculated as 1757.92 (1625.55–1901.07). The LC_{50} reported here compares favorably with Dubois' (1976) value of 1210 PIB/ml using NPV decontaminated by sodium omadine and washed with distilled water. The data were extrapolated to LC_2, or the concentration required to kill one larva, and gave a value with 5% fiducial limits of 6.76 (6.25–7.31) PIB/ml.

A plot of daily mortality, averaged over two replicates, at three different doses is shown in Figure 2. The incubation time before the virus expresses itself seems dependent on dosage, taking nine days at 250 and 2500 PIB/ml, and seven days at 25000 PIB/ml. It could also be inferred that the rate of mortality is greatest at the highest concentration when compared to that at 250 and 2500 PIB/ml. The data also show that the peak mortality period was 10–12 days at 25000 PIB/ml, 14–16 days at 2500 PIB/ml, and 16–18 days at 250 PIB/ml. These data are consistent with Bird's observations (Cunningham and Entwistle 1981) that as concentration decreased the time for the expression of 100% mortality increased.

Though extrapolation of laboratory results to field situations is a hazardous undertaking, it nevertheless furnishes a basis for mortality predictions. For instance, the regression equa-

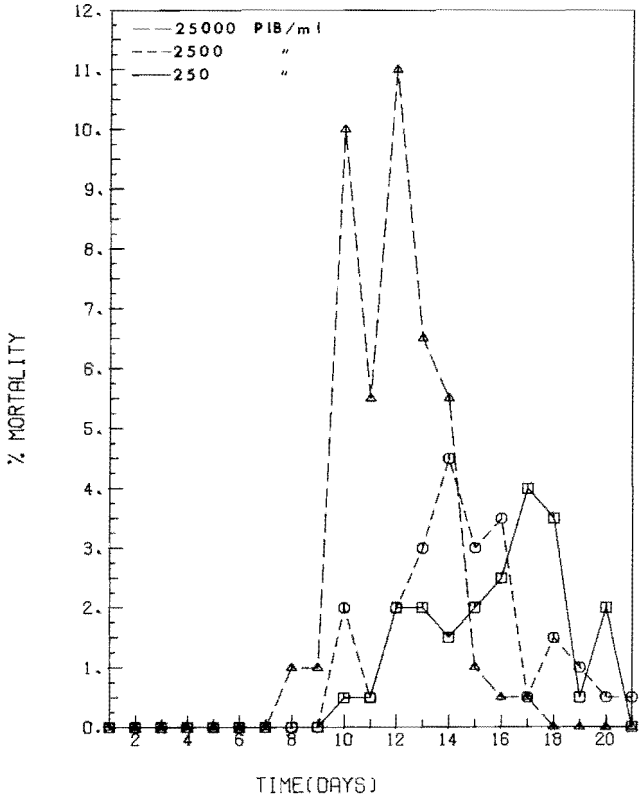

Fig. 2. Plot of percent mortality versus time induced at three concentrations of nucleopolyhedrosis virus of *Neodiprion sertifer*.

tion for the probit mortality versus concentration predicts an LC_{99} of 7.59×10^5 PIB/ml which compares favorably with 100% mortality observed in field plots sprayed with virus at a concentration of 4.6×10^5 PIB/ml. Also, from our field studies, peak mortality was observed after 15 days which is well within the range observed in the laboratory at 2500–25000 PIB/ml.

Thus, depending on our concern, whether it be preserving beneficials, minimizing environmental load of the virus, or furnishing future sources for virus epizootics, it is possible to manipulate the NPV of *N. sertifer* to achieve these ends.

ACKNOWLEDGMENT

The authors gratefully acknowledge the technical assistance of Odette Mohamed.

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