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Time Trends in Mortality of *Conophthorus ponderosae* (Hopkins) Exposed to Insecticide Residues

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The protection of the female cones of *Pinus monticola* (Dougl. ex D. Don) from attack by *Conophthorus ponderosae* (Hopkins) requires that the beetles be killed before they enter the cone. This encourages consideration of the distributions of length of time to death and how these distributions are changed with different concentrations of various candidate insecticides. In this study a three-parameter Weibull function was employed to depict the distributions for data originally collected for a probit analysis. The time to achieve 90% mortality as defined by the Weibull function was taken as a dependent variable, and a regression evaluation was used to define relationships with concentration for selected insecticides. The mode $Y = b_0 + b_1 \cdot X^{-1}$, in which $Y = LT_{90}$ and X = insecticide concentration in g litre^{-1}, proved useful when a sufficient range of concentrations was included in the testing.

1. Introduction

Beetles of the genus *Conophthorus* are among the major pests of female cones of *Pinus* species. These beetles overwinter as adults in cones or branch tips, emerge in spring, and attack the developing second-year female cones. Female beetles bore into cones through the petiole or basal scales, completely severing the conductive tissue in as few as 30 min. As soon as the conductive tissue is severed, the cone is functionally dead and will not continue to develop. After laying the initial complement of eggs in a gallery along the cone axis, the female may leave the cone and attack one or more additional cones. Several broods per season, but only one generation per year, are usual.[1–3]

For effective control, female beetles must be killed or incapacitated before they can sever the conductive tissue or injure the developing cone. The control strategy with the highest probability of success would use a contact insecticide that acts rapidly to kill the female beetles or to render them ineffective, and has a substantial residual life, or can be reapplied one or more times during the flight season of the beetle.

To identify fast-acting insecticides, a laboratory bioassay was done to determine the response of *Conophthorus ponderosae* (Hopkins) (synonymous with *C. monticolae*) to residues of 11 insecticide formulations.[4] Probit analysis was used to develop response lines based on mortality counts made after 12 and 24 h. The LC_{90} after 12 h was used to select application rates for a field experiment to protect the cone crop in a western white pine [*Pinus monticola* (Dougl. ex D. Don)] seed orchard from attacks by *C. ponderosae*.[5]

During the laboratory experiment, observations were made in addition to those after 12 and 24 h to quantify time trends in beetle mortality. The three-parameter Weibull function was used to describe the frequency distribution of time to change-of-state (from alive to moribund or dead). Throughout this paper, this change-of-state will be referred to simply as death, but the term is intended to cover the moribund condition.

Originally developed for use in reliability testing, the Weibull function has more recently been adopted for use in seed viability testing,[6] for describing insect growth and development,[7] for evaluating age-specific mortality of birds and mammals,[8] and in insect toxicology.[9] Whereas probit

analysis estimates the dose of insecticide required to cause a given level of mortality at a fixed time, the Weibull function was used to estimate the proportion that died by a given time with a fixed dose. Specifically, the time to death was viewed as a random variable with a Weibull probability distribution. By this approach, a function $F(x)$, expressing the proportion that have died by a given time x since treatment, is defined. For the three-parameter Weibull function,

$$F(x) = 1 - \exp\{-[(x - a)/b]^c\} \qquad \text{for } x \geq a$$

The three parameters can be interpreted as follows: (*i*) the *a* (location) parameter is the earliest time beyond which the probability of death rises above zero; (*ii*) the *b* (scale) parameter is the additional time beyond time *a*, during which approximately the first 63% of the deaths occur; and (*iii*) the *c* (shape) parameter is concerned with the symmetry of the distribution. More background on the Weibull distribution, with particular reference to entomological experiments, is available.[9]

2. Methods and materials

2.1. Insecticides and bioassay procedure

Insecticides used in this experiment were chlorpyrifos ('Dursban', 0.48 kg a.i. litre^{-1} e.c.); diazinon ('Spectracide', 0.48 kg a.i. litre^{-1} e.c. and 'Knoxout' 2FM, microencapsulated at 23% a.i.); fenitrothion ('Sumithion', 0.96 kg a.i. litre^{-1} e.c.); gamma-HCH ('Isotox', 0.20 kg a.i. litre^{-1} e.c.); parathion-methyl ('Pencap-M', microencapsulated at 22% a.i.); permethrin ('Pounce', 0.38 kg a.i. litre^{-1} e.c.); resmethrin (microencapsulated at 3% a.i.); and fenvalerate ('Pydrin', 0.29 kg a.i. litre^{-1} e.c.).

All insecticides were formulated in distilled water. For each insecticide formulation, eight concentrations (Table 1) were prepared individually in small volumes (5–10 ml). One millilitre of each concentration was applied to a separate piece of filter paper (Whatman) in the lid of a 90×15 mm plastic Petri dish, and allowed to dry. Filter papers treated with the same insecticides were placed in a plastic bag and stored in a freezer (−3.6°C) until used.

Adult male and female beetles were collected in cones from the white pine seed orchard in Sandpoint, Idaho, USA. Beetles were removed from the cones by hand, placed in Petri dishes lined with moistened filter paper, and held for 1 to 2 days at 5°C until sufficient numbers had been collected to complete one replication. Twenty beetles were placed on the treated filter paper within the Petri dish lid. A wide-mouth canning jar lid (8.5 cm diam.) with a wire screen insert was placed on top of the filter paper to contain the beetles on the treated filter paper and also to provide ventilation. To prevent insecticide vapours from accumulating in the air space immediately above the treated filter paper, the beetles were exposed to the insecticide residues in an operating fume hood.

Table 1. Concentration of nine insecticide formulations used in the experiments

Insecticide	Concentration (g.a.i. litre^{-1})[a]							
	A	B	C	D	E	F	G	H
Resmethrin	0.036	0.060	0.084	0.108	0.18	0.24	0.30	0.48
Permethrin	0.036	0.096	0.12	0.18	0.24	–	–	–
Fenvalerate	0.048	0.060	0.084	0.096	–	–	–	–
Gamma-HCH	0.024	0.072	0.108	0.30	0.48	0.72	1.20	–
Chlorpyrifos	0.072	0.096	0.24	0.42	0.60	0.72	–	–
Fenitrothion	0.60	0.84	0.96	1.44	1.80	2.04	2.40	–
Diazinon	0.24	0.42	0.48	0.60	0.84	0.96	1.20	1.80
Diazinon (encap.)	0.108	0.18	0.24	0.30	0.36	–	–	–
Parathion-methyl	0.24	0.30	0.34	0.36	0.42	0.48	0.54	0.60

[a] Experiments were conducted by selecting the concentrations originally as lb per 100 US gallons of water. Some of the concentrations resulted in mortality patterns that precluded their use in the three-parameter Weibull function analysis.

Dead and moribund insects were removed and their number recorded 2, 4, 6, 8, 10, 12, 16, 20 and 24 h after placement on the paper. A beetle was considered moribund (irreversibly approaching death) when it could no longer right itself and walk.

2.2. Experimental design and data analysis

The experiment was replicated three times with replicates blocked in time. Thus, all treatments within a replication were evaluated before the next replicate was initiated. For each of the 76 Petri dishes (eight concentrations of nine formulations, and four controls), the number of newly dead or moribund beetles was recorded for each observation period. The three parameters of the Weibull distribution for time to death were estimated for each replication of every insecticide and concentration and controls. The estimation procedure was based on a maximum likelihood approach for censored samples and is implemented in a computer program (GERM), originally designed for quantification of seed germination response, but usable for this application. [GERM is a computer program designed to summarise data of seed germination tests with an option for fitting the Weibull function. Copies of the program are available from T. R. Dell, Southern Forest Experiment Station, USDA, Forest Service, Room T-10210, Postal Services Building, 701 Loyola Avenue, New Orleans, Louisiana, 70113, USA.]

These three parameters were used to estimate five additional response variables, the times required to achieve 25, 50, 75, 90 and 95% mortality.

Each of the response variables for each insecticide formulation was subjected to an analysis of variance with a randomised complete-block design to determine whether there was any significant difference in these responses attributable to blocks or the concentration of insecticide. Specifically, the null hypothesis of no difference in mean values of the particular response variable associated with the fixed concentration was tested in each case. The hypothesis, concerning the blocking factor that related to repeating the entire set of treatments at three different times, was similar, but the main intention was one of error reduction.

The Weibull parameters were used also to generate data points for cumulative distribution functions (cumulative mortality versus time) for each concentration of each formulation. These distributions are graphed mainly for illustrative purposes (Figures 1 and 2). Estimates of the LT_{90} (the length of time required to reach 90% mortality) were regressed as a function of the concentration (g litre^{-1}) for each formulation by the curvilinear equation $Y = b_o + b_1 \cdot X^{-1}$, in which Y = the LT_{90} and X = the concentration.

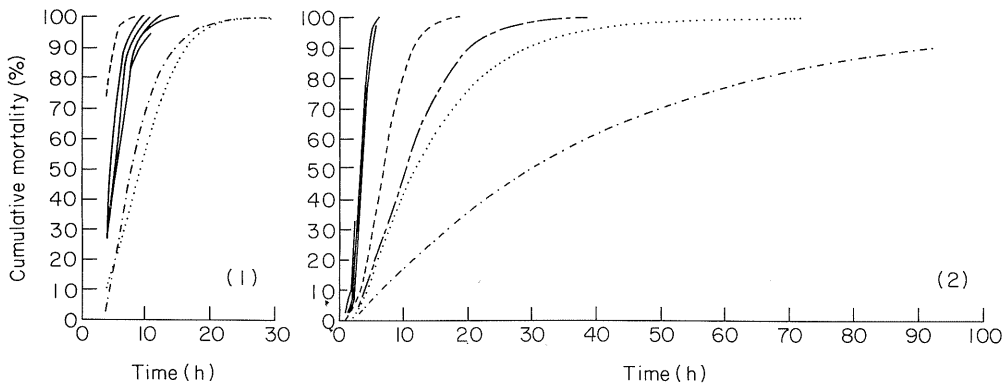

Figure 1. Cumulative mortality versus time for adult *Conophthorus ponderosae* exposed to various concentrations of resmethrin: (----) 0.48 g litre^{-1}; (———) 0.084, 0.108, 0.18, 0.24 and 0.30 g litre^{-1}; (–·–·–·) 0.036 g litre^{-1}; and (.....) 0.060 g litre^{-1}.

Figure 2. Cumulative mortality versus time for adult *Conophthorus ponderosae* exposed to various concentrations of gamma-HCH: (———) 0.48, 0.72 and 1.20 g litre^{-1}; (-----) 0.30 g litre^{-1}; (———) 0.108 g litre^{-1}; (.....) 0.072 g litre^{-1}; and (–·–·–·) 0.024 g litre^{-1}.

3. Results and discussion

3.1. Estimated parameters

The concentrations selected primarily to determine concentration-response lines by the probit method are obviously not always suitable for estimating time trends with the Weibull function. Ideally, concentrations selected for probit analysis should cause mortalities in the upper and lower zones of response: between 10 and 30%, and between 70 and 95%.[10,11] Clustering the concentrations in these ranges reduces the variation associated with the extremes of a concentration-response line, such as at the LC_{10} or LC_{90}.[12] However, concentrations for studies to be evaluated with the three-parameter Weibull function must cause both some mortality at not less than four different times, and at least 30% mortality by the end of the trial. As a result, not all of the concentrations of every insecticide were usable (Table 1). Data for carbaryl and encapsulated fenitrothion, and for some concentrations of the remaining nine formulations were suitable for probit analysis,[4] but were unusable for time-trend evaluations with the Weibull function and, therefore, are not reported here.

Cumulative distribution functions illustrate the variations in time trends in mortality for each concentration of the insecticide formulations. Some formulations, such as resmethrin (Figure 1) and parathion-methyl, were fast-acting at all concentrations tested. Other formulations, such as gamma-HCH (Figure 2), permethrin, and fenvalerate displayed distinct differences in speed of action as a function of concentration.

By analysis of variance, it was determined that the LT_{75}, LT_{90} and LT_{95} response variables were estimated with enough precision to detect differences ($P<0.05$) between concentrations and blocks. For all formulations except fenvalerate and parathion-methyl, statistically significant differences were observed between LT_{90} values associated with concentration. The LT_{90} is a particularly useful response variable from a practical standpoint because of the concern to eliminate as much of the population as fast as possible. All further discussion, therefore, will concern the relationship between the concentration and the time to 90% mortality (LT_{90}).

3.2. Prediction equations

Initial plotting of the data permitted evaluation of the various model equations for fit to the data. The equation selected was $Y = b_0 + b_1 \cdot X^{-1}$, in which $Y =$ the LT_{90} and $X =$ the concentration (Figure 3). For three of the nine formulations [fenvalerate, parathion-methyl, and diazinon (encap.)], a meaningful regression could not be defined. The concentrations tested for these formulations did not cover a sufficient range to evaluate a trend. Possibly this problem could be rectified with better concentration selection or further replication. Although the cumulative distribution functions (Figures 1 and 2) permit a visual estimate of the LT_{90}, the regression equation allows more precise estimates for any concentration between the highest and the lowest included in the regression.

The equation $Y = b_0 + b_1 \cdot X^{-1}$ demonstrated an important concept; there is a concentration above which an increase no longer appreciably decreases the time to achieve 90% mortality. The regression equation expresses the approach to an asymptote (b_0) as the concentration is increased. Asymptotes of LT_{90} values between 2.3 and 7.0 h were obtained for formulations with significant regressions in this series of bioassays, gamma-HCH being the fastest-acting formulation.

4. Conclusions

Use of the three-parameter Weibull function and curvilinear regression adds the dimension of time to the evaluation of insecticides. Although probit analysis estimates a dosage or concentration at which a treatment will achieve a given level of mortality, the Weibull function provides estimates of the time trend to any given level of mortality for a particular dosage or concentration. Coupling the Weibull estimates with curvilinear regression allows estimates of the amount of time required to achieve a given level of mortality for a wide range of dosages or concentrations, and allows an investigator to select the fastest-acting insecticide formulation. For control of insects such as *Conophthorus ponderosae*, for which the time to achieve maximum mortality is critical, time trends

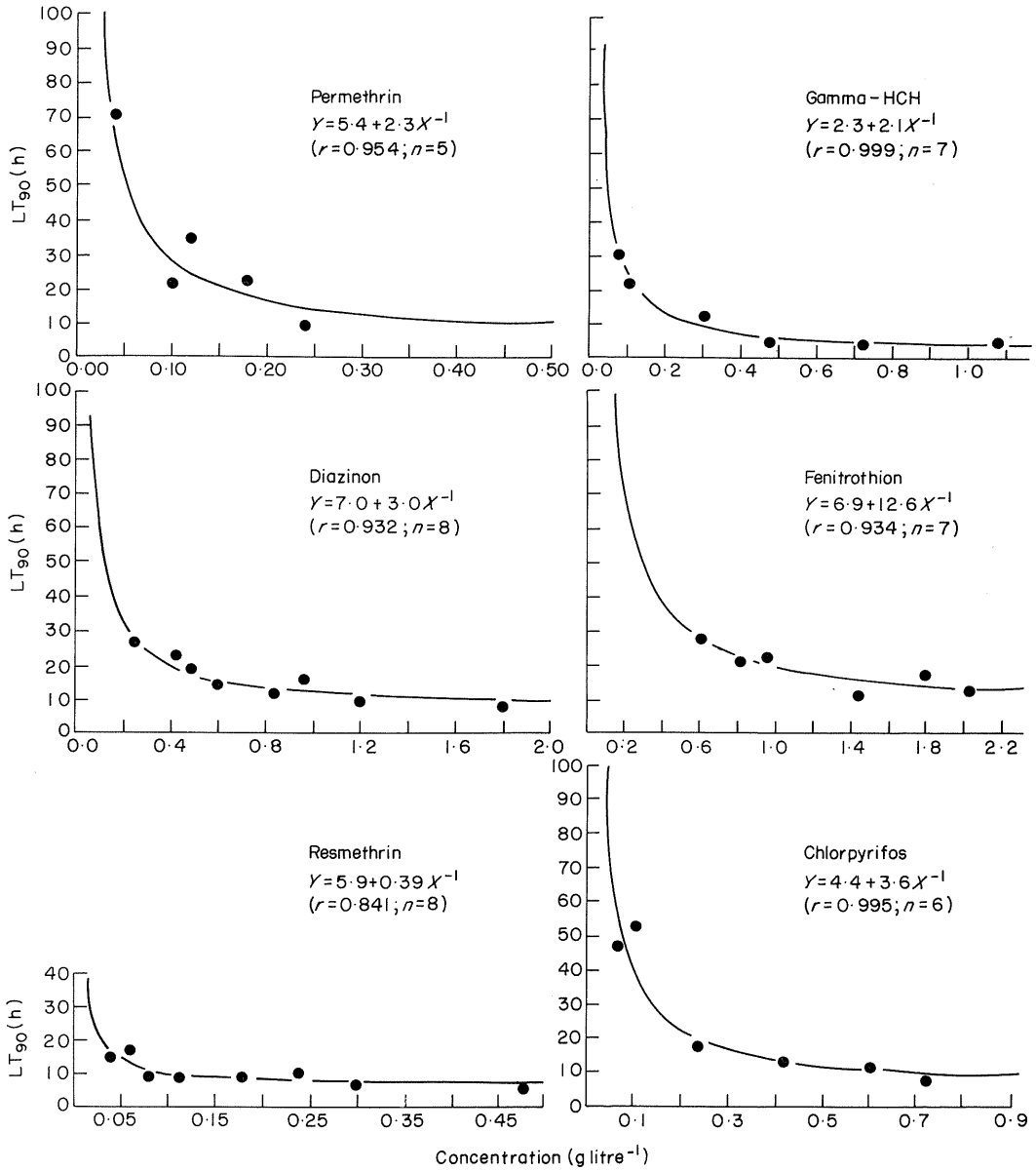

Figure 3. Regression of the LT_{90} (time to 90% mortality) as a function of the concentration (g.a.i. litre^{-1}) of six insecticide formulations. *Conophthorus ponderosae* adults were exposed to residues of insecticide formulations for 24 h. LT_{90} values were estimated by Weibull parameters.

in mortality are a useful tool when selecting effective insecticide formulations and concentrations for definitive field testing. If speed of action is not critical, then the conventional technique of probit analysis should suffice.

Note

This publication does not contain recommendations for the pesticide uses reported, nor does it imply that they have been registered by the appropriate governmental agencies.

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