The influence of application
ent irrigation on the fate and
phos for control of Japanese
rabaeidae) larvae in turfgrass.
165–470.
The economics of improving
ev. Entomol. 21: 45–60.
alculation of economic-injury
esholds for pest management.
297–303.
son. 1986. Models for age
with distributed maturation
247–262.
eires, D. W. Onstad, B. H.
ley. 1985. Timing and ef-
le treatments against the San
Diaspididae). J. Econ. Ento-

Cumulative insect-days as an
n. J. Econ. Entomol. 76: 375–

ve timing of an insecticide. J.
83–1085.
ers, T. L. Wagner, D. K. Loh,
. Hu, P. E. Pulley & R. N.
puter-aided decision making
ing in pest management sys-
the southern pine beetle (Co-
. Econ. Entomol. 77: 1073–

eissig, W. L. Roelofs, M. R.
naker. 1987. Timing treat-
t (Diptera: Tephritidae) con-
e traps baited with synthetic
. Entomol. 80: 1057–1063.
ct of timing of application on
hos, isazophos, and diazinon
leoptera: Scarabaeidae) grubs
ol. 78: 172–180.
elopments in computer-based
systems. Annu. Rev. Entomol.

1 21 March 1989; accepted 1

Choosing the Optimal Diagnostic Dose for Monitoring Insecticide Resistance

W. ROSS HALLIDAY[1] AND KENNETH P. BURNHAM[2,3]

Stored-Product Insects Research and Development Laboratory, USDA–ARS, Savannah, Georgia 31403

J. Econ. Entomol. 83(4): 1151–1159 (1990)

ABSTRACT We developed a model to investigate the best conditions for conducting diagnostic dose tests to monitor insecticide resistance when dose–response lines of the susceptible and heterozygous strains overlap. In the model, χ^2 analysis and a one-tailed Z test were used to test the accuracy or power of the experiment. Seven independent factors (slope, resistance factor, frequency of resistance, inheritance of resistance, dose, number of susceptible strain insects, and number of test strain insects) contributed to a test's potential accuracy. Only dose and number of insects treated can be altered by the scientist to improve a test's accuracy. With two exceptions—dose and sample size—the accuracy of the test will be higher as the numerical value of any factor increases. Under our conditions, accuracy decreased as dose approached 100% mortality of the susceptible strain. This relationship suggests that, in the absence of a discriminating dose that has been shown to distinguish genotypes, use of slightly lower doses that do not kill an extremely high proportion of susceptible insects may be preferable.

KEY WORDS Insecta, resistance, diagnostic dose, statistical test power

THE APPEARANCE of insecticide resistance in a population is usually detected with one or more of three techniques. The traditional approach uses complete dose–response tests with 4–5 doses that produce 10–90% mortality. Resistance is expressed in terms of the ratio of the LD_{50} or LD_{95} of the resistant strain to that of the susceptible strain. Alternatively, one dose is often used and the mortalities of the susceptible and test strains are compared. This approach is called the discriminating or diagnostic dose test. The term discriminating dose is properly used when enough genetic and toxicological evidence has accumulated that shows that a dose causes a different response between genotypes. Diagnostic dose is a less rigorous term and is used when one wants to monitor resistance but is less certain that the dose does separate genotypes. The third choice for detecting resistance is to use one of the small-scale biochemical assays or techniques from molecular biology that have been adapted to measure the frequency of specific resistance mechanisms in populations (Hemingway et al. 1986, Brown & Brogdon 1987). These elegant assays are currently of limited value in monitoring the initial development of resistance, because years of research are required for selection of homozygous resistant strains and assay optimization and validation.

An extensive body of literature about the statistical treatment of data for probit analysis of susceptible strains was reviewed by Finney (1971). Toxicities of different xenobiotics can be compared with probit regression. Investigations of the requirements for reliable estimation of dose–response regressions for insecticides have emphasized criteria for precise comparisons. Robertson et al. (1984), for example, investigated optimal sample size and dose selection necessary to produce precise lethal dose estimates in typical bioassays. Although the concept of discriminating doses has been used for many years (see Brown & Pal 1971), its statistical limitations have been investigated only recently.

The scientist who suspects that resistance is present must choose between estimation of complete dose–response lines or use of a single-dose test. Disadvantages of complete dose–response lines include being time-consuming and insensitive to slight changes in resistance gene frequencies, especially at the LD_{50}. Because of the wide fiducial limits at the LD_{95} and higher, determining whether resistance exists based on differences at the LD_{95} can be ambiguous or misleading. Roush & Miller (1986) described some of the advantages of the discriminating dose technique. The primary advantage is speed: Because fewer individuals must be tested, more populations can be tested. They divided diagnostic dose tests into two categories (perfect and nonperfect) depending on whether the dose killed all susceptible insects and no resistant ones (perfect) or killed both genotypes (nonperfect).

In many instances, perfect discriminating doses have provided useful data for genetic studies. Most notably, this has occurred when organophosphorus or cyclodiene insecticides have been used because

[1] Current address: Ricera, Inc., P.O. Box 1000, Painesville, Ohio 44077.
[2] USDA–ARS, North Carolina State University, Raleigh, N.C. 27695.
[3] Current address: Colorado Cooperative Fisheries and Wildlife Research Unit, Colorado State University, Fort Collins, Colo. 80523.

resistance levels are generally high and inherited in a codominant to dominant manner. For most situations, some caution must be used before the technique is applied. Unfortunately, the genetics and toxicology of resistance are understood for few species. Therefore, the researcher does not know whether a discriminating dose exists. Extrapolation from work on other species is sometimes used as an indicator of the level of resistance, type of resistance and mode of inheritance which might be expected in a homozygous strain. Extrapolation between laboratory strains and field strains is tenuous because of natural variation in sensitivity between populations. Should a field population be less sensitive than a laboratory strain, lower mortality might occur at a supposed discriminating dose when in fact the survival would be due to natural variation in response within the field population. Based on these reasons alone, use of the diagnostic dose approach to monitor resistance might not be wise if that resistance is not well understood.

Dennehy et al. (1983) indicated the dangers of arbitrarily choosing a dose several times higher than the LD_{99}. For mites resistant to dicofol, use of such a dose resulted in an underestimate of resistance because a large proportion of the resistant mites also were killed by this dose. By lowering the dose and changing the test technique, they were better able to estimate the resistant population.

Roush & Miller (1986) briefly considered the subject of low versus high diagnostic doses. Their concern was that high doses inevitably have a higher estimation error. A lower dose would then be better based on its intrinsically improved accuracy. However, this effect is mitigated by the increased sample size.

Neither Dennehy et al. (1983) nor Roush & Miller (1986) addressed the central question of how to determine the correct dose with which to test a putatively resistant strain. In a perfect scenario, all doses that kill all susceptible insects exclusively are equivalent. Under the nonperfect scenario, choosing the dose is less straightforward because an accurate estimation of the susceptible strain's mortality at the discriminating dose is required.

In this paper, we describe an approach for improving the probability of detecting resistance based on finding the best diagnostic dose. This approach measures the statistical confidence, or power, we could have in detecting a difference in mortality of the susceptible and test strains that might be due to resistance. Statistically, the accuracy or power of the test is calculated at various conditions that can be changed. For testing two strains, large-sample statistical theory assures us that the most powerful tests are the χ^2 test for a two-tailed test, or the Z test for a one-tailed test (Lehmann 1959). The actual power of these optimal tests, as influenced by all relevant factors, is the subject of this paper.

The power of a statistical test can be defined in a number of ways. According to Mendenhall & Schaeffer (1973), power $= 1 - \beta$, where β is the probability of accepting the null hypothesis (H_0: mortality of the susceptible strain = mortality of the test strain) when it is false. In this sense, power is the probability that the test will reject the null hypothesis and is really only of interest when H_0 is false. However, power is defined when the null hypothesis is true, in which case power equals the α level (where α is the probability of rejecting a true hypothesis). A much more valuable use of power occurs when H_0 is false. In this case, power is the probability of correctly accepting the alternative hypothesis (H_{alt}: mortality of the susceptible strain $\neq$ mortality of test strain), i.e., a difference in mortality exists between the two strains that might be due to resistance. To use the model we want it to predict conditions under which power will be maximized.

The Model

The model we used to calculate the power of diagnostic dose tests is based directly on the 2×2 contingency table in which the number of surviving and dead susceptible insects are compared with the numbers from the test strain with either the χ^2 test (df = 1) or the Z test. The power of either of these tests is easily computed using SAS-PC Version 6.03 (SAS Institute 1987, 70). When H_0 is false, the χ^2 statistic follows a noncentral χ^2 distribution (df = 1) with a noncentrality parameter, λ. In SAS-PC, the noncentral χ^2 distribution is an interactive function, i.e., for any value of λ, SAS will calculate power.

To find λ, we calculated the expected number of dead and alive insects for the treatment and used these numbers in the formula for the χ^2 statistic. The validity of this procedure to compute χ^2 test power has been shown by Drost et al. (1989). To compare the two-tailed χ^2 test statistic with the one-tailed Z test favored by Roush & Miller (1986), we noted that Z is the signed square root of the χ^2 test and H_{alt} is rejected if Z exceeds the critical value of 1.645 (for $\alpha = 0.05$). The probability distribution of the Z test is the normal distribution with mean $\sqrt{\lambda}$ and a standard deviation of 1. Thus, the power of the one-tailed Z test is the probability that such a random variable exceeds 1.645.

Mortality of the susceptible strain was based on the calculated logit regression, given the slope and the LD_{50}. We characterized the resistance by calculating logit regression lines for fully resistant strains relative to the susceptible strains. Next, the mode of inheritance of the resistance was established by placing the logit regression line between the homozygous susceptible and resistant lines. Finally, a test strain was synthesized in which a resistance gene existed in Hardy–Weinberg equilibrium. Mortality in the synthetic test strain at various doses was compared with mortality in the susceptible strain. Mortality of both strains was calculated at doses producing 0–99.9% mortality of the sus-

: = 1 − β, where β is the
g the null hypothesis (H_0:
tible strain = mortality of
s false. In this sense, power
he test will reject the null
only of interest when H_0
ır is defined when the null
ıich case power equals the
probability of rejecting a
ıch more valuable use of
is false. In this case, power
ectly accepting the alterna-
ıortality of the susceptible
ıst strain), i.e., a difference
ween the two strains that
nce. To use the model we
litions under which power

Model

to calculate the power of
ɔased directly on the 2 × 2
ıich the number of surviv-
ɜ insects are compared with
test strain with either the
Z test. The power of either
mputed using SAS-PC Ver-
1987, 70). When H_0 is false,
ı noncentral χ^2 distribution
rality parameter, λ. In SAS-
istribution is an interactive
ılue of λ, SAS will calculate

ated the expected number
ɔcts for the treatment and
the formula for the χ^2 sta-
ıis procedure to compute χ^2
own by Drost et al. (1989).
iled χ^2 test statistic with the
d by Roush & Miller (1986),
ɜigned square root of the χ^2
d if Z exceeds the critical
0.05). The probability dis-
is the normal distribution
ındard deviation of 1. Thus,
iled Z test is the probability
riable exceeds 1.645.
ɜeptible strain was based on
ression, given the slope and
rized the resistance by cal-
ɔn lines for fully resistant
usceptible strains. Next, the
ıf the resistance was estab-
ɔgit regression line between
ıtible and resistant lines. Fi-
synthesized in which a re-
ı Hardy–Weinberg equilib-
ynthetic test strain at various
ith mortality in the suscep-
f both strains was calculated
99.9% mortality of the sus-

List 1. Nomenclature and definitions of factors affecting the power of diagnostic dose tests[a]

Toxicological

Δ (Delta) = magnitude of resistance for the homozygous resistant strain on a logarithmic scale.
σ (Sigma) = reciprocal of the slope of the dose response line.

Genetic

p = frequency of the resistant allele in a test population.
γ (Gamma) = dominance of the resistance gene; 0 = completely recessive, 1 = completely dominant.

Operational

n_s = number of susceptible insects.
n_t = number of insects from the test population.
n = total number of insects ($=n_s + n_t$).
d = dose producing mortality in susceptible strain.

[a] After Georghiou & Taylor (1977).

ceptible strain. Survival of the two populations was compared statistically as described above. This procedure was repeated as each parameter of interest was changed sequentially.

A convenient way to visualize the dose is to view it in terms of toxic equivalents required to kill a certain proportion of the susceptible population. For computational simplicity, we assumed that slopes for the regression lines are equal. For ease of presenting the results, we classified variables affecting the power into three groups (operational, toxicological, and genetic) following the scheme of Georghiou & Taylor (1977), who listed similar factors that influence the rate of resistance development. Parameters that we considered are listed and defined in List 1 according to whether they are toxicological, genetic, or operational. According to Georghiou & Taylor (1977), only operational factors can be manipulated to make resistance develop faster or slower. Likewise, in our analysis only operational factors can be changed by researchers to increase the test's power. These factors are the number of susceptible insects tested (n_s), the number of test strain insects tested (n_t) and the dose (d [mortality of the susceptible strain]). The genetic factors are frequency of the resistance gene (p) and dominance of the resistance (gamma, γ). Toxicological factors are resistance factor (Δ, delta) and slope of the dose–response line. In one sense, genetic and toxicological factors are inherent to the insect and insecticide and cannot be changed. However, the magnitude of these factors certainly varies within insecticide classes and might offer some chance for manipulation. We examined the contribution of toxicological and genetic factors to determine if some situations were more likely to produce tests of high power.

Results

Our calculations are presented in two ways. As mentioned earlier, mortality in the test strains was determined relative to the mortality in the susceptible strain. In this manner, power could be directly

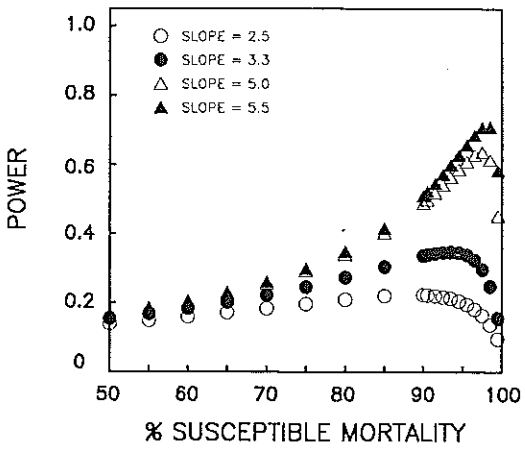


Fig. 1. Effect of changing slope on power of diagnostic dose test.

related to dose. The second method was to plot maximal power (MaxPower) as a dependent variable. MaxPower is the highest power found for a specified set of conditions over the range of doses producing 0–99.5% mortality of the susceptible strain. MaxPower provides useful comparisons of the effects of multiple variables. As shown in Fig. 1, MaxPower for a slope of 3.3 was 0.35. We refer to the optimal dose that produces MaxPower as d_{opt}. As will be shown later, d_{opt} varies depending on the magnitude of the parameters. The following results are based on selected parameter values chosen to illustrate each relationship.

Comparison of χ^2 and One-Tailed Z Tests. Of obvious interest is the effect of the statistical test used. In earlier versions of our program, we used a χ^2 test to generate power because our interest then was in d_{opt} more than the value of power. In an earlier draft of this manuscript, two anonymous reviewers indicated that a one-tailed Z test would be better because the χ^2 test is two tailed. When monitoring populations for resistance, we are not interested in significance of results in which the mortality of the test strain is greater than that of the susceptible strain. In our opinion, use of a one-tailed Z test is preferable.

In this series of calculations, each parameter was set and the power calculated over a range of concentrations from 0 to 99.5% mortality of the susceptible strain; maximum power was recorded. Six series were done to see whether relationships observed were consistent. As shown in Table 1, the maximum power depends on the test statistic. As expected MaxPower was higher in all cases when the Z test statistic was used. However, the effect was small, especially at very high powers. The dose producing MaxPower, d_{opt}, remained constant for the two test statistics (a result that statistical theory can show will always be true).

Effect of Toxicological Factors. The first toxicological factor that we examined was slope, the

Table 1. Comparison of maximum power (MaxPower) and optimal dose (d_{opt}) obtained using either a χ^2 or one-tailed Z test statistic

Slope	Dominance[a]	Resistance level[b]	Frequency of resistance	n[c]	MaxPower		d_{opt}	
					χ^2	Z test	χ^2	Z test
2.0	0.1	10.0	0.05	200	0.051	0.059	62.0	62.0
3.0	0.1	10.0	0.05	200	0.052	0.064	65.0	65.0
3.0	0.5	10.0	0.05	200	0.087	0.140	81.0	81.0
3.0	0.5	100.0	0.05	200	0.245	0.353	94.0	94.0
3.0	0.5	100.0	0.10	200	0.619	0.731	93.0	93.0
3.0	0.5	100.0	0.10	2,000	0.999	0.999	94.0	94.0

[a] Based on gamma (γ).
[b] Based on delta (Δ), where 10^{Δ} = resistance level.
[c] Based on $n_s = n_t$.

reciprocal of sigma. We investigated the effect of slope on power by examining changes over the entire range of doses at a given slope (Fig. 1). The conditions for this test were $n = 200$; $n_s = n_t = 100$; $\Delta = 1.0$; $P = 0.05$; and $\gamma = 0.90$. Slopes ranged from 2.5 to 5.5. Slope had a distinct effect on power (Fig. 1). Three important trends in this figure are apparent. First, the test's power increased with higher slopes. For example, at the dose producing 95% mortality of the susceptible strain, power is 0.66 for a slope of 5.5 and 0.20 for a slope of 2.5. The second important trend was the consistent decrease in the power between the optimal dose and 100% mortality in the susceptible strain. This decrease was primarily due to the fact that fewer susceptible individuals survived the dose. Consequently, the statistical power of the test was reduced. This decrease in power suggests that the accepted procedure of establishing a discriminating dose of twice or four times the LD_{99} or $LD_{99.9}$ will actually sometimes cause the wrong conclusion to be made whether resistance exists in the test population. The third trend is that as slope increases, so does the optimal dose. In these calculations, the optimal dose ranged from 89.0 for a slope of 2.5 to 98.1 for a slope of 5.5. This means that the optimal dose for conducting diagnostic dose tests depends upon the variables unique to that system.

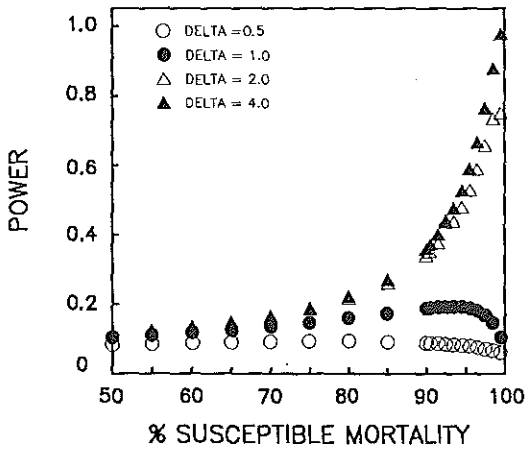


Fig. 2. Effect of changing Δ on power of diagnostic dose tests.

The parameter Δ is directly related to the resistance factor (the ratio of the LD_{50} of the fully resistant strain to the LD_{50} of the susceptible strain). In our model, all values were transformed so that $\Delta = 1.0$ would actually correspond with a resistance factor of 10.0 when the LD_{50}'s were based on the logarithm of dose.

Delta had a large effect on power (Fig. 2). The conditions of this test were $P = 0.01$; $n = 2{,}000$; $n = n_s = 1000$; $\gamma = 0.9$; and slope = 3.0. At low Δ power remained low. Under the conditions of this test, an apparent threshold existed above which the power increased dramatically. This occurred when Δ increased from 1 to 2 (Fig. 2). Power decreased as dose approached 100% susceptible mortality in the same manner as was seen in Fig. 1 except when resistance equaled 10,000 ($\Delta = 4.0$).

Effect of Genetic Factors on Power. We define p as the frequency of the resistance gene in the test population. All parameters were held constant as before and the frequency of the resistance gene in the test population was then incrementally changed. The initial parameters for Fig. 3A were slope = 3.00; $n = 200$; $n_s = n_t = 100$; $\Delta = 2.0$; $\gamma = 0.5$. Hardy–Weinberg equilibrium was assumed. We also ran a number of tests and analyses of extensions of the parameters already mentioned (slope and Δ). In these additional tests, we set the resistance gene frequency equal to 0.01, 0.05, 0.10 and calculated MaxPower for a series of slopes (Fig. 3B) or Δ (Fig. 3C). Conditions were set as follows for slope effects: $n = 2{,}000$; $n_s = n_t = 1{,}000$; $\Delta = 1.0$; and $\gamma = 0.90$. We chose the same range of realistic slopes as in Fig. 1. Frequency of the resistance gene played a major role in determining the power. When power was plotted against dose for three frequencies (Fig. 3A), we found that the relationship between power and dose was similar to that previously seen for changes in the slope and resistance level. As dose increased, power increased until MaxPower was reached, after which power decreased as the dose approached 100% susceptibl

ained using either a χ^2 or one-

	d_{opt}	
it	χ^2	Z test
9	62.0	62.0
4	65.0	65.0
0	81.0	81.0
3	94.0	94.0
1	93.0	93.0
9	94.0	94.0

r a slope of 5.5. This means
: for conducting diagnostic
ion the variables unique to

directly related to the resis-
o of the LD_{50} of the fully
D_{50} of the susceptible strain).
es were transformed so that
correspond with a resistance
ie LD_{50}'s were based on the

ffect on power (Fig. 2). The
were $P = 0.01$; $n = 2{,}000$; n_t
and slope = 3.0. At low Δ,
Under the conditions of this
hold existed above which the
atically. This occurred when
2 (Fig. 2). Power decreased
)0% susceptible mortality in
as seen in Fig. 1 except when
000 ($\Delta = 4.0$).

ictors on Power. We defined
f the resistance gene in the
rameters were held constant
uency of the resistance gene
on was then incrementally
parameters for Fig. 3A were
; $n_s = n_t = 100$; $\Delta = 2.0$; $\gamma =$
; equilibrium was assumed.
ber of tests and analyses as
ameters already mentioned
e additional tests, we set the
ency equal to 0.01, 0.05, or
axPower for a series of slopes
3C). Conditions were set as
s: $n = 2{,}000$; $n_s = n_t = 1{,}000$;
). We chose the same range
in Fig. 1. Frequency of the
l a major role in determining
ver was plotted against dose
(Fig. 3A), we found that the
power and dose was similar
l for changes in the slope and
se increased, power increased
reached, after which power
approached 100% susceptible

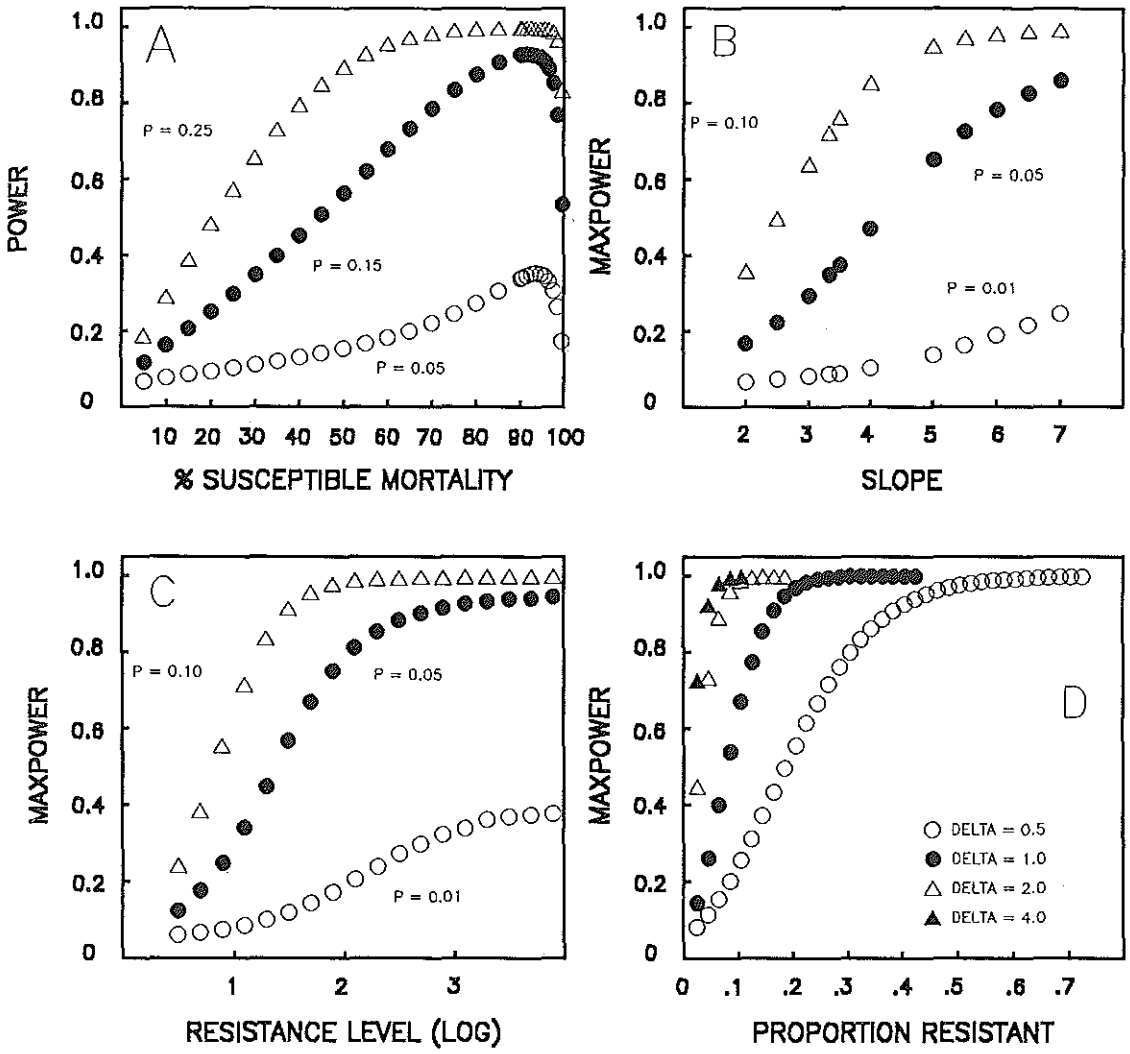


Fig. 3. Relationship between P, power, and MaxPower. (A) Effect of increasing dose on power. (B) Effect on MaxPower of different slopes of the dose response line. (C) Effect of different resistance levels on MaxPower. (D) Effect of changing gene frequency on MaxPower at four resistance levels.

strain mortality. When MaxPower reached very high levels, the power curve reached a plateau (Fig. 3A, $P = 0.25$). The data in Fig. 3A reiterate the earlier finding (Table 1) that as the frequency of the resistance gene increases, the optimal dose decreases.

MaxPower increases as slope increases for three different resistance gene frequencies (Fig. 3B). This figure suggests that the dose–response line should have as high a slope as possible. A low slope will severely limit accuracy of a test. Fig. 3C demonstrates the effects of increasing resistance levels. For high resistance levels >100 (i.e., $\Delta > 2.0$), slight increases in the frequency of the resistance gene will rapidly increase the accuracy of the test. This figure also shows that, under some conditions, the maximal chance the diagnostic dose would have of detecting resistance is unacceptably low. When $P = 0.01$, MaxPower reached a plateau at 0.38 and at $P = 0.05$ it reached a plateau at 0.94 (Fig. 3C). In Fig. 3D, the relationship between proportion resistant and MaxPower at different resistance levels is shown. The results in this figure reiterate the findings in Fig. 2: as resistance increases, so does the probability of detecting it with a diagnostic dose test.

Fig. 3A–D indicate that gene frequency can play a major role in determining the potential to discover resistance. When gene frequency changes slightly, a large change in the power can occur under some conditions. Why power increases as gene frequency increases seems intuitively obvious. When the frequency of resistant individuals increases in a population, the number of survivors

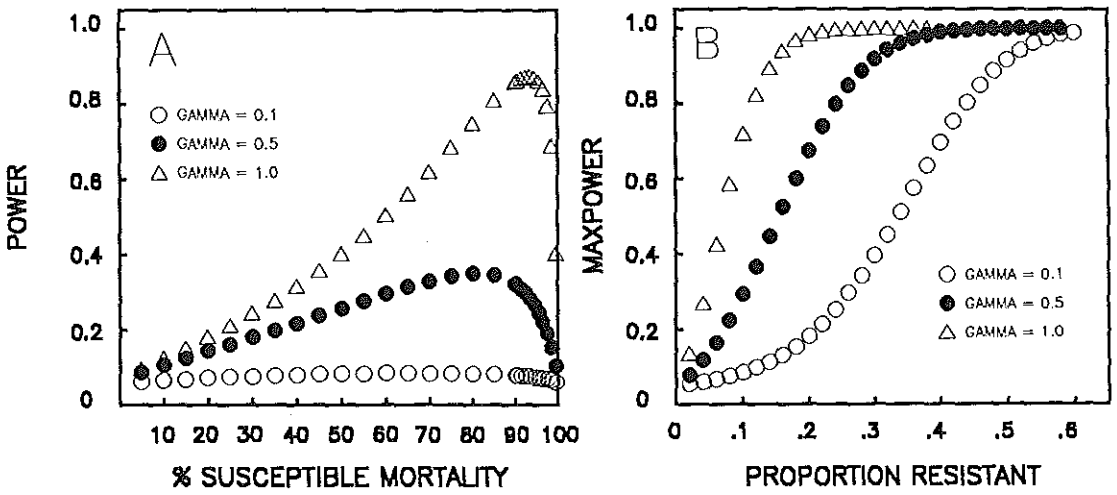


Fig. 4. Relationships between γ, power, MaxPower, and P. (A) Effect of changing dose on power at three values of γ. (B) Effect of gene frequency changes on MaxPower.

from the test population will increase concomitantly. The larger the sample size, the better the statistical accuracy will be. Decreased accuracy can be explained by fewer susceptible survivors being available for calculating the χ^2 value.

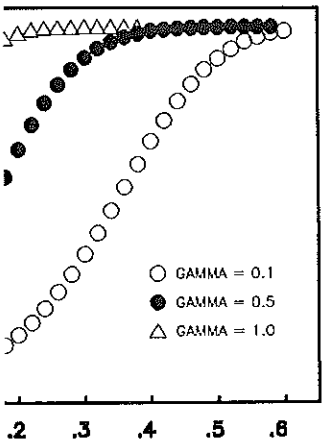
The second genetic component we investigated was dominance. For simplicity of calculations, we used a factor called γ instead of the traditional degree of dominance (D) of Stone (1968). Instead of ranging from -1 to 1 like D, γ ranges from 0 to 1. A value of 0 indicates complete recessiveness of the trait, whereas 0.5 and 1.0 indicate intermediate and complete dominance, respectively. D and γ are linearly related as shown in equation 1:

$$(D + 1)/2 = \gamma \qquad (1)$$

The degree of dominance of resistance influences accuracy of diagnostic dose tests in much the same manner as the parameters previously examined (Fig. 4). The conditions of this test were $P = 0.05$; $n = 2{,}000$; $n_s = n_t = 1{,}000$; slope = 3.0; and $\Delta = 1.0$. The conditions for Fig. 4B were the same as Fig. 4A except that $n = 200$, $n_s = 110$, and $n_t = 90$. At values of γ that indicate resistance is recessive, power is much lower than had the resistance been dominant. Fig. 4A indicates that, at d_{opt}, power increases from 0.084 to 0.35 to 0.87 as γ changes from 0.10 to 0.50 to 1.0. Fig. 4B shows the relationship between MaxPower, γ, and P. This figure reiterates earlier results that showed increasing the gene frequency results in a higher power. It also demonstrates the advantage of having a dominant trait. To obtain a value of power of 0.9 (a reasonable level of accuracy in a test), the recessive trait in this example must be present at about three times the frequency of a dominant one (Fig. 4B).

Effect of Operational Factors on Power. Sample size is one of the most important considerations in designing any experiment. In diagnostic dose tests, sample size depends on the number of susceptible insects (n_s) and the number of insects from the test strain (n_t). We examined the relationship between sample size and power when n_s and n_t were equal, and the effect of changing the ratio of susceptible to resistant insects. Results of calculations in which n ($=n_s + n_t$) varied from 200 to 2,000 and n_t and n_s were equal are shown in Fig. 5A. Other parameters were set at $P = 0.05$, $\Delta = 1.0$, $\gamma = 0.9$, and slope = 3.0. Trials run with differing sample sizes produced results analogous to those seen earlier, i.e., power peaked at d_{opt} and was higher for increasing sample sizes (Fig. 5A). Under these conditions, MaxPower increased from approximately 0.293 when only 200 individuals were tested to approximately 0.966 for 2,000 individuals. This result seems intuitively obvious. As sample size increases, so does the statistical accuracy of the test. The optimal dose was unaffected by changing the sample size.

Roush & Miller (1986) suggested that testing a large number of susceptible insects would provide a better estimate of the true dose being used. This raised the question of whether changing the ratio of susceptible to resistant insects might also affect power. As shown in Fig. 5B, an optimal ratio exists but it becomes important only as power becomes relatively large. In the calculations when $P = 0.01$ power changed very little over a large range of ratios. When $P = 0.05$, power decreased relatively uniformly. Slight departures from the 1:1 ratio did not drastically decrease power. When many more insects of either type were tested, power was lower than it would have been had roughly equal numbers been tested. The highest power occurred when $n_s/(n_s + n_t) = 0.55$. The magnitude of the difference in power between a 1:1 and a 11:9 ratio was very small (0.791 versus 0.795 for $P = 0.05$). The asymmetry suggests that, to have a test with the



ing dose on power at three values

on the number of susceptible
umber of insects from the test
ined the relationship between
er when n_s and n_t were equal,
nging the ratio of susceptible
esults of calculations in which
rom 200 to 2,000 and n_t and
wn in Fig. 5A. Other param-
= 0.05, $\Delta = 1.0$, $\gamma = 0.9$, and
n with differing sample sizes
logous to those seen earlier,
t d_{opt} and was higher for in-
; (Fig. 5A). Under these con-
ncreased from approximately
0 individuals were tested to
for 2,000 individuals. This re-
obvious. As sample size in-
tatistical accuracy of the test.
s unaffected by changing the

986) suggested that testing a
eptible insects would provide
he true dose being used. This
f whether changing the ratio
stant insects might also affect
ig. 5B, an optimal ratio exists,
rtant only as power becomes
e calculations when $P = 0.01$,
little over a large range of
5, power decreased relatively
artures from the 1:1 ratio did
ase power. When many more
were tested, power was lower
een had roughly equal num-
highest power occurred when
The magnitude of the differ-
en a 1:1 and a 11:9 ratio was
rsus 0.795 for $P = 0.05$). The
that, to have a test with the

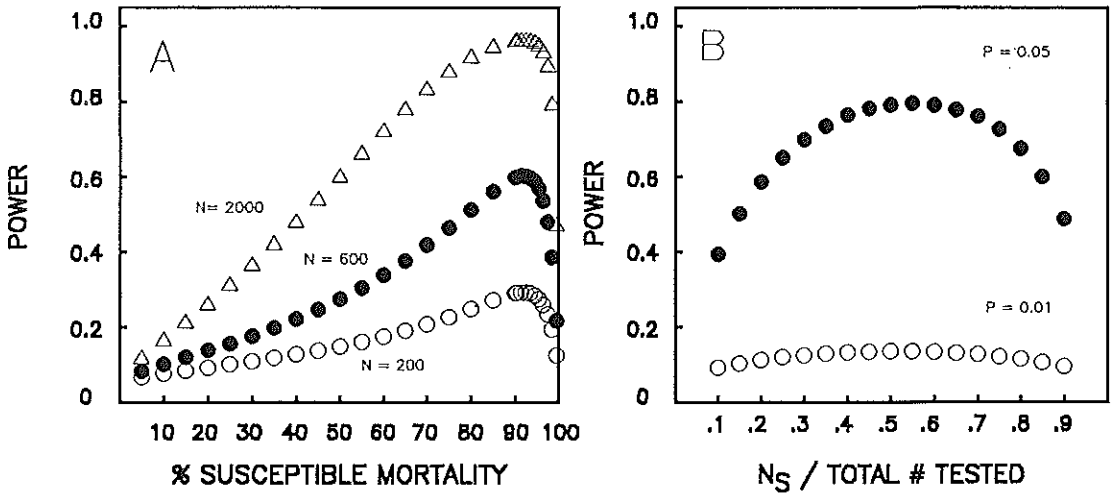


Fig. 5. (A) Effect of n on power. (B) Effect of unequal numbers of susceptible, n_s, and test strain insects on power.

maximum power, researchers should test slightly more insects from the susceptible strain than insects from the test population.

Examples from Published Data

This kind of analysis requires dose–response data for susceptible, fully resistant, and heterozygous strains. Knowing the resistance factor, slope, and dominance, the researcher can then alter the remaining variables to determine the optimum dose, the frequency of the resistance gene necessary in the population to be detectable, and the number of insects that must be tested. The results of this kind of analysis have been presented in Table 2. Published values of the level of resistance, slope of the dose–response line, and dominance (γ) were used as starting points. Power was calculated for three resistance gene frequencies (0.01, 0.05, and 0.10) based on testing either 100 or 1,000 susceptible and test insects. The resulting maximum power and dose are listed.

As described earlier, optimal dose and total sample were unrelated since the dose at which maximum power occurs remains constant despite changes in total sample size. Data from the work on *Bacillus thuringiensis* (Berliner) resistance in Indianmeal moth, *Plodia interpunctella* (Hübner), by McGaughey & Beeman (1988) suggest that use of diagnostic dose tests for monitoring resistance will not be very effective until the resistance gene reaches fairly high levels. Table 2 illustrates this point. Maximum power will reach the 0.95 power level when $n = 2{,}000$ and the resistance gene frequency is between 0.05 and 0.10. The best dose to use kills 85.0% of the susceptible strain. The situation would be even bleaker for monitoring resistance to pyrethrins in *Sitophilus granarius* (L.).

Table 2. Published values for slope, resistance level, and dominance for three insect–insecticide systems and the calculated values of power of diagnostic dose tests run with either 200 or 2,000 total insects at three gene frequencies[a]

Species	Insecticide	Slope	Resistance	Dominance	P	MaxPower when		% Mortality at d_{opt}	
						n = 200[b]	n = 2,000[b]	Susceptible	Test strain
P. interpunctella	*B. thuringiensis*	2.5	87.1	0.370	0.01	0.067	0.116	86.0	85.3
					0.05	0.170	0.707	85.0	81.3
					0.10	0.380	0.995	85.0	77.6
S. granarius	Pyrethrins	4.2	16.7	0.124	0.01	0.055	0.067	66.0	65.7
					0.05	0.080	0.188	70.0	68.4
					0.10	0.128	0.486	76.0	72.8
C. quinquefasciatus	Bifenthrin	4.2	18.6	0.675	0.01	0.104	0.336	97.0	96.1
					0.05	0.475	0.999	96.0	90.7
					0.10	0.860	0.999	94.0	83.7

[a] References are *Plodia interpunctella*, McGaughey & Beeman (1988); *Sitophilus granarius*, Prickett (1980); *Culex quinquefasciatus*, Halliday & Georghiou (1985).
[b] Based on $n_s/(n_s + n_t) = 0.55$.

This resistance is characterized by low dominance and low resistance level (Prickett 1980) and is thought to be due to a single gene, probably a mixed function oxidase. Even under the best conditions of $P = 0.1$ and a sample size of 2,000, the most power would be is 0.49. Should one intend to monitor resistance to bifenthrin, a pyrethroid, in *Culex quinquefasciatus* Say, the southern house mosquito, the chances of detecting resistance are much higher. Resistance in this species is monofactorial as shown in both genetic crosses and metabolism experiments (Halliday 1983). In this situation, characterized by a fairly high slope, an intermediately dominant trait, and a low resistance level, power attains high levels (>0.95) when the gene frequency is between 0.01 and 0.05. If it is known that the gene frequency in the population is very high, (i.e., >10%), the range of doses that will produce power of 0.999 is large (from 60.0 to 99.5%).

Conclusions

Diagnostic dose tests for monitoring resistance must be used with caution. Our results have shown relationships between various factors and how they influence the power of a diagnostic dose to correctly determine whether resistance exists in a population. Our model, which was designed to calculate the optimal dose to use in such experiments, revealed seven factors that interacted to influence the power. These could be conveniently classified as toxicological, genetic, or operational. As each factor changed, so did power but to varying degrees. The optimal dose increased as slope, resistance level, and dominance increased. The optimal dose decreased as resistance frequency increased and was unaffected by changes in sample size.

What does this analysis mean for those currently using or thinking of using diagnostic doses? It does not mean that all diagnostic dose experiments will produce incorrect results. Should such a test produce a significant difference in mortality between a susceptible and field strain, then this work is extraneous. There is a trend to conduct diagnostic dose tests in which the dose is chosen rather arbitrarily by multiplying the LD_{99} or $LD_{99.9}$ by a factor of two or three (Suckling et al. 1987, Subramanyam et al. 1989). Clearly this strategy will result in the failure to detect resistance under some circumstances.

Application of this analysis in cases where resistance is not indicated would be interesting. An after-the-fact analysis of the test conditions might reveal whether the chances of detecting resistance had been high. If so, the original conclusion of there being no resistance in the field population would be strengthened. The true value of this kind of analysis probably resides in its ability to predict a priori the best dose to use in such assays and how likely the test is to reveal differences if they exist. The results presented here stress the need to find improved systems to monitor resistance in its early stages. Such systems might be derived from dose–response procedures, such as the use of two or three doses that kill between 50 and 95% of the susceptible strain or from improvements in molecular biology technology in developing probes for resistance.

Acknowledgment

We thank David G. Heckel, Dept. of Biological Sciences, Clemson University, Clemson, S.C., for helpful comments and suggestions about earlier drafts of this manuscript.

References Cited

Brown, A. W. A. & R. Pal. 1971. Insecticide resistance in arthropods. World Health Organization, Geneva, Switzerland.

Brown, T. M. & W. G. Brogdon. 1987. Improved detection of insecticide resistance through conventional and molecular techniques. Annu. Rev. Entomol. 32: 145–162.

Dennehy, T. J., J. Grannett & T. F. Leigh. 1983. Relevance of slide-dip and residual bioassay comparisons to detection of resistance in spider mites. J. Econ. Entomol. 70: 653–658.

Drost, F. C., W. C. M. Kallenberg, D. S. Moore & J. Oosterhoit. 1989. Power approximations to multinomial tests of fit. J. Am. Stat. Assoc. 84: 130–144.

Finney, D. J. 1971. Probit analysis, 3rd ed. Cambridge University Press, Cambridge, England.

Georghiou, G. P. & C. E. Taylor. 1977. Operational influences in the evolution of insecticide resistance. J. Econ. Entomol. 70: 653–658.

Halliday, W. R. 1983. The interrelationship between DDT- and pyrethroid-resistance in the southern house mosquito, *Culex quinquefasciatus* Say. Ph.D. dissertation, University of California, Riverside.

Halliday, W. R. & G. P. Georghiou. 1985. Cross-resistance and dominance relationships of pyrethroids in a permethrin-selected strain of *Culex quinquefasciatus* (Diptera: Culicidae). J. Econ. Entomol. 78: 1227–1232.

Hemingway, J., C. Smith, K. G. I. Jayawardena & P. R. J. Herath. 1986. Field and laboratory detection of the altered acetylcholinesterase resistance genes which confer organophosphate and carbamate resistance in mosquitoes (Diptera: Culicidae). Bull. Entomol. Res. 76: 559–565.

Lehmann, E. L. 1959. Testing statistical hypotheses. Wiley, New York.

McGaughey, W. H. & R. W. Beeman. 1988. Resistance to *Bacillus thuringiensis* in colonies of Indianmeal moths and almond moth (Lepidoptera: Pyralidae). J. Econ. Entomol. 81: 28–33.

Mendenhall, W. & R. L. Schaefer. 1973. Mathematical statistics and applications. Duxbury, North Scituate, Mass.

Prickett, A. J. 1980. The cross-resistance spectrum of *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) heterozygous for pyrethrin resistance. J. Stored-Prod. Res. 16: 19–25.

Robertson, J. L., K. C. Smith, N. E. Savin & R. J. Lavigne. 1984. Effects of dose selection and sample

ıonitor resistance in its early
ıight be derived from dose–
uch as the use of two or three
n 50 and 95% of the suscep-
improvements in molecular
developing probes for resis-

ɔwledgment

Ieckel, Dept. of Biological Sci-
ity, Clemson, S.C., for helpful
ɔns about earlier drafts of this

'ences Cited

J. 1971. Insecticide resistance
l Health Organization, Geneva,

. Brogdon. 1987. Improved
ide resistance through conven-
techniques. Annu. Rev. Ento-

ınnett & T. F. Leigh. 1983.
lip and residual bioassay com-
of resistance in spider mites. J.
353–658.

Kallenberg, D. S. Moore & J.
Power approximations to mul-
[. Am. Stat. Assoc. 84: 130–144.
obit analysis, 3rd ed. Cambridge
mbridge, England.

E. Taylor. 1977. Operational
ɔlution of insecticide resistance.
): 653–658.

The interrelationship between
l-resistance in the southern house
nquefasciatus Say. Ph.D. disser-
California, Riverside.

P. Georghiou. 1985. Cross-
ance relationships of pyrethroids
cted strain of *Culex quinquefas-*
licidae). J. Econ. Entomol. 78:

ith, K. G. I. Jayawardena & P.
. Field and laboratory detection
'lcholinesterase resistance genes
ɔphosphate and carbamate resis-
(Diptera: Culicidae). Bull. Ento-
35.

. Testing statistical hypotheses.

R. W. Beeman. 1988. Resis-
:uringiensis in colonies of Indi-
mond moth (Lepidoptera: Pyral-
mol. 81: 28–33.

. L. Schaefer. 1973. Mathe-
d applications. Duxbury, North

The cross-resistance spectrum of
(L.) (Coleoptera: Curculionidae)
rethrin resistance. J. Stored-Prod.

C. Smith, N. E. Savin & R. J.
fects of dose selection and sample

size on the precision of lethal dose estimates in dose–mortality regression. J. Econ. Entomol. 77: 833–837.

Roush, R. T. & G. L. Miller. 1986. Considerations for design of insecticide resistance monitoring programs. J. Econ. Entomol. 79: 293–298.

SAS Institute. 1987. SAS user's guide: statistics, version 6. SAS Institute, Cary, N.C.

Stone, B. F. 1968. A formula for determining the degree of dominance of cases of monofactorial inheritance to chemicals. Bull. W.H.O. 38: 325–326.

Subramanyam, B., P. K. Harein & L. K. Cutkomp. 1989. Organophosphate resistance in adults of red flour beetle (Coleoptera: Tenebrionidae) and sawtoothed grain beetle (Coleoptera: Cucujidae) infesting barley stored on farms in Minnesota. J. Econ. Entomol. 82: 989–995.

Suckling, D. M., D. J. Rogers, P. W. Shaw, C. H. Wearing, D. R. Penman & R. B. Chapman. 1987. Monitoring azinphosmethyl resistance in the light brown apple moth (Lepidoptera: Tortricidae) in New Zealand. J. Econ. Entomol. 80: 733–738.

Received for publication 18 April 1989; accepted 3 November 1989.

ARTICLE INFORMATION:
Title: choosing the optimal diagnostic dose for monitoring insecticide resistance
Authors: halliday burnham
Pub Name: Journal of economic entomology
Std Num: 00220493
Volume: 83
Issue:
Pages: 1151-1159
Supplement:
Pub Date: 1990