Analyzing haplodiploid inheritance of insecticide resistance in whitefly biotypes


Summary:

This article describes the analysis of haplodiploid inheritance of insecticide resistance in whitefly biotypes. The authors present evidence for both maternal and paternal effects on resistance traits, highlighting the complexity of inheritance patterns in haplodiploid organisms.

Keywords:

Haplodiploidy, insecticide resistance, whitefly, inheritance, maternal, paternal effects.

DOI: 10.1017/S0007485308006330

How to cite this article:


Request Permissions: Click here
Analyzing haplodiploid inheritance of insecticide resistance in whitefly biotypes

D.W. Crowder1 *, A.R. Horowitz2, B.E. Tabashnik1, T.J. Dennehy1, I. Denholm3, K. Gorman3, and Y. Carrière1
1Department of Entomology, 410 Forbes Building, University of Arizona, Tucson, AZ, 85721, USA: 2Department of Entomology, ARO, Gilat Research Center, M.P. Negev, Israel: 3Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

Abstract

We developed new methods for analyzing inheritance of insecticide resistance in haplodiploid arthropods and applied them to elucidate resistance of the whitefly Bemisia tabaci (Gennadius) to an insect growth regulator, pyriproxyfen. Two invasive biotypes of this devastating crop pest, the B biotype in Arizona and the Q biotype in Israel, have evolved resistance to pyriproxyfen. Here, we incorporated data from laboratory bioassays and crossing procedures exploiting haplodiploidy into statistical and analytical models to estimate the number of loci affecting pyriproxyfen resistance in strains of both biotypes. In tests with models of one to ten loci, the best fit between expected and observed mortality occurred with a two-locus model for the B biotype strain (QC-02) and for one- and two-locus models for the Q biotype strain (Pyri-R). The estimated minimum number of loci affecting resistance was 1.6 for the B biotype strain and 1.0 for the Q biotype strain. The methods used here can be applied to insecticide resistance and other traits in haplodiploid arthropods.

Keywords: Bemisia tabaci, inheritance, pyriproxyfen, biotype, statistical genetics, haplodiploid

(Accepted 14 July 2008)

Introduction

Evolution of insecticide resistance is a global problem that provides some of the most compelling examples of adaptation by natural selection (Denholm & Rowland, 1992; Onstad, 2007; Tabashnik et al., 2008). Although nearly all techniques for analyzing inheritance of insecticide resistance focus on diploid insects, haplodiploidy is a key factor influencing evolution of resistance and other traits in some important arthropod pests, including spider mites, thrips and whiteflies (Denholm et al., 1998; Carrière, 2003; Crowder et al., 2006). Here, we develop techniques for estimating the number of genes contributing to insecticide resistance in haplodiploid arthropods and apply them to the whitefly, Bemisia tabaci (Gennadius), one of the world’s most adaptable and invasive agricultural pests (Byrne & Bellows, 1991; Brown et al., 1995).

In haplodiploid species such as B. tabaci that reproduce by arrhenotoky, unmated females produce only haploid male offspring, and mated females produce haploid male and diploid female offspring (Bull, 1983; Byrne & Devonshire, 1996; Heimpel & de Boer, 2008). The inheritance of insecticide resistance in arrhenotokous species can be estimated by analyzing the response of F2 male progeny of hybrid F1 females (e.g. Brown et al., 1991; Herron & Rophail, 1993; Horowitz et al., 2003). This type of analysis can also be applied to haplodiploid species with males that develop from fertilized diploid eggs that lose their paternal chromosomes in development (Heimpel & de Boer, 2008).
**Materials and methods**

**Strains**

The susceptible strain (YUMA 04-S) of the B biotype was derived from a cotton field in Yuma, AZ in 2004 and has since been reared on cotton plants without exposure to insecticides. The resistant strain (QC-02) of the B biotype was derived from cotton fields in Queen Creek, AZ in 2002. Two selections with a pyriproxyfen concentration of 0.1 μg ml⁻¹ resulted in a 1000-fold increase in resistance in this strain (Dennehy et al., 2004). Since April 2003, this strain has been reared on cotton plants sprayed with 1.0 μg ml⁻¹ of pyriproxyfen.

The susceptible strain (ALM-1) of the Q biotype was collected from tomato fields near Almeria, Spain in 1994, and the resistant strain (Pyri-R) was collected in southwestern Israel from a rose greenhouse in 1992 (Horowitz et al., 2003). Soon after it was collected, bioassays showed that Pyri-R was highly resistant to pyriproxyfen (>500-fold resistance). Since 1992, this strain has been reared on cotton plants sprayed with 20 μg ml⁻¹ of pyriproxyfen.

**Insect types**

We tested susceptible males, resistant males and F₂ male progeny of F₁ females from reciprocal crosses between the strains. For the reciprocal crosses, groups of ten virgin females of one strain (susceptible or resistant) were paired with 15 males of the other strain in a 20-ml scintillation vial containing agar and a cotton leaf disk to mate for 48 h. F₁ females resulting from these crosses were used in bioassays. The responses of F₂ male progeny of F₁ females were pooled, as maternal effects were not associated with pyriproxyfen resistance in either resistant strain tested (Horowitz et al., 2004, Crowder et al., 2008).

**Bioassays**

For B biotype strains, excised cotton seedlings with one true leaf were dipped for 20 s in 0–320 μg ml⁻¹ of formulated pyriproxyfen (Knack™ 0.86 EC; Valent USA). Ten three-day-old virgin females (susceptible, resistant, or F₁) were aspirated into Petri dishes, with stems held in water (Crowder et al., 2008). The virginity of adult females was assured by sexing and isolating females as nymphs (Horowitz et al., 2003). Seedlings with adults were held in growth chambers (27°C, 50% RH, 16:8 h light:dark) for 48 h, after which adults were removed, eggs were counted and seedlings were placed in 20-ml scintillation vials containing tap water. To assess mortality, live nymphs were counted seven days after oviposition. This bioassay was conducted from March to April 2007. For each insect type, 4–8 replicates were performed with each concentration.

The bioassay methods for Q biotype strains (conducted in 1999 and 2000) were similar to those for the B biotype with the following modifications: (i) bioassays were conducted in clip-cages on whole cotton plants at the 3–4 node stages; (ii) adults laid eggs on untreated leaves for 48 h, after which eggs were counted and leaves were dipped for ten seconds in 0–100 μg ml⁻¹ of formulated pyriproxyfen; and (iii) egg hatch was checked after ten days. The response of F₂ male progeny of virgin F₁ females was tested the same as for the B biotype. However, for the Q biotype, the response of susceptible and resistant male progeny from virgin females were not tested as in the B biotype, only the response of susceptible and resistant strains (pooled males and females) was measured. Thus, for all subsequent analyses, statistical models for the Q biotype were based on the response of the susceptible and resistant strains.

**Data analysis**

Mortality observed at each pyriproxyfen concentration was corrected for control mortality (Abbott, 1925). Probit analysis (PROC PROBIT: SAS Institute, 2002) was used to estimate slopes of the concentration-mortality lines and their standard errors, as well as the LC₅₀ values and their 95% fiducial limits. LC₅₀ values were considered significantly different if their 95% fiducial limits did not overlap. Resistance ratios of each insect type were calculated as their LC₅₀ divided by the LC₅₀ of the susceptible strain of the same biotype.

**Tests of monogenic and polygenic models**

We modified models for a diploid species (Tabashnik, 1991; Tabashnik et al., 1992) to estimate the number of loci affecting resistance in a haplodiploid pest based on the response of F₂ males. In models with one, two, five or ten loci affecting resistance, we assumed that each locus had one allele for susceptibility (S) and one for resistance (R). Models calculated expected mortality of F₂ males at each concentration tested. In the one-locus model, the genotype of F₁ females was S and 50% of F₂ males were S and 50% were R. The expected mortality of F₂ males at concentration x = (50%×mortality of S at x + 50%×mortality of R at x). The LC₅₀ and slopes for susceptible (S) or resistant (R) males were based on the bioassay data.

In the two-locus model, the F₂ male progeny of F₁ females (genotype S₁R₁S₁R₂) were 25% S₁S₂, 25% S₁R₂, 25% R₁S₁ and
Effective number of loci affecting resistance

We used Lande’s (1981) method, adapted for haplodiploid species (Jones, 2001), to estimate the minimum number of loci \( n_L \) involved in resistance in both biotypes as follows:

\[
n_L = \frac{\left( \mu_{P_S} - \mu_{P_R} \right)^2}{4\sigma_S^2} < n
\]  

(1)

where \( \mu_{P_S} \) and \( \mu_{P_R} \) are the log(LC50) for resistant and susceptible strains, respectively. The actual number of genes is \( n \). The extra genetic variance segregating in \( F_2 \) males beyond that in \( F_1 \) females, \( \sigma_S^2 \), was estimated as:

\[
\sigma_S^2 = \sigma_{F_2}^2 - \frac{1}{2} \sigma_{F_1}^2 + \frac{1}{4} \sigma_{P_R}^2 + \frac{1}{4} \sigma_{P_R}^2
\]  

(2)

where \( \sigma_S^2 \), \( \sigma_{F_2}^2 \), \( \sigma_{F_1}^2 \), \( \sigma_{P_R}^2 \), and \( \sigma_{P_R}^2 \) were, respectively, the phenotypic variances of \( F_2 \) males, \( F_1 \) females, the susceptible strain and the resistant strain. Each variance was estimated as the inverse of the squared slope (Tabashnik et al., 1992).

\begin{table}[h]
\centering
\caption{Responses to pyriproxyfen for the B and Q biotypes of \textit{B. tabaci}.}
\begin{tabular}{lllll}
\hline
Insect type & \( n \) & Slope ± SE & LC50 (95\% FL) (\( \mu g \) ml\(^{-1} \)) & RR \\
\hline
\textit{B} Biotype & & & & \\
Susceptible male & 1005 & 0.59 ± 0.070 & 0.0061 (0.0023–0.013) & 1 \\
Resistant male & 1391 & 1.9 ± 0.70 & 14 (8.7–21) & 2300 \\
\( F_2 \) male & 3860 & 0.58 ± 0.079 & 0.77 (0.39–1.7) & 130 \\
\textit{Q} Biotype & & & & \\
Susceptible strain & 1837 & 2.3 ± 0.13 & 0.0020 (0.0018–0.0023) & 1 \\
Resistant strain & 3230 & 1.8 ± 0.083 & 14 (10–17) & 6900 \\
\( F_2 \) male & 9031 & 0.50 ± 0.064 & 0.16 (0.061–0.33) & 80 \\
\hline
\end{tabular}
\end{table}
For the B biotype, the LC\textsubscript{50} and slopes for parental strains were based on empirical data for males, as Crowder et al. (2008) observed no differences between males and females of either the QC-02 or Yuma 04-S strains. The variance for F\textsubscript{1} females was measured in assays for the B (Crowder et al., 2008) and Q biotypes (Horowitz et al., 2003).

Results

Resistance levels

Relative to their susceptible counterparts, males of the resistant QC-02 strain (B biotype) were highly resistant to pyriproxyfen, as were individuals of the resistant Pyri-R strain (Q biotype) (table 1, fig. 1). The LC\textsubscript{50} values for F\textsubscript{2} males were intermediate between the susceptible and resistant individuals for both biotypes (table 1, fig. 1).

Indirect tests of monogenic and polygenic models

B biotype

The average absolute difference between observed and expected mortality was lowest for the two-locus model (8.79\%) and highest for the one-locus model (13.9\%) (table 2, fig. 2). After accounting for concentration (\(t\textsubscript{35} = 1.99, P = 0.054\)), the two-locus model had a significantly better fit to the observed data than the one-locus model (\(t\textsubscript{35} = 2.30, P = 0.028\)) and was marginally better than the five- (\(t\textsubscript{35} = 1.41, P = 0.16\)) and ten-locus models (\(t\textsubscript{35} = 1.68, P = 0.10\)). The ranking of models was consistent in tests with various values for slope and resistance ratio (table 3). For the ten concentrations tested, significant deviation of observed versus expected mortality occurred at five concentrations for the two-locus model, nine concentrations for the one-locus model, and eight concentrations for the five- and ten-locus models (table 2).

For two-locus models, the fit to the observed data improved in two of the additive models with unequal effects of loci (table 4). The best fit was obtained with a major locus conferring 468-fold resistance and a minor locus conferring fivefold resistance (fig. 3). The average absolute difference between observed and expected mortality with this model was 7.52\%, compared to 8.79\% in a model with equal effects of two loci. One of the four models with epistasis was a better fit to the observed data than the additive and equal two-locus model (table 5). In this model (model C), the
The average absolute difference between observed and expected mortality was 7.45% (fig. 3). For the ten concentrations tested, significant deviation of observed versus expected mortality occurred at four concentrations for the model with a major locus conferring 468-fold resistance and a minor locus conferring fivefold resistance and two concentrations with model C, indicating that both models fit the observed data at more concentrations than the model with equal effects of two loci (fig. 3).

Q biotype

The average absolute difference between observed and expected mortality was lowest for models of one (8.13%) and two loci (8.02%) and highest for models of five (12.7%) and ten loci (15.2%) (table 2, fig. 4). After accounting for concentration ($t_{63} = -2.84, P = 0.0061$), the one-locus model provided a significantly better fit to the observed data than the five ($t_{63} = 1.86, P = 0.067$) and ten-locus models ($t_{63} = 2.38, P = 0.021$) but was not different from the two-locus model ($t_{63} = 0.25, P = 0.80$). The ranking of models was consistent in tests with various values for slope and resistance ratio (table 3). For the 17 concentrations tested, significant deviation of observed versus expected mortality occurred at ten and nine concentrations for the one- and two-locus models, respectively, and 16 concentrations for the five- and ten-locus models (table 2).

For two-locus models, the fit to the observed data improved in three of the additive models with unequal effects of loci (table 4). The best fit occurred with a major locus conferring 343-fold resistance and a minor locus conferring 20-fold resistance. The average absolute difference between observed and expected mortality with this model was 7.18%, compared to 8.02% in a model with equal effects of two loci. None of the models with epistasis provided a better fit to the observed data than the additive and equal two-locus model (table 5).

Effective number of loci affecting resistance

The LC$_{50}$ and slope (± SE) of the probit lines for F$_2$ males, susceptible and resistant individuals for the B and Q biotypes are shown in table 1. The slope of F$_1$ females was 1.2 ± 0.29 for the B biotype (Crowder et al., 2008) and 1.5 ± 0.11 for the Q biotype (Horowitz et al., 2003). Based on these data, the estimated minimum number of loci affecting resistance was 1.6 in the B biotype and 1.0 in the Q biotype, consistent with model results.

Discussion

Our approach represents a new method to examine the inheritance of insecticide resistance in a haplodiploid
arthropod. In haplodiploid species, if resistance is controlled by two alleles at a single locus (S for susceptibility and R for resistance), the F2 male progeny of virgin F1 females (genotype SR) are expected to be 50% S and 50% R. Analyzing the response of F2 males from virgin F1 females eliminates the need for backcrosses or crosses between F1 males and females to examine the mode of inheritance.

Table 3. Effects of number of loci, slope and resistance ratio on the absolute value of the mean difference (%) between observed and expected mortality across ten pyriproxyfen concentrations for F2 males of the B biotype of B. tabaci.

<table>
<thead>
<tr>
<th>Slope</th>
<th>B biotype</th>
<th>One locus</th>
<th>Two loci</th>
<th>Five loci</th>
<th>Ten loci</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.59</td>
<td>13.4</td>
<td>9.48</td>
<td>11.4</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>1.23</td>
<td>14.3</td>
<td>10.7</td>
<td>12.6</td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td>1.87</td>
<td>16.1</td>
<td>11.0</td>
<td>13.7</td>
<td>15.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Resistance ratio = 1170</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.59</td>
<td>14.2</td>
<td>9.36</td>
<td>11.3</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>1.23</td>
<td>14.9</td>
<td>10.5</td>
<td>12.4</td>
<td>13.4</td>
<td></td>
</tr>
<tr>
<td>1.87</td>
<td>17.0</td>
<td>10.9</td>
<td>13.2</td>
<td>15.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Resistance ratio = 2340</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.59</td>
<td>13.4</td>
<td>9.48</td>
<td>11.4</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>1.23</td>
<td>16.0</td>
<td>10.4</td>
<td>12.4</td>
<td>13.1</td>
<td></td>
</tr>
<tr>
<td>1.87</td>
<td>18.3</td>
<td>10.7</td>
<td>12.7</td>
<td>15.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Resistance ratio = 4680</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Fit between observed and expected mortality for additive two-locus models with various relative contributions of each locus.

<table>
<thead>
<tr>
<th>Contribution of locus 1</th>
<th>Contribution of locus 2</th>
<th>% Mean differencea</th>
</tr>
</thead>
<tbody>
<tr>
<td>B biotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48x</td>
<td>48x</td>
<td>8.79</td>
</tr>
<tr>
<td>20x</td>
<td>117x</td>
<td>13.5</td>
</tr>
<tr>
<td>10x</td>
<td>234x</td>
<td>8.99</td>
</tr>
<tr>
<td>5x</td>
<td>468x</td>
<td>7.52</td>
</tr>
<tr>
<td>2x</td>
<td>1169x</td>
<td>8.16</td>
</tr>
<tr>
<td>Q biotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>83x</td>
<td>83x</td>
<td>8.02</td>
</tr>
<tr>
<td>20x</td>
<td>343x</td>
<td>7.18</td>
</tr>
<tr>
<td>10x</td>
<td>686x</td>
<td>7.20</td>
</tr>
<tr>
<td>5x</td>
<td>1372x</td>
<td>7.45</td>
</tr>
<tr>
<td>2x</td>
<td>3430x</td>
<td>8.23</td>
</tr>
</tbody>
</table>

a Mean absolute difference between observed and expected mortality across all concentrations.

Table 5. Fit between observed and expected mortality for two-locus models with epistasis.

<table>
<thead>
<tr>
<th>Model</th>
<th>Resistance of S1R2 relative to S1S1</th>
<th>Resistance of R1S2 relative to S1S1</th>
<th>% Mean differencea</th>
</tr>
</thead>
<tbody>
<tr>
<td>B biotype</td>
<td>Standardb</td>
<td>48x</td>
<td>48x</td>
</tr>
<tr>
<td>A</td>
<td>1x</td>
<td>1x</td>
<td>24.0</td>
</tr>
<tr>
<td>B</td>
<td>1x</td>
<td>48x</td>
<td>15.8</td>
</tr>
<tr>
<td>C</td>
<td>48x</td>
<td>2340x</td>
<td>7.45</td>
</tr>
<tr>
<td>D</td>
<td>2340x</td>
<td>2340x</td>
<td>11.4</td>
</tr>
<tr>
<td>Q biotype</td>
<td>Standardb</td>
<td>83x</td>
<td>83x</td>
</tr>
<tr>
<td>A</td>
<td>1x</td>
<td>1x</td>
<td>14.8</td>
</tr>
<tr>
<td>B</td>
<td>1x</td>
<td>83x</td>
<td>8.10</td>
</tr>
<tr>
<td>C</td>
<td>83x</td>
<td>6860x</td>
<td>10.2</td>
</tr>
<tr>
<td>D</td>
<td>6860x</td>
<td>6860x</td>
<td>18.3</td>
</tr>
</tbody>
</table>

a Mean absolute difference between observed and expected mortality across all concentrations.

b Standard represents case where both loci contribute equally to resistance (no epistasis).
In diploid species, backcrosses are used to examine the mode of inheritance of a trait (e.g. Tabashnik, 1991; Tabashnik et al., 1992; Alves et al., 2006). Backcrosses between F1 females and males of a parental strain, or crosses between F1 males and females, can also be used to examine the mode of inheritance in a haplodiploid species if the response of F2 males and females can be assessed independently (Goka, 1998; Van Leeuwen et al., 2004). In these cases, the response of F2 females can be analyzed based on the principles for diploids, and F2 males can be analyzed as described above. Thus, data for F2 females and males could both be used to test for mode of inheritance independently. However, for species such as B. tabaci, where the response of F2 females can be analyzed based on the principles for diploids, and F2 males can be analyzed as described above. Thus, data for F2 females and males could both be used to test for mode of inheritance independently. However, for species such as B. tabaci, where the response of females to compounds acting on immature stages cannot be assessed independently of males, analysis of the response of F2 males from virgin F1 females provides a more robust test.

While the backcross method can be used in a haplodiploid species as described above, it has also been incorrectly applied (Omer et al., 1995; Tan et al., 1996). In these studies, F1 females were backcrossed to males of a parental strain, and the response of all F2 progeny (males and females) were analyzed based on the methods for a diploid species. However, haploid males cannot be analyzed the same as diploid males. Because the genotypes in haploid males and diploid females differ, pooling the data for F2 progeny is not an appropriate test.

Our results suggest that resistance is controlled by more than one locus in the QC-02 (B biotype) strain and by one or two loci in the Pyri-R (Q biotype) strain. These results were consistent for a broad spectrum of model parameters. However, even in the best fit models, we observed significant deviation between observed and expected mortality for at least 50% of concentrations tested in both the QC-02 and Pyri-R strains. For the QC-02 strain, our results suggest that the effects of different loci on resistance are unequal and additive (major and minor loci) or non-additive (epistasis). The low slope of the concentration-mortality curve for the Yuma 04-S strain may also have confounded results, as Tabashnik (1991) showed that models are less effective when parental strains have relatively low slopes. For the Pyri-R strain, the deviation between observed and expected mortality may be explained by variation in the concentration-mortality curve for F2 males. For this strain, increasing the sample size to reduce variation may have improved the fit between observed and expected mortality under the one- and two-locus models. Still, results using Lande’s equation (modified for haplodiploids) support the conclusion that resistance is controlled by more than one locus in the QC-02 strain and one or two loci in the Pyri-R strain.

Our results are supported by biochemical and molecular work that indicate that at least two loci are involved in pyriproxyfen resistance in the QC-02 strain (Xianchun Li, 2004).
personal communication). Our results also support those of Horowitz et al. (2003), who suggested that resistance in the Pyri-R strain was controlled by a single locus. They fitted curves to bioassay data for the response of $F_2$ males to pyriproxyfen and observed a broad plateau at 50% mortality, indicating that resistance was monogenic (Horowitz et al., 2003). Their approach differed from the present one in that the test of monogenic resistance was based on visual inspection of the data to see if a plateau of 50% mortality for $F_2$ males occurred at concentrations intermediate to the dose-response curves for the parental strains.

While our results support a conclusion that the mode of inheritance of resistance to pyriproxyfen may differ between the B and Q biotype strains, it is unclear if this difference is responsible for the more rapid evolution of pyriproxyfen resistance in field populations of the Q biotype in Israel compared to the B biotype in Arizona (Horowitz et al., 1999, 2002; Li et al., 2003; Dennehy et al., 2004, 2005). Although the number of loci affecting resistance may differ between the QC-02 strain from Arizona and the Pyri-R strain from Israel, the intensity of resistance was similar; and resistance in both strains is inherited as a partially-dominant, autosomal trait (Horowitz et al., 2003; Crowder et al., 2007, 2008). However, strains of the Q biotype have recently been introduced to the U.S. on ornamental and greenhouse crops (Dennehy et al., 2005). These strains are more resistant to pyriproxyfen and other insecticides than either the QC-02 or Pyri-R strains (Dennehy et al., 2005; T.J. Dennehy, unpublished data). Thus, the mode of inheritance of resistance in introduced strains of the Q biotype may differ from that reported here. Molecular genetic analyses can provide further insight into the mechanisms of resistance in both biotypes by identifying genes associated with resistance.

Results from the tests of models with various numbers of loci and the modified Lande’s equation are based on the assumptions that the logarithm of tolerance is normally distributed for each genotype, that each locus had only two alleles (S or R) and that the parental strains were homozygous for susceptibility or resistance (Lande, 1981; Tabashnik, 1991; Tabashnik et al., 1992). Violations of these assumptions could limit the precision of both methods (Tabashnik et al., 1992). The low slope observed in the YUMA 04-S susceptible strain (B biotype) indicates high phenotypic variation, suggesting that this strain may not be completely homozygous for susceptibility. This could have reduced our ability to distinguish between results with the one and multi-locus models. However, despite this uncertainty, our results strongly suggest that resistance in the QC-02 strain is not monogenic.

We modified our standard models from those of Tabashnik (1991) to account for differences in slope between the susceptible and resistant strains of the B biotype. The threefold differences in the slope of concentration-mortality curves between susceptible and resistant males could have added bias to our models if we had averaged the slope of parental strains in indirect tests, as in Tabashnik (1991). Instead, we used slopes estimated from bioassays of each parental strain and assumed that the slope of concentration-mortality curves for intermediate genotypes were additive. However, qualitative results as to the best-fit models were similar in the sensitivity analysis where we assumed slope of concentration-mortality curves of all genotypes were the average of the parental strains. Results for the Q biotype strain were less dependent on slope than the B biotype strain due to the similarity in slope between susceptible and resistant strains of the Q biotype.

Although the statistical and analytical methods presented here cannot produce definitive conclusions as to the mode of inheritance of pyriproxyfen resistance in strains of the B and Q biotypes, they allowed for testing of alternatives to a monogenic hypothesis. Without such alternatives, there is a high probability of incorrectly accepting or rejecting a monogenic hypothesis (Preisler et al., 1990; Tabashnik, 1991; Tabashnik et al., 1992). The results from such tests can be used as a guide for molecular analysis of resistance, as relative estimates of the number of loci affecting resistance can be obtained. However, studies that link molecular data with bioassay data are needed to provide further tests of the model’s predictive power. The methods presented here can be applied to studies of insecticide resistance in other haplodiploid species, including mites, ticks, thrips and hymenopterans. Through a more detailed statistical analysis of hypotheses, using data commonly collected in inheritance studies, this type of analysis can improve the rigor of studies investigating the mode of inheritance of insecticide resistance or other traits.

Acknowledgements

Thanks to Christa Ellers-Kirk for growing seedlings for the bioassays. The research was funded in part by the United States Environmental Protection Agency under the Science to Achieve Results Graduate Fellowship Program, grant FP-91648901-0, an Achievement Rewards for College Scientists scholarship and NRCGP grant 2005-00925. This publication is not officially endorsed by EPA and may not reflect the views of the agency. Rothamsted Research receives grant-funded support from the Biotechnology and Biological Sciences Research Council of the UK.

References


crosses between the A and B biotypes of *Bemisia tabaci*. *Insect Science and its Application* 14, 255–266.


