Analyzing haplodiploid inheritance of insecticide resistance in whitefly biotypes

D.W. Crowder, A.R. Horowitz, B.E. Tabashnik, T.J. Dennehy, I. Denholm, K. Gorman and Y. Carrière

Analyzing haplodiploid inheritance of insecticide resistance in whitefly biotypes

D.W. Crowder[1]*, A.R. Horowitz[2], B.E. Tabashnik[1], T.J. Dennehy[1], I. Denholm[3], K. Gorman[3], and Y. Carrière[1]

[1]Department of Entomology, 410 Forbes Building, University of Arizona, Tucson, AZ, 85721, USA: [2]Department of Entomology, ARO, Gilat Research Center, M.P. Negev, Israel: [3]Rothamsted 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.

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 F_2 male progeny of hybrid F_1 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).

*Author for correspondence
Fax: 520-621-1150
E-mail: dcrowder@ag.arizona.edu

B. tabaci is a species complex, with numerous biotypes that differ in biological and genetic characteristics (Brown *et al.*, 1995; Perring, 2001). The B and Q biotypes are considered the most harmful by virtue of polyphagy, propensity for resistance and global spread by human transport of infected plant material (Denholm *et al.*, 1998; Horowitz *et al.*, 2005). Resistance to the insect growth regulator, pyriproxyfen, a selective insecticide effective against *B. tabaci* in many countries including the USA and Israel (Ishaaya & Horowitz, 1992; Horowitz & Ishaaya, 1996; Palumbo *et al.*, 2001), has evolved in the B and Q biotypes (Horowitz *et al.*, 1999, 2002; Dennehy *et al.*, 2004, 2005).

Barriers to interbreeding have been found between biotypes of *B. tabaci*, including the B and Q biotypes (Costa *et al.*, 1993; De Barro & Hart, 2000; Rothamsted Research, unpublished data). Information on the genetics of resistance in these biotypes can therefore be used to explore parallels in their architecture and molecular basis and improve resistance management for this pest. Here, we incorporate data from laboratory bioassays and crossing experiments exploiting the unique attributes of haplodiploidy into statistical and analytical models to estimate the number of loci affecting pyriproxyfen resistance in strains of both biotypes.

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 $0.1\,\mu g\,ml^{-1}$ 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 $1.0\,\mu g\,ml^{-1}$ 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 $20\,\mu g\,ml^{-1}$ of pyriproxyfen.

Insect types

We tested susceptible males, resistant males and F_2 male progeny of F_1 females from reciprocal crosses between the strains. For the reciprocal crosses, groups of ten virgin females of one strain (susceptible or resistant) were placed 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. F_1 females resulting from these crosses were used in bioassays. The responses of F_2 male progeny of F_1 females were pooled, as maternal effects were not associated with pyriproxyfen resistance in either resistant strain tested (Horowitz *et al.*, 2003; Crowder *et al.*, 2008).

Bioassays

For B biotype strains, excised cotton seedlings with one true leaf were dipped for 20 s in 0–$320\,\mu g\,ml^{-1}$ of formulated pyriproxyfen (Knack[TM] 0.86 EC; Valent USA). Ten three-day-old virgin females (susceptible, resistant, or F_1) 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–$100\,\mu g\,ml^{-1}$ of formulated pyriproxyfen; and (iii) egg hatch was checked after ten days. The response of F_2 male progeny of virgin F_1 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 LC_{50} values and their 95% fiducial limits. LC_{50} values were considered significantly different if their 95% fiducial limits did not overlap. Resistance ratios of each insect type were calculated as their LC_{50} divided by the LC_{50} 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 F_2 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 F_2 males at each concentration tested. In the one-locus model, the genotype of F_1 females was SR and 50% of F_2 males were S and 50% were R. The expected mortality of F_2 males at concentration x = (50% × mortality of S at x + 50% × mortality of R at x). The LC_{50} and slopes for susceptible (S) or resistant (R) males were based on the bioassay data.

In the two-locus model, the F_2 male progeny of F_1 females (genotype $S_1R_1S_2R_2$) were 25% S_1S_2, 25% S_1R_2, 25% R_1S_2 and

Table 1. Responses to pyriproxyfen for the B and Q biotypes of *B. tabaci*.

Insect type	n	Slope $\pm$ SE	LC$_{50}$ (95% FL) (μg ml^{-1})	RR
B Biotype				
Susceptible male	1005	0.59 $\pm$ 0.070	0.0061 (0.0023–0.013)	1
Resistant male	1391	1.9 $\pm$ 0.70	14 (8.7–21)	2300
F$_2$ male	3860	0.58 $\pm$ 0.079	0.77 (0.39–1.7)	130
Q Biotype				
Susceptible strain	1837	2.3 $\pm$ 0.13	0.0020 (0.0018–0.0023)	1
Resistant strain	3230	1.8 $\pm$ 0.083	14 (10–17)	6900
F$_2$ male	9031	0.50 $\pm$ 0.064	0.16 (0.061–0.33)	80

25% R$_1$R$_2$. In this model, the LC$_{50}$ and slope for S$_1$S$_2$ and R$_1$R$_2$ were those measured in susceptible and resistant males, respectively. The log(LC$_{50}$) and slope for S$_1$R$_2$ and R$_1$S$_2$ was the mean of the log(LC$_{50}$) and slope in susceptible and resistant males (Tabashnik, 1991). In the five- and ten-locus models, the genotype frequency of F$_2$ males was based on the binomial distribution. In these cases, the LC$_{50}$ and slope of the most susceptible and resistant genotypes (homozygous at all loci) was the LC$_{50}$ and slope for susceptible and resistant males. The log(LC$_{50}$) and slope for intermediate genotypes were additive between the log(LC$_{50}$) and slopes in susceptible and resistant males (Tabashnik, 1991; Tabashnik *et al.*, 1992). For example, the log(LC$_{50}$) for genotype S$_1$S$_2$S$_3$R$_4$R$_5$ = (3 $\times$ log[LC$_{50}$][susceptible male] + 2 $\times$ log[LC$_{50}$][resistant male])/5 and the slope = (3 $\times$ slope[susceptible male] + 2 $\times$ slope[resistant male])/5. For all genotypes, expected mortality at each concentration was estimated based on the LC$_{50}$ and slope for that genotype.

Modeling was done in SAS (SAS Institute, 2002). For models of five and ten loci, the effects of loci were equal and additive on a logarithmic scale (Tabashnik *et al.*, 1992). For two loci, we tested effects of loci equal and additive (as above), plus four additive models with unequal effects of each locus (major and minor locus) and four models with epistasis (nonadditive interactions between loci) (Tabashnik *et al.*, 1992). In the models with epistasis, the phenotypes of S$_1$R$_2$ and R$_1$S$_2$ varied among models.

We used 2 $\times$ 2 contingency tables to test for significant deviation between observed and expected mortality at each concentration (Sokal & Rohlf, 1995). In addition, we calculated the average absolute difference between observed and expected mortality as the mean difference between observed and expected mortality (absolute values) across all concentrations. We used multiple regression to determine the effects of the modeled number of loci and concentration (log transformed) on the average absolute difference between observed and expected mortality (log transformed). As empirical estimates of LC$_{50}$ and slopes have inherent uncertainty, we also performed a sensitivity analysis by varying the slope and resistance ratios in models. Unlike the methods above, in these tests we assumed that the slope for all genotypes were equal.

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_E) involved in resistance in both biotypes as follows:

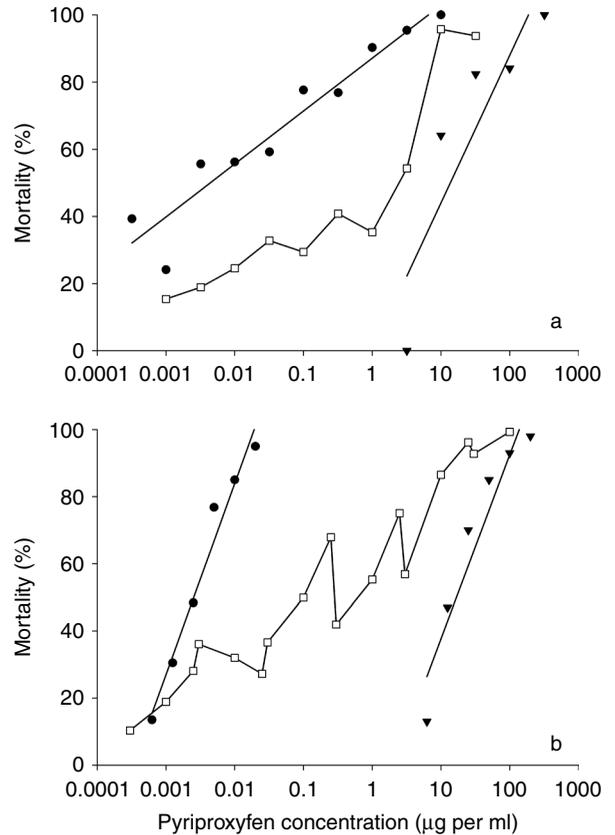$$n_e = (\mu_{P_2} - \mu_{P_1})^2 / (4\sigma_S^2) \leq n \qquad (1)$$

Fig. 1. Mortality caused by pyriproxyfen for the (a) B biotype and (b) Q biotypes. For the B biotype (a), responses shown are for males only. For the Q biotype (b), responses shown are for the susceptible and resistant strains (males and females combined) and F$_2$ males (–●–, susceptible; –▼–, resistant; –□–, F$_2$).

where μ_{P_2} and μ_{P_1} are the log(LC$_{50}$) 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, σ_s^2, was estimated as:

$$\sigma_S^2 = \sigma_{F_2}^2 - \left(\frac{1}{2}\sigma_{F_1}^2 + \frac{1}{4}\sigma_{P_1}^2 + \frac{1}{4}\sigma_{P_2}^2\right) \qquad (2)$$

where σ_s^2, $\sigma_{F_2}^2$, $\sigma_{F_1}^2$, $\sigma_{P_1}^2$ and $\sigma_{P_2}^2$ 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).

D.W. Crowder *et al.*

Table 2. Tests for deviation between observed and expected mortality (df = 1) for F_2 males of the B and Q biotypes of *B. tabaci* with monogenic and additive polygenic models.

Concn (μg ml^{-1})	Genetic model							
	One locus		Two loci		Five loci		Ten loci	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P
B Biotype								
0.0010	5.8	0.016[a]	0.30	0.58	11	0.0011[a]	16	< 0.0001[a]
0.0032	11	0.0011[a]	0.54	0.46	10	0.0015[a]	18	< 0.0001[a]
0.010	10	0.0013[a]	0.65	0.42	4.9	0.026[a]	10	0.0012[a]
0.032	3.2	0.073	0.0030	0.95	1.2	0.27	2.4	0.12
0.10	33	< 0.0001[a]	23	< 0.0001[a]	17	< 0.0001[a]	15	< 0.0001[a]
0.32	17	< 0.0001[a]	16	< 0.0001[a]	17	< 0.0001[a]	17	< 0.0001[a]
1.0	47	< 0.0001[a]	61	< 0.0001[a]	74	< 0.0001[a]	76	< 0.0001[a]
3.2	11	0.0012[a]	32	< 0.0001[a]	48	< 0.0001[a]	58	< 0.0001[a]
10	66	< 0.0001[a]	22	< 0.0001[a]	7.6	0.0057[a]	2.4	0.13
32	26	< 0.0001[a]	3.6	0.057	1.4	0.23[a]	0.98	0.0064[a]
Mean difference (%)[b]		13.9		8.79		11.3		13.2
Q Biotype								
0.00030	1.4	0.24	3.1	0.078	5.0	0.025[a]	5.6	0.018[a]
0.0010	0.089	0.77	6.2	0.013[a]	21	< 0.0001[a]	24	< 0.0001[a]
0.0025	0.02	0.89	8.5	0.0035[a]	25	< 0.0001[a]	33	< 0.0001[a]
0.0030	0.97	0.33	11	0.0012[a]	24	< 0.0001[a]	30	< 0.0001[a]
0.010	7.6	0.0060[a]	9.5	0.0020[a]	27	< 0.0001[a]	44	< 0.0001[a]
0.025	32	< 0.0001[a]	0.60	0.43	1.4	0.24	5.3	0.021[a]
0.030	4.8	0.029[a]	0.60	0.44	4.1	0.044[a]	7.0	0.0080[a]
0.10	0.0010	0.97	2.6	0.11	4.7	0.031[a]	8.3	0.0039[a]
0.25	12	0.0006[a]	7.2	0.0075[a]	5.3	0.022[a]	4.0	0.044[a]
0.30	3.6	0.059	10	0.0016[a]	16	< 0.0001[a]	3.1	0.078
1.0	4.2	0.041[a]	67	< 0.0001[a]	65	< 0.0001[a]	20	< 0.0001[a]
2.5	30	< 0.0001[a]	1.0	0.31	15	0.0001[a]	36	< 0.0001[a]
3.0	0.094	0.76	27	< 0.0001[a]	58	< 0.0001[a]	81	< 0.0001[a]
10	58	< 0.0001[a]	0.12	0.73	44	< 0.0001[a]	94	< 0.0001[a]
25	37	< 0.0001[a]	8.2	0.0043[a]	5.6	0.018[a]	14	0.0002[a]
30	7.8	0.0051[a]	0.037	0.85	11	0.0008[a]	16	< 0.0001[a]
100	12	0.0006[a]	1.4	0.24	5.3	0.021[a]	10	0.0015[a]
Mean difference(%)[b]		8.13		8.02		12.7		15.2

[a] Probability values indicating significant differences between the observed and expected mortality ($P < 0.05$).
[b] Mean difference was calculated as the mean of the difference between observed and expected mortality (absolute values) across all concentrations tested.

For the B biotype, the LC$_{50}$ 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_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$_{50}$ values for F_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 ($t_{35} = 1.99$, $P = 0.054$), the two-locus model had a significantly better fit to the observed data than the one-locus model ($t_{35} = 2.30$, $P = 0.028$) and was marginally better than the five- ($t_{35} = 1.41$, $P = 0.16$) and ten-locus models ($t_{35} = 1.68$, $P = 0.10$). 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
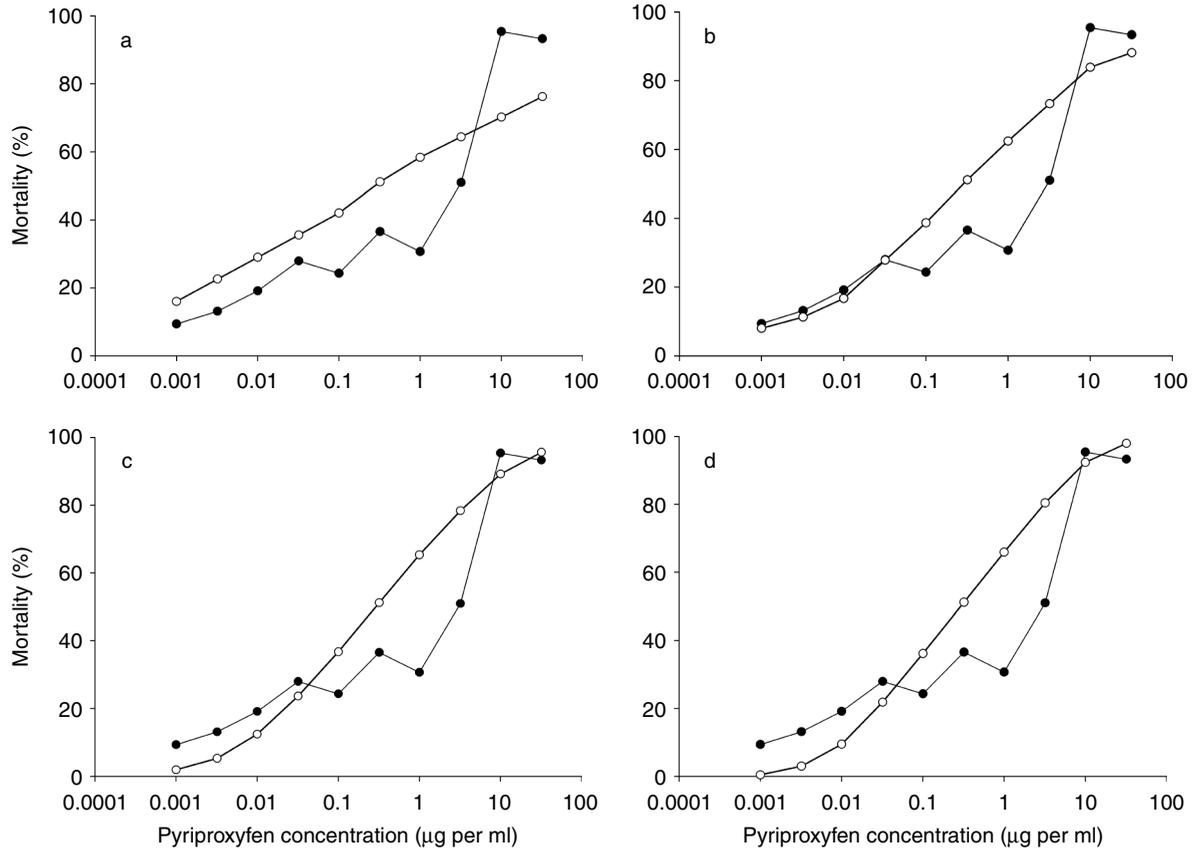
Fig. 2. Observed vs. expected mortality at each of ten pyriproxyfen concentrations tested for F_2 males of the B biotype of *B. tabaci* ((a) –●–, observed; –○–, one locus; (b) –●–, observed; –○–, two loci; (c) –●–, observed; –○–, five loci; (d) –●–, observed; –○–, ten loci).

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 ($\pm SE$) 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

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 F_2 males of the B biotype of *B. tabaci*.

Slope	B biotype			
	One locus	Two loci	Five loci	Ten loci
	Resistance ratio = 1170			
0.59	13.4	9.48	11.4	10.9
1.23	14.3	10.7	12.6	13.6
1.87	16.1	11.0	13.7	15.9
	Resistance ratio = 2340			
0.59	14.2	9.36	11.3	10.9
1.23	14.9	10.5	12.4	13.4
1.87	17.0	10.9	13.2	15.5
	Resistance ratio = 4680			
0.59	13.4	9.48	11.4	10.9
1.23	16.0	10.4	12.4	13.1
1.87	18.3	10.7	12.7	15.2
Slope	Q biotype			
	One locus	Two loci	Five loci	Ten loci
	Resistance ratio = 3430			
1.82	8.23	9.53	14.4	16.1
2.05	8.35	9.69	14.7	16.7
2.28	8.60	9.85	15.0	17.0
	Resistance ratio = 6860			
1.82	8.64	8.76	13.5	15.7
2.05	8.89	8.85	13.8	16.2
2.28	9.07	9.00	14.0	16.6
	Resistance ratio = 12,290			
1.82	9.78	8.46	12.7	15.3
2.05	10.1	8.50	13.0	15.8
2.28	10.3	8.57	13.2	16.1

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

Contribution of locus 1	Contribution of locus 2	% Mean difference[a]
B biotype		
48×	48×	8.79
20×	117×	13.5
10×	234×	8.99
5×	468×	7.52
2×	1169×	8.16
Q biotype		
83×	83×	8.02
20×	343×	7.18
10×	686×	7.20
5×	1372×	7.45
2×	3430×	8.23

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

arthropod. In haplodiploid species, if resistance is controlled by two alleles at a single locus (S for susceptibility and R for resistance), the F_2 male progeny of virgin F_1 females (genotype SR) are expected to be 50% S and 50% R. Analyzing the response of F_2 males from virgin F_1 females eliminates the need for backcrosses or crosses between F_1 males and females to examine the mode of inheritance.
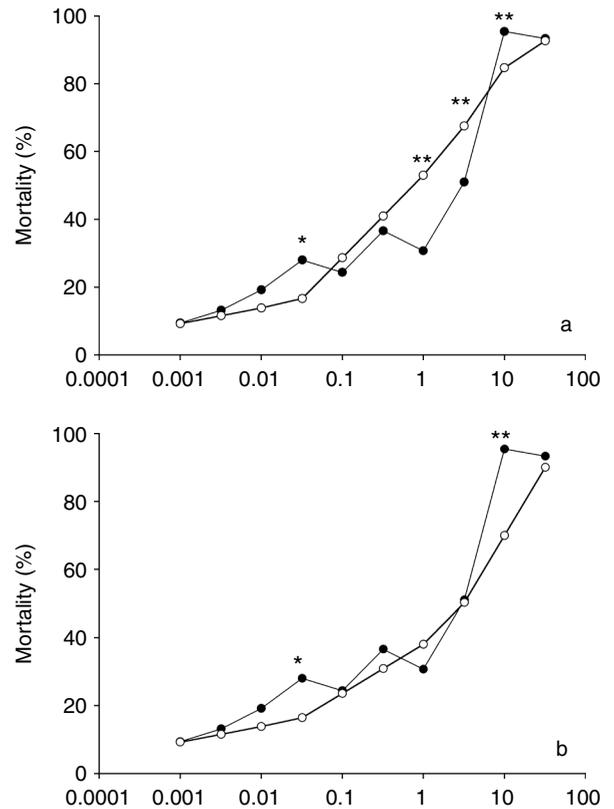

Fig. 3. Observed vs. expected mortality for F_2 males of the B biotype with (a) a model with a major locus conferring 468-fold resistance and a minor locus conferring five-fold resistance and (b) epistasis model C (resistance additive at one locus and dominant at a second locus). 2×2 contingency table analyses were used to test for significant deviation between observed and expected mortality at each concentration (*: $P < 0.05$; **: $P < 0.001$) (–●–, observed; –○–, expected).

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

Model	Resistance of S_1R_2 relative to S_1S_1	Resistance of R_1S_2 relative to S_1S_1	% Mean difference[a]
B biotype			
Standard[b]	48×	48×	8.79
A	1×	1×	24.0
B	1×	48×	15.8
C	48×	2340×	7.45
D	2340×	2340×	11.4
Q biotype			
Standard[b]	83×	83×	8.02
A	1×	1×	14.8
B	1×	83×	8.10
C	83×	6860×	10.2
D	6860×	6860×	18.3

[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).
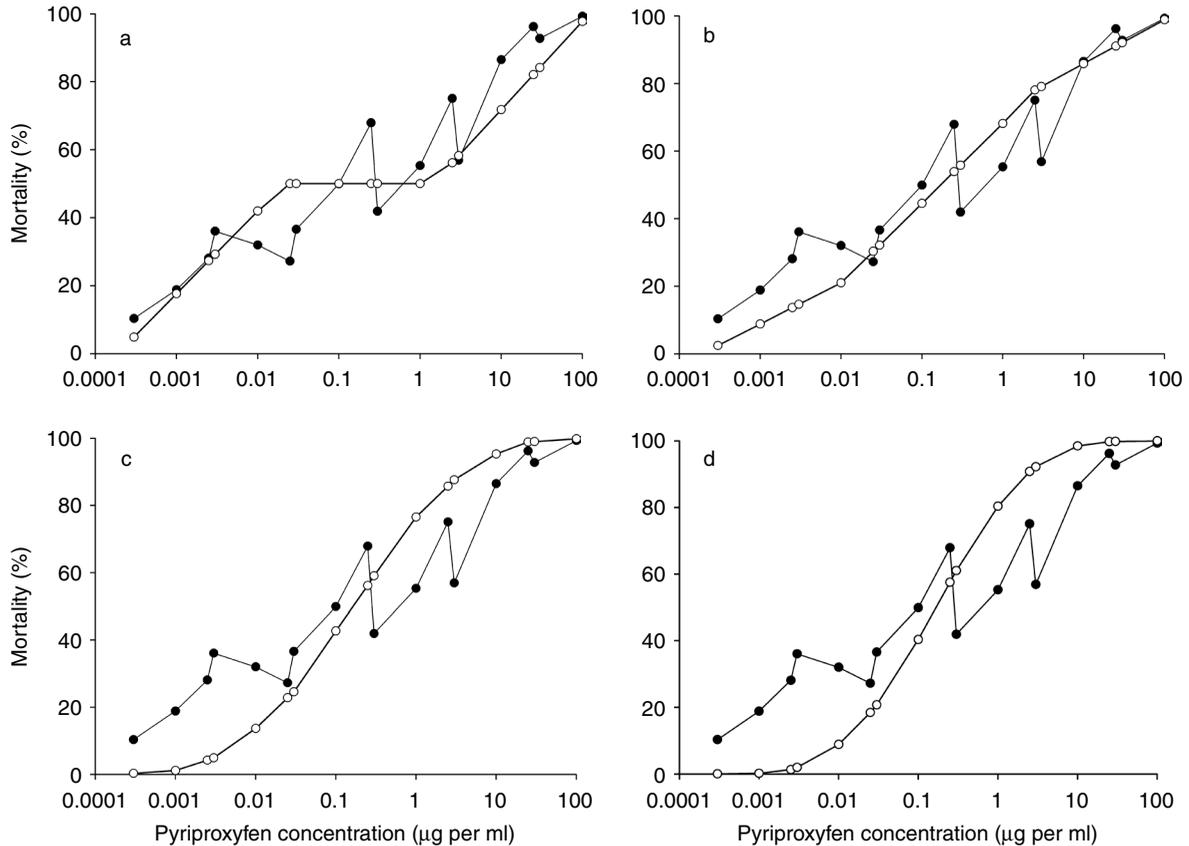
Fig. 4. Observed vs. expected mortality at each of 17 pyriproxyfen concentrations tested for F$_2$ males of the Q biotype of *B. tabaci* ((a) –●–, observed; –○–, one locus; (b) –●–, observed; –○–, two loci; (c) –●–, observed; –○–, five loci; (d) –●–, observed; –○–, ten loci).

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 F$_1$ females and males of a parental strain, or crosses between F$_1$ males and females, can also be used to examine the mode of inheritance in a haplodiploid species if the response of F$_2$ males and females can be assessed independently (Goka, 1998; Van Leeuwen *et al.*, 2004). In these cases, the response of F$_2$ females can be analyzed based on the principles for diploids, and F$_2$ males can be analyzed as described above. Thus, data for F$_2$ 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 F$_2$ males from virgin F$_1$ 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, F$_1$ females were backcrossed to males of a parental strain, and the response of all F$_2$ 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 F$_2$ 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 F$_2$ 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,

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

References

Abbott, W.S. (1925) A method for computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**, 265–267.

Alves, A.P., Spencer, T.A., Tabashnik, B.E. & Siegfried, B.D. (2006) Inheritance of resistance to the Cry1Ab *Bacillus thuringiensis* toxin in *Ostrinia nubilalis* (Lepidoptera: Crambidae). *Journal of Economic Entomology* **99**, 494–501.

Brown, E.J., Cave, F.E. & Hoy, M.A. (1991) Mode of inheritance of azinphosmethyl resistance in a laboratory-selected strain of *Trioxys pallidus*. *Entomologia Experimentalis et Applicata* **63**, 229–236.

Brown, J.K., Frohlich, D.R. & Rosell, R.C. (1995) The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? *Annual Review of Entomology* **40**, 511–534.

Bull, J.J. (1983) *Evolution of Sex Determining Mechanisms*. 316 pp. Menlo Park, Benjamin/Cummings.

Byrne, D.N. & Bellows, T.S. (1991) Whitefly biology. *Annual Review of Entomology* **36**, 431–457.

Byrne, F.J. & Devonshire, A.L. (1996) Biochemical evidence of haplodiploidy in the whitefly *Bemisia tabaci*. *Biochemical Genetics* **34**, 93–107.

Carrière, Y. (2003) Haplodiploidy, sex, and the evolution of pesticide resistance. *Journal of Economic Entomology* **96**, 1626–1640.

Costa, H.S., Brown, J.K., Sivasupramaniam, S. & Bird, J. (1993) Regional distribution, insecticide resistance, and reciprocal

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

Crowder, D.W., Carrière, Y., Tabashnik, B.E., Ellsworth, P.C. & Dennehy, T.J. (2006) Modeling evolution of resistance to pyriproxyfen by the sweetpotato whitefly (Hemiptera: Aleyrodidae). *Journal of Economic Entomology* **99**, 1396–1406.

Crowder, D.W., Dennehy, T.J., Ellers-Kirk, C., Yafuso, C., Ellsworth, P.C., Tabashnik, B.E. & Carrière, Y. (2007) Field evaluation of resistance to pyriproxyfen in *Bemisia tabaci* (B biotype). *Journal of Economic Entomology* **100**, 1650–1656.

Crowder, D.W., Ellers-Kirk, C., Yafuso, C., Dennehy, T.J., Degain, B.A., Harpold, V.S., Tabashnik, B.E. & Carrière, Y. (2008) Inheritance of resistance to pyriproxyfen in *Bemisia tabaci* (Hemiptera: Aleyrodidae) males and females (B biotype). *Journal of Economic Entomology* **101**, 927–932.

De Barro, P.J. & Hart, P.J. (2000) Mating interactions between two biotypes of the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Australia. *Bulletin of Entomological Research* **90**, 103–112.

Denholm, I. & Rowland, M.W. (1992) Tactics for managing pesticide resistance in arthropods – theory and practice. *Annual Review of Entomology* **37**, 91–112.

Denholm, I., Cahill, M., Dennehy, T.J. & Horowitz, A.R. (1998) Challenges with managing insecticide resistance in agricultural pests, exemplified by the whitefly *Bemisia tabaci*. *Philosophical Transactions of the Royal Society of London Series B* **353**, 1757–1767.

Dennehy, T.J., Degain, B.A., Harpold, V.S. & Brink, S.A. (2004) Whitefly resistance to insecticides in Arizona: 2002 and 2003 results. pp. 1926–1938 in *Proceedings of the Beltwide Cotton Conference*. Memphis, National Cotton Council, 5–9th January 2004, San Antonio TX.

Dennehy, T.J., Degain, B.A., Harpold, V.S., Brown, J.K., Morin, S., Fabrick, J.A. & Nichols, R.L. (2005) New challenges to management of whitefly resistance to insecticides in Arizona. The University of Arizona Cooperative Extension Vegetable Report. http://ag.arizona.edu/pubs/crops/az1382/az1382_2.pdf.

Goka, K. (1998) Mode of inheritance of resistance to three new acaricides in the Kanzawa spider mite, *Tetranychus kanzawai* Kishida (Acari: Tetranychidae). *Experimental and Applied Acarology* **22**, 699–708.

Heimpel, G.E. & de Boer, J.G. (2008) Sex determination in the hymenoptera. *Annual Review of Entomology* **53**, 209–230.

Herron, G.A. & Rophail, J. (1993) Genetics of hexythiazox resistance in two spotted spider mite, *Tetranychus urticae* Koch. *Experimental and Applied Acarology* **17**, 423–431.

Horowitz, A.R. & Ishaaya, I. (1996) Chemical control of *Bemisia* – management and application. pp. 537–556 in Gerling, D. & Mayer, R.T. (*Eds*) *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management*. Andover, MA, Intercept.

Horowitz, A.R., Mendelson, Z., Cahill, M., Denholm, I. & Ishaaya, I. (1999) Managing resistance to the insect growth regulator, pyriproxyfen, in *Bemisia tabaci*. *Pesticide Science* **55**, 272–276.

Horowitz, A.R., Kontsedalov, S., Denholm, I. & Ishaaya, I. (2002) Dynamics of insecticide resistance in *Bemisia tabaci*: a case study with an insect growth regulator. *Pest Management Science* **58**, 1096–1100.

Horowitz, A.R., Gorman, K., Ross, G. & Denholm, I. (2003) Inheritance of pyriproxyfen resistance in the whitefly, *Bemisia tabaci* (Q biotype). *Archives of Insect Biochemistry and Physiology* **54**, 177–186.

Horowitz, A.R., Kontsedalov, S., Khasdan, V. & Ishaaya, I. (2005) Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. *Archives of Insect Biochemistry and Physiology* **58**, 216–225.

Ishaaya, I. & Horowitz, A.R. (1992) Novel phenoxy juvenile hormone analog (pyriproxyfen) suppresses embryogenesis and adult emergence of sweetpotato whitefly (Homoptera, Aleyrodidae). *Journal of Economic Entomology* **85**, 2113–2117.

Jones, C.D. (2001) Extension of the Castle-Wright effective factor estimator to sex linkage and haplodiploidy. *Journal of Heredity* **92**, 274–276.

Lande, R. (1981) The minimum number of genes contributing to quantitative variation between and within populations. *Genetics* **99**, 541–553.

Li, A.Y., Dennehy, T.J. & Nichols, R.L. (2003) Baseline susceptibility and development of resistance to pyriproxyfen in *Bemisia argentifolii* (Homoptera: Aleyrodidae) in Arizona. *Journal of Economic Entomology* **96**, 1307–1314.

Omer, A.D., Tabashnik, B.E. & Johnson, M.W. (1995) Inheritance of dicrotophos resistance in greenhouse whitefly. *Entomologica Experimentalis et Applicata* **77**, 177–181.

Onstad, D.W. (2007) *Insect Resistance Management: Biology, Economics, and Prediction*. 305 pp. New York, Elsevier Science.

Palumbo, J.C., Horowitz, A.R. & Prabhaker, N. (2001) Insecticidal control and resistance management for *Bemisia tabaci*. *Crop Protection* **20**, 739–765.

Perring, T.M. (2001) The *Bemisia tabaci* species complex. *Crop Protection* **20**, 725–737.

Preisler, H.K., Hoy, M.A. & Robertson, J.A. (1990) Statistical analysis of modes of inheritance for pesticide resistance. *Journal of Economic Entomology* **83**, 1649–1655.

SAS Institute (2002) SAS, Ver. 8. 2nd edn. SAS Institute, Cary, NC, USA.

Sokal, R.R. & Rohlf, F.J. (1995) *Biometry*. 887 pp. New York, W.H. Freeman & Company.

Tabashnik, B.E. (1991) Determining the mode of inheritance of pesticide resistance with backcross experiments. *Journal of Economic Entomology* **84**, 703–712.

Tabashnik, B.E., Schwartz, J.M., Finson, N. & Johnson, M.W. (1992) Inheritance of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology* **85**, 1046–1055.

Tabashnik, B.E., Gassmann, A.J., Crowder, D.W. & Carrière, Y. (2008) Insect resistance to Bt crops: evidence versus theory. *Nature Biotechnology* **26**, 199–202.

Tan, W.-J., Riley, D.R. & Wolfenbarger, D.A. (1996) Quantification and genetic analysis of bifenthrin resistance in the silverleaf whitefly. *Southwestern Entomologist* **21**, 265–275.

Van Leeuwen, T., Stillatus, V. & Tirry, L. (2004) Genetic analysis and cross-resistance spectrum of a laboratory-selected and chlorfenapyr resistant strain of two-spotted spider mite (Acari: Tetranychidae). *Experimental and Applied Acarology* **32**, 249–261.