Research Article

Received: 22 May 2008 Revised: 23 August 2008 Accepted: 26 August 2008 Published online in Wiley Interscience: 18 December 2008

(www.interscience.wiley.com) DOI 10.1002/ps.1674

# Lack of fitness costs associated with pyriproxyfen resistance in the B biotype of *Bemisia tabaci*

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## Abstract

**BACKGROUND: The insect growth regulator pyriproxyfen has provided effective control of the whitefly *Bemisia tabaci* Gennadius in many countries. Here, whether or not fitness costs were associated with pyriproxyfen resistance in a laboratory-selected resistant strain (QC02-R) of the B biotype was determined.**

**RESULTS: Mortality caused by pyriproxyfen and fitness traits over time were measured in unselected and selected hybrid strains, which were created by crossing individuals of the resistant strain with individuals of a susceptible strain. Fitness costs were not associated with resistance in QC02-R, as mortality caused by pyriproxyfen did not increase over time in unselected hybrid strains and fitness traits were similar in unselected and selected hybrid strains. Using a new method to examine the inheritance of resistance, based on data from fitness cost experiments, it was estimated that pyriproxyfen resistance is controlled by two loci in the QC02-R strain.**

**CONCLUSION: The lack of fitness costs associated with pyriproxyfen resistance could promote the evolution of resistance in field populations with similar traits to QC02-R.**


**Keywords:** *Bemisia tabaci*; fitness cost; pyriproxyfen; biotype; number of loci; insecticide resistance

## 1 INTRODUCTION

The whitefly *Bemisia tabaci* Gennadius is one of the world's most adaptable and invasive pests of agriculture.[1,2] Selective insecticides, such as the insect growth regulator pyriproxyfen, have improved control of *B. tabaci* in many countries.[3,4] The most serious threat to the continued effectiveness of pyriproxyfen is the evolution of resistance in *B. tabaci*. Laboratory bioassays tracking the evolution of resistance in the B biotype of *B. tabaci* in Arizona over the last decade indicate declines in susceptibility in some regions.[5–7]

Fitness costs can affect evolution of insecticide resistance.[8–14] Fitness costs occur when, in the absence of exposure to insecticide, individuals with resistance alleles have lower fitness than individuals without resistance alleles. Such costs can occur when resistant mechanisms divert energy and resources away from other fitness traits or when resistance mechanisms alter the normal functioning of target sites.[9,12]

As with diploid insects, models of haplodiploid insects such as *B. tabaci* suggest that fitness costs can delay or prevent the evolution of insecticide resistance when refuges from insecticide exposure are present.[15–17] If such costs occur with pyriproxyfen resistance, rotation of pyriproxyfen with other insecticides or spatial and temporal refuges from pyriproxyfen exposure could help delay the onset of resistance. In pyriproxyfen-resistant strains of *B. tabaci*, fitness costs occur in the Q biotype,[17] but they have not been evaluated previously in the B biotype.

Here, the authors investigated whether fitness costs are associated with resistance in a laboratory-selected pyriproxyfen-resistant strain of the B biotype of *B. tabaci*. Individuals of the resistant strain were crossed with a pyriproxyfen-susceptible strain to create hybrid strains that were reared with or without selection with pyriproxyfen. To determine whether fitness costs were associated with resistance, susceptibility to pyriproxyfen, fecundity and development time were measured over 27 generations in each of the hybrid strains. A method was also developed that compared observed mortality in the fitness cost experiment with theoretical distributions to analyze the number of loci affecting resistance in the QC02-R strain.

## 2 MATERIALS AND METHODS

### 2.1 Insect strains

All *B. tabaci* strains were of the B biotype. The pyriproxyfen-susceptible strain (YM04-S, called Yuma 04-S previously)[18,19] was derived from a cotton field in Yuma, AZ, in 2004, and was reared on cotton plants without exposure to insecticides. The pyriproxyfen-resistant strain (QC02-R, called QC-02 previously)[18,19] was derived from cotton fields in Queen Creek, AZ, in 2002. Laboratory selection with pyriproxyfen over 6 months resulted in an increase in the $LC_{50}$

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of QC02-R from 0.0057 to 6.3 μg mL$^{-1}$ of pyriproxyfen (>1000-fold increase).[6] Starting in April 2003, this strain was reared on cotton plants sprayed with 1.0 μg mL$^{-1}$ of pyriproxyfen. The QC02-R strain had over 600-fold resistance to pyriproxyfen compared with the YM04-S strain at the time the experiments were initiated.[18,19] Resistance in this strain is inherited as a partially to completely dominant trait and is likely affected by at least two loci.[18–20]

Hybrid strains were created in December 2005 by crossing individuals of the YM04-S and QC02-R strains. Groups of ten virgin females from one strain were placed in a 20 mL vial containing agar and a leaf disk to mate with 15 males of the other strain for 48 h, after which it was assumed that all females had mated. Over a period of 7 days, 100 YM04-S females and 120 QC02-R males were transferred to a population cage containing 18 cotton plants at the 3–4 node stage. In parallel, 113 QC02-R females and 123 YM04-S males were transferred to a second cage with 18 cotton plants. After an additional 8 days, all adults were removed from cages, after which plants with $F_1$ immatures were split into two hybrid strains (AZH1 and AZH2). For the AZH1 strain, nine plants with $F_1$ progeny of YM04-S females × QC02-R males were randomly selected and placed in a population cage with nine randomly selected plants with progeny of QC02-R females × YM04-S males. The remaining 18 plants (nine from each cross) were placed in a second cage to create the AZH2 strain. These strains were reared at 25 °C with a 14 h photoperiod on cotton plants without exposure to insecticides.

Two hybrid strains were created that were selected for pyriproxyfen resistance. Nine plants sprayed with 0.1 μg mL$^{-1}$ of pyriproxyfen were placed into the population cage of each hybrid strain (AZH1 and AZH2) when $F_5$ adults were present. After 8 days, adults were removed from the plants placed in the AZH1 or AZH2 cages and were transferred to an uninfested cage to create the AZH1-R and AZH2-R strains respectively. For simplicity, the generation in selected strains is reported as in unselected strains (i.e. the first generation in the selected hybrid strains is reported as the $F_6$ generation). The AZH1-R and AZH2-R strains were maintained in the same way as the AZH1 and AZH2 strains, except cotton plants were sprayed with 0.1 μg mL$^{-1}$ of pyriproxyfen before being added to cages. After four generations of selection with 0.1 μg mL$^{-1}$ of pyriproxyfen, after which the AZH1-R and AZH2-R strains had reached high population densities, the concentration of pyriproxyfen used to spray plants was increased to 1.0 μg mL$^{-1}$ in the $F_9$ generation to increase the selection pressure on the strains and remove any remaining susceptible individuals.

To maintain discrete generations in hybrid colonies, six uninfested cotton plants were added to a cage for 7 days in each generation. After 7 days, all adults were removed and the plants were transferred to an uninfested population cage. This procedure was repeated, so 12 cotton plants containing eggs laid over 14 days were the start of the next generation. After an additional 7 days, during which time adults began to emerge, the procedure was repeated for the next generation.

### 2.2 Bioassays

Bioassays to measure susceptibility to pyriproxyfen in the AZH1 and AZH2 strains were conducted in the $F_2$, $F_6$, $F_{10}$, $F_{14}$, $F_{19}$ and $F_{27}$ generations. Bioassays of the AZH1-R and AZH2-R strains were conducted in the $F_{10}$, $F_{14}$, $F_{19}$ and $F_{27}$ generations. Excised cotton seedlings with one leaf were dipped for 20 s in a diagnostic concentration of pyriproxyfen (3.2 μg mL$^{-1}$) that killed 95–100% of susceptible males and 0–10% of resistant males[19] or in deionized water (control).

A single virgin female from a hybrid strain was aspirated into a petri dish containing a pyriproxyfen-treated seedling, with the stem held in water. The virginity of adult females was assured by sexing and isolating females as pupae. Seedlings thus arranged were held in growth chambers (27 °C, 50% RH, 14 : 10 h light : dark) for 48 h. After 48 h, the adult was removed, eggs were counted, and seedlings were inserted into 20 mL vials containing tap water. To assess mortality, live nymphs (all haploid males) were counted 7 days after oviposition. Forty replicates were set up for each hybrid strain in each generation tested, but only the progeny of females collected alive after 48 h were considered in the analysis. At least 17 females for each strain in every generation met the criteria (mean 26, range 17–38), and all of these laid at least ten eggs.

As an internal standard, mortality caused by pyriproxyfen in the YM04-S and QC02-R strains was measured by aspirating five virgin females from a strain onto a treated seedling for 48 h, and mortality was assessed as above. Four replicates with each strain were conducted in each generation tested.

To measure control mortality, groups of five virgin females from each strain were aspirated onto a seedling that had been dipped for 20 s in deionized water. Mortality was assessed as above. Four replicates with each strain were conducted in every generation tested.

### 2.3 Life history traits

Fecundity and developmental time were measured for individuals in the hybrid strains in the $F_{10}$, $F_{14}$, $F_{19}$ and $F_{27}$ generations. Fecundity was the number of eggs laid by a virgin female in 48 h (same methods as in Section 2.2). Only females collected alive after 48 h were considered in the analysis (17–38 females met this criteria for each strain in each generation). To measure developmental time, 15–20 mating pairs from each strain were placed in a petri dish on a cotton seedling to lay eggs for 48 h, after which seedlings were kept in growth chambers (27 °C, 50% RH, 14 : 10 h light : dark). Beginning 14 days after egg laying, the number of male and female adults on each seedling was checked daily for 2 weeks to determine the mean developmental time from egg to adult. Twelve replicates were conducted with each strain in each generation.

### 2.4 Data analysis

Mortality observed at each pyriproxyfen concentration was corrected for control mortality.[21] Multiple regression[22] was used, with generation, strain and their interaction as factors, to determine whether susceptibility to pyriproxyfen (arcsine square root transformed mortality) varied over time in the YM04-S and QC02-R strains. If both generation and the interaction of generation and strain were not significantly different from zero, this would indicate that bioassay methods were consistent across generations and that the strains were not contaminated over the course of the experiment.

Multiple regression was also used to test whether susceptibility to pyriproxyfen varied over time between the AZH1 and AZH2 strains. The authors analyzed the effect of three explanatory variables (generation, strain and their interaction) on susceptibility to pyriproxyfen (arcsine square root transformed mortality). If costs favoring susceptible alleles are present, susceptibility should increase over time in both strains. In contrast, if both generation



  

and the interaction of generation and strain are not significant, this will indicate no costs. Linear regression was used to examine further whether susceptibility to pyriproxyfen changed over time in each strain.

Three-way ANOVA was used, with generation, treatment (unselected or selected), strain nested within treatment, the interaction of treatment and generation and the interaction of strain and generation as factors, to determine if the change in susceptibility to pyriproxyfen from generations $F_{10}$ to $F_{27}$ differed between the unselected and selected hybrid strains. ANOVA was used rather than multiple regression because the association between generation and mortality in the selected hybrid strains was not linear. If selection for resistance is effective, susceptibility should decrease over time in the AZH1-R and AZH2-R strains. However, susceptibility should remain stable (without costs) or increase (with costs) in AZH1 and AZH2, resulting in a significant interaction of treatment and generation. One-way ANOVA was used to examine further how susceptibility to pyriproxyfen changed over time in each strain.

If resistance entails fitness costs, life history traits should be negatively affected in AZH1-R compared with AZH1 and in AZH2-R compared with AZH2, and the magnitude of costs should increase over time.[10] In generations $F_{10}$, $F_{14}$, $F_{19}$ and $F_{27}$, three-way ANOVA (with generation, treatment, strain nested within treatment, the interaction of treatment and generation and the interaction of strain and generation as factors) and linear contrasts were used to determine whether fecundity (square root transformed) and developmental time differed between the AZH1 and AZH1-R strains and between the AZH2 and AZH2-R strains. Data on developmental time for males and females were pooled, as no significant differences occurred between the sexes in any generation tested ($P > 0.35$ for all comparisons). All analyses were performed in JMP.[23]

### 2.5 Number of loci affecting resistance

Kolmogorov–Smirnov two-sample tests[22] were used to compare the observed mortality in the AZH1 and AZH2 strains with expected distributions, with models where resistance was affected by one, two, three or five loci. In each generation tested, the observed distribution was based on mortality caused by pyriproxyfen for progeny of each virgin female from AZH1 and AZH2. For the expected distributions, it was assumed that each locus had two alleles (S for susceptibility, R for resistance). The allele frequencies in each generation were estimated by assuming that seedlings with >90% mortality caused by the diagnostic concentration of pyriproxyfen had progeny of a susceptible female, seedlings with <10% mortality had progeny of a resistant female and other seedlings had progeny of a hybrid female.[19]

With all models, an expected distribution of 1000 females was created, the genotypes of which were in Hardy–Weinberg equilibrium based on the estimated S and R allele frequencies. For example, in a model with one locus and an R allele frequency of 0.5, 250 of the females were SS, 500 were RS and 250 were RR. In the one-locus model, the mortality caused by pyriproxyfen for male progeny of each SS or RR female (male genotype S or R respectively) was a random number drawn from a normal distribution with a mean of 96 or 7% respectively, and an SD of 4. The means represent the average mortality over all generations in YM04-S and QC02-R respectively, and the standard deviation was the average standard deviation of mortality across all generations in YM04-S (SD = 3.2) and QC02-R (SD = 4.9). Mortality caused by pyriproxyfen for male progeny of RS females (50% R, 50% S) was a random number drawn from a distribution with a mean of 51.5% (50% R × 7% mortality of R + 50% S males × 96% mortality of S) and an SD of 4. For models with two, three or five loci, the mortality caused by pyriproxyfen for male progeny of the most susceptible or resistant female genotypes (homozygous at all loci) was the same as for SS and RR females respectively. For intermediate male genotypes, mortality caused by pyriproxyfen was additive between the extremes, which assumes that all loci contribute equally to resistance. For example, in the five-locus model, the mortality of $S_1S_2S_3R_4R_5$ was a random number drawn from a normal distribution with mean 60.4% ($0.4 \times 7 + 0.6 \times 96$) and SD 4. Cases where effects of loci were unequal (i.e. major and minor loci) were not modeled, and epistasis was not considered.

## 3 RESULTS

### 3.1 Changes in susceptibility to pyriproxyfen over time

Neither generation ($t_{44} = -1.45$, $P = 0.15$) nor the interaction of generation and strain ($t_{44} = 1.74$, $P = 0.089$) affected susceptibility to pyriproxyfen in the YM04-S and QC02-R strains, although the QC02-R strain had higher mortality than usual in the $F_{10}$ generation (Fig. 1). The lack of temporal change in susceptibility of these strains, which served as internal standards, suggests that the bioassay methods were consistent across generations.

In the multiple regression model, neither generation ($t_{311} = -1.63$, $P = 0.11$) nor the interaction of generation and strain ($t_{311} = -1.35$, $P = 0.18$) affected susceptibility to pyriproxyfen in the AZH1 and AZH2 strains. This indicates a lack of fitness costs in both strains (Fig. 1). In addition, overall susceptibility to pyriproxyfen did not differ significantly between the AZH1 and AZH2 strains ($t_{311} = 0.41$, $P = 0.68$). These results were supported by individual simple linear regression models, where susceptibility did not change significantly over time in the AZH1 ($t_{154} = -1.94$, $P = 0.054$) or AZH2 ($t_{157} = -0.18$, $P = 0.86$) strains (Fig. 1).

The temporal pattern of change in susceptibility to pyriproxyfen from generation $F_{10}$ to $F_{27}$ differed significantly between the unselected and selected hybrid strains (three-way ANOVA, $F_{3,385} = 5.44$, $P = 0.0011$) (Fig. 1). Susceptibility in the AZH1 (one-way ANOVA, $F_{3,97} = 0.29$, $P = 0.83$) and AZH2 strains ($F_{3,97} = 0.29$, $P = 0.84$) did not change significantly from generations $F_{10}$ to

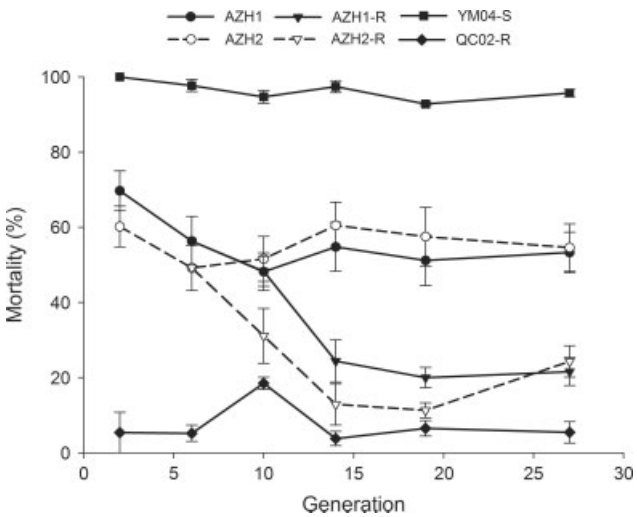


**Figure 1.** Mortality caused by 3.2 µg mL$^{-1}$ of pyriproxyfen (± SE) over time in males of six strains.

  

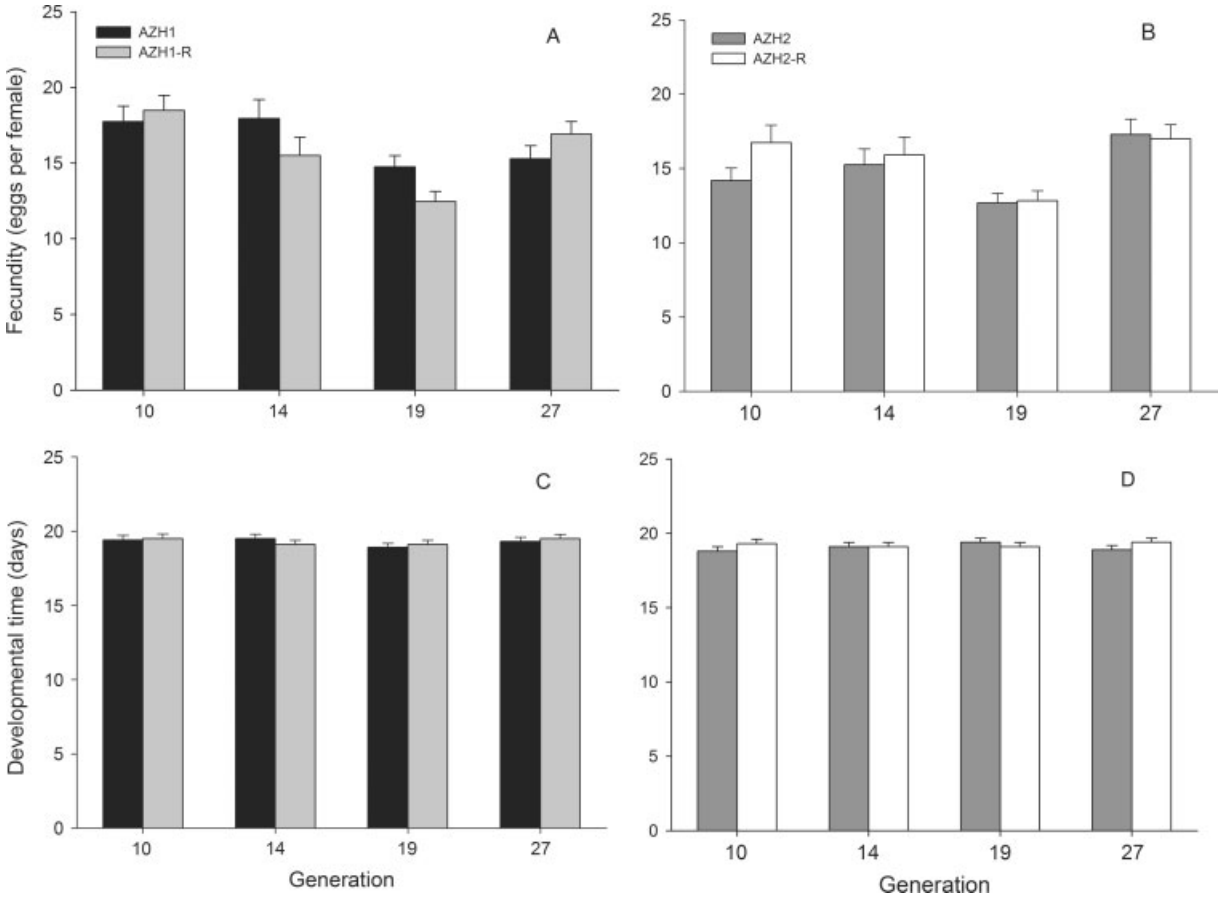


**Figure 2.** Fecundity (± SE) in the (A) AZH1 and AZH1-R and (B) AZH2 and AZH2-R strains; developmental time (± SE) in the (C) AZH1 and AZH1-R and (D) AZH2 and AZH2-R strains. Fecundity is the average number of eggs laid by females in 48 h (back-transformed), and developmental time is the average number of days to complete development from egg to adult at 27 °C.

$F_{27}$, which provides support of a lack of costs in both strains. Susceptibility decreased significantly from generation $F_{10}$ to $F_{27}$ in the AZH1-R (one-way ANOVA, $F_{3,105} = 9.57$, $P < 0.0001$) and AZH2-R strains ($F_{3,86} = 3.32$, $P = 0.024$), indicating that selection increased the frequency of resistance in both strains (Fig. 1).

### 3.2 Life history traits

Overall, fecundity did not differ significantly between the AZH1 and AZH1-R strains ($t_{383} = 0.88$, $P = 0.38$) (Fig. 2a), or between the AZH2 and AZH2-R strains ($t_{383} = -0.63$, $P = 0.39$) (Fig. 2b). Additionally, there was no significant difference in fecundity between the AZH1 and AZH1-R strains or between the AZH2 and AZH2-R strains in any single generation ($P > 0.10$ for all contrasts) (Figs 2a and b). Overall, developmental time also did not differ significantly between the AZH1 and AZH1-R strains ($t_{176} = 1.0$, $P = 0.30$) (Fig. 2c), or between the AZH2 and AZH2-R strains ($t_{176} = 0.34$, $P = 0.73$) (Fig. 2d). Additionally, there was no significant difference in developmental time between the AZH1 and AZH1-R or between the AZH2 and AZH2-R strains in any single generation ($P > 0.10$ for all contrasts) (Figs 2c and d).

### 3.3 Number of loci affecting resistance

Based on the observed data, the estimated R allele frequency, which was assumed to be the same at all loci in the models, was 0.45, 0.49, 0.50, 0.44, 0.51 and 0.47 in the $F_2$, $F_6$, $F_{10}$, $F_{14}$, $F_{19}$ and $F_{27}$ generations respectively. Based on these R allele frequencies, the two-locus model provided the best fit to the observed data for unselected hybrid strains (pooled AZH1 and AZH2) (Table 1). With the two-locus model, significant deviation between the observed and expected distributions occurred in two of the six generations tested. With the one-, three- or five-locus models, significant deviation between observed and expected distributions occurred in four, five and six, respectively, of the six generations tested (Table 1).

**Table 1.** Results of Kolmogorov–Smirnov two-sample tests (*D*-statistic and *P*-value) for the goodness of fit between observed mortality in unselected hybrid strains (AZH1 and AZH2) and expected mortality with models of 1–5 loci

| | Model | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | One locus | | Two loci | | Three loci | | Five loci | |
| Generation | *D* | *P* | *D* | *P* | *D* | *P* | *D* | *P* |
| 2 | 0.21 | 0.009 | 0.21 | 0.010 | 0.27 | <0.0001 | 0.28 | <0.0001 |
| 6 | 0.13 | 0.33 | 0.18 | 0.084 | 0.22 | 0.018 | 0.25 | 0.004 |
| 10 | 0.14 | 0.27 | 0.11 | 0.59 | 0.16 | 0.19 | 0.20 | 0.037 |
| 14 | 0.22 | 0.016 | 0.23 | 0.012 | 0.25 | 0.007 | 0.22 | 0.016 |
| 19 | 0.21 | 0.038 | 0.19 | 0.077 | 0.25 | 0.007 | 0.30 | 0.001 |
| 27 | 0.19 | 0.035 | 0.16 | 0.13 | 0.21 | 0.011 | 0.25 | 0.001 |



  

As a test of the model assumptions, if there are no fitness costs, average mortality in the pooled AZH1 and AZH2 strains should be halfway between the observed mortality in the YM04-S and QC02-R strains. This occurred in five of six generations tested (one-sample *t*-test, $\alpha = 0.10$). Thus, the assumption that loci were additive in the models was appropriate.

## 4 DISCUSSION

Fitness costs associated with pyriproxyfen resistance were not detected in the QC02-R strain of the B biotype of *B. tabaci*, as susceptibility to pyriproxyfen did not change over time in the unselected hybrid strains. Life history traits also did not differ between unselected or selected hybrid strains, providing further support of a lack of costs. Simulation models indicate that fitness costs in *B. tabaci* can significantly delay the evolution of resistance to pyriproxyfen when refuges of crops not treated with pyriproxyfen are used as part of a resistance management strategy.[15–17] Thus, a lack of fitness costs in field populations with similar resistance traits to QC02-R could decrease the impact of insecticide rotations or refuges of crops not treated with pyriproxyfen. However, costs might occur with other pyriproxyfen-resistant strains or populations of the B biotype or under different environmental conditions.

The present results differ from a pyriproxyfen-resistant strain (Pyri-R) of the Q biotype of *B. tabaci*, where resistance decreased eightfold over 13 generations without selection.[17] The large fitness costs observed in that study mainly affected development time, as development time was progressively reduced in the resistant strain after selection with pyriproxyfen ceased.[17] Although the Pyri-R and QC02-R strains share many similarities,[18,19,24] evidence suggests that resistance to pyriproxyfen may be primarily controlled by one locus in Pyri-R[20,24] and two or more loci in QC02-R.[20] Results from the new method developed here also suggest that pyriproxyfen resistance is conferred primarily by two loci in the QC02-R strain. Thus, although this method is based on several assumptions, results agreed with those of more standard approaches.

Large fitness costs are considered most likely to be associated with single genes of large phenotypic effect, as is expected with insecticide resistance.[10,12,25,26] While major genes are often the first to become fixed during the course of adaptation, subsequent mutations of smaller effects can reduce the negative pleiotrophic effects of major mutations, including those associated with insecticide resistance.[27–30] The loci affecting resistance in the QC02-R strain, or genetic modifiers not yet identified, could modify costs associated with any single resistance gene. The lack of fitness costs could also be explained if none of the resistance genes in QC02-R has deleterious effects or if the experimental conditions masked such effects. In particular, costs might be magnified under harsh field conditions relative to optimal laboratory conditions.[14]

If manifested in the field, several traits of the QC02-R strain could promote the evolution of resistance to pyriproxyfen in heavily treated populations. These traits include the lack of fitness costs seen here, partially dominant inheritance of resistance under field conditions[18] and no differences in susceptibility to pyriproxyfen between males and females.[18,19] These traits could threaten sustainability of the resistance management strategy for pyriproxyfen, especially in Arizona where the registration of pyriproxyfen has recently been expanded to include crops other than cotton.

## ACKNOWLEDGEMENTS

Thanks to Christine Yafuso for help in creating the hybrid strains and Tim Dennehy for providing the susceptible and resistant strains. 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 NRICGP grant 2005-00925. This publication is not officially endorsed by EPA and may not reflect the views of the agency.

## REFERENCES

1 Byrne DN and Bellows TS, Whitefly biology. *Annu Rev Entomol* **36**:431–457 (1991).
2 Brown JK, Frohlich DR and Rosell RC, The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? *Annu Rev Entomol* **40**:511–534 (1995).
3 Horowitz AR and Ishaaya I, Chemical control of *Bemisia* – management and application, in *Bemisia 1995: Taxonomy, Biology, Damage, Control and Management*, ed. by Gerling D and Mayer RT. Intercept, Andover, UK, pp. 537–556 (1996).
4 Palumbo JC, Horowitz AR and Prabhaker N, Insecticidal control and resistance management for *Bemisia tabaci*. *Crop Prot* **20**:739–765 (2001).
5 Li AY, Dennehy TJ and Nichols RL, Baseline susceptibility and development of resistance to pyriproxyfen in *Bemisia argentifolii* (Homoptera: Aleyrodidae) in Arizona. *J Econ Entomol* **96**:1307–1314 (2003).
6 Dennehy TJ, Degain BA, Harpold VS and Brink SA, Whitefly resistance to insecticides in Arizona: 2002 and 2003 results, in *Proc Belt Cotton Conf*, San Antonio, TX, 5–9 January 2004. National Cotton Council, Memphis, TN, pp. 1926–1938 (2004).
7 Dennehy TJ, Degain BA, Harpold VS, Brown JK, Morin S, Fabrick JA, *et al*, New challenges to management of whitefly resistance to insecticides in Arizona. *The University of Arizona Cooperative Extension Vegetable Report*. [Online]. University of Arizona (2005). Available: http://ag.arizona.edu/pubs/crops/az1382/az1382_2.pdf [30 October 08].
8 Roush RT and McKenzie JA, Ecological genetics of insecticide and acaricide resistance. *Annu Rev Entomol* **32**:361–380 (1987).
9 Carrière Y, Deland JP, Roff DA and Vincent C, Life history costs associated with the evolution of insecticide resistance. *Proc Roy Soc Lond B* **58**:35–45 (1994).
10 Carrière Y and Roff DA, Change in genetic architecture resulting from the evolution of insecticide resistance: a theoretical and empirical analysis. *Heredity* **75**:618–629 (1995).
11 McKenzie JA, *Ecological and Evolutionary Aspects of Insecticide Resistance*. RG Landes Co., Austin, TX (1996).
12 Coustau C, Chevillon C and ffrench-Constant R, Resistance to xenobiotics and parasites: can we count the cost? *Trends Ecol Evol* **15**:378–383 (2000).
13 Carrière Y and Tabashnik BE, Reversing insect adaptation to transgenic insecticidal plants. *Proc Roy Soc Lond B* **268**:1475–1480 (2001).
14 Gassmann AJ, Carrière Y and Tabashnik BE, Fitness costs of insect resistance to *Bacillus thuringiensis*. *Annu Rev Entomol* **54**:147–163 (2009).
15 Carrière Y, Haplodiploidy, sex, and the evolution of pesticide resistance. *J Econ Entomol* **96**:1626–1640 (2003).
16 Crowder DW, Carrière Y, Tabashnik BE, Ellsworth PC and Dennehy TJ, Modeling evolution of resistance to pyriproxyfen by the sweetpotato whitefly (Hemiptera: Aleyrodidae). *J Econ Entomol* **99**:1396–1406 (2006).
17 Wilson M, Moshitzky P, Laor E, Ghanim M, Horowitz AR and Morin S, Reversal of resistance to pyriproxyfen in the Q biotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Pest Manag Sci* **63**:761–768 (2007).
18 Crowder DW, Dennehy TJ, Ellers-Kirk C, Yafuso C, Ellsworth PC, Tabashnik BE, *et al*, Field evaluation of resistance to pyriproxyfen in *Bemisia tabaci* (B biotype). *J Econ Entomol* **100**:1650–1656 (2007).
19 Crowder DW, Ellers-Kirk C, Yafuso C, Dennehy TJ, Degain BA, Harpold VS, *et al*, Inheritance of resistance to pyriproxyfen in *Bemisia tabaci* (Hemiptera: Aleyrodidae) males and females (B biotype). *J Econ Entomol* **101**:927–932 (2008).

  

20 Crowder DW, Horowitz AR, Tabashnik BE, Dennehy TJ, Denholm I, Gorman K, *et al*, Analyzing haplodiploid inheritance of insecticide resistance in whitefly biotypes. *Bull Entomol Res*, (in press).
21 Abbott WS, A method for computing the effectiveness of an insecticide. *J Econ Entomol* **18**:265–267 (1925).
22 Sokal RR and Rohlf FJ, *Biometry*. WH Freeman and Co., New York, NY (1995).
23 *JMP 5.1, Version 5*. SAS Institute, Cary, NC (2004).
24 Horowitz AR, Gorman K, Ross G and Denholm I, Inheritance of pyriproxyfen resistance in the whitefly, *Bemisia tabaci* (Q biotype). *Arch Insect Biochem Phys* **54**:177–186 (2003).
25 Macnair MR, Why the evolution of resistance to anthropogenic toxins normally involves major gene changes: the limits to natural selection. *Genetica* **84**:213–219 (1991).
26 Orr HA The population genetics of adaptation: the distribution of factors fixed during adaptive evolution. *Evolution* **52**:935–949 (1998).
27 McKenzie JA and Purvis A, Chromosomal localization of fitness modifiers of diazon resistance genotypes of *Lucilia cuprina*. *Heredity* **53**:625–634 (1984).
28 Davidse LC, Benzimidazole fungicides: mechanism of action and biological impact. *Annu Rev Phytopathol* **24**:43–65 (1986).
29 McKenzie JA and O'Farrell K, Modification of developmental instability and fitness – malathion resistance in the Australian sheep blowfly, *Lucilia cuprina*. *Genetica* **89**:67–76 (1993).
30 Raymond M, Berticat C, Weill M, Pasteur N and Chevillon C, Insecticide resistance in the mosquito *Culex pipiens*: what have we learned about adaptation. *Genetica* **112**:287–296 (2001).



  