FIELD PERFORMANCE AND BIOLOGICAL FITNESS IMPLICATIONS OF AN INSECTICIDE ROTATION STRATEGY WITH RESISTANT GERMAN COCKROACHES

J. GRIFFITH, A. ADAMS, A. CLEVERLY, J.-N. CHABREL
AgrEvo Environmental Health, Berkhamsted, UK

F. BLAZEK
AgrEvo Environmental Health, Prague, Czech Republic

Abstract—Field and laboratory studies were initiated in 1993 to investigate the effect(s) of specific insecticide use strategies on resistant populations of German cockroach, *Blattella germanica*. Infestation levels were monitored using sticky traps, and these data formed the basis for the estimates of treatment efficacy at each trials site.

The resistant status of each population was gauged, initially, using field test kits at a discriminating dose, and then determined by topically applying insecticides to males of the F1 generation after field-collected cockroaches had been reared in the laboratory. Further tests were done to establish the resistance of strains following various periods of time in culture, without insecticide pressure, to establish the stability of the resistance. The factor of resistance (FOR) to both pyrethroids and organophosphates (OPs) declined, markedly, over 4–12 generations in the 4 most resistant strains studied. In 3 other strains that were less resistant, the trend for declining FOR was present, but the magnitude of the change was lower.

Field sites were chosen to help demonstrate the effect of continuous use of one or another active ingredient, or the alternate use of those 2 insecticides. A key feature of this study was the measurement of the biological fitness (reproductive potential) of the field strains, since a successful "rotation" strategy requires that the resistance to any given compound (or chemical class) declines when that compound is not in use, because resistance to it carries a fitness cost. Simulation demographic studies were used to compare the fitness of different strains. In every case, insecticide resistance was associated with a significant fitness cost, such that the reproductive potential of the field strains from 2 of the sites was more than 10 times less than the reference susceptible strain. These data are discussed in terms of the rationale and justification for insecticide rotation as a practical resistance management strategy for *Blattella*.

INTRODUCTION

Numerous studies of cockroach (*Blattella germanica*) resistance have been documented and published in recent years. Often field strains are compared with laboratory – reared susceptible, reference strains using methods such as topical application (eg, Atkinson *et al,* 1991) or exposure to a residue in a jar or vial (eg, Cochran, 1989). Each study constitutes a snapshot which might provide information about which insecticides could control the population in the field, and, perhaps which mechanism (s) was responsible for the resistance. In the absence of information showing the development of resistance in a population, and any changes in resistance profile associated with changes in control tactics (such as use of a different chemical class of insecticide), it is difficult to recommend specific resistance management strategies on anything other than theoretical grounds.

Theoretical models and criteria for successful implementation of insecticide mixtures and rotation management strategies have been published (eg, Denholm and Rowland, 1992). There are also a few instances from crop protection, where resistance management has successfully extended the useful life of the products being applied, one of the best examples being the control of *Helicoverpa armigera* in cotton (Forrester *et al.*, 1993). Key differences between that case and managing resistance of *B. germanica* in the urban environment are that for cockroaches control may be practised repeatedly throughout the year, dispersal (potential for immigration of susceptible genes) is comparatively limited and control must satisfy an aesthetic, not a more readily quantifiable economic, injury level.

During 1993, AgrEvo EH (then Roussel Uclaf Environmental Health) initiated field studies to investigate the practical implications of a simple rotation strategy against *B. germanica* populations. This resistance management tactic requires that gene frequencies conferring resistance to a given

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compound, or chemical class, will decline when the selecting agent is removed because resistant genotypes are at some fitness disadvantage (Denholm and Rowland, 1992). Actual control levels are being monitored and in addition the field populations are sampled annually and sent to us for assessment of both their resistance factors and biological fitness (reproductive parameters). These data are compared with the results obtained from succeeding generations of the samples collected prior to commencing the study. The sub -strains are being maintained without insecticide pressure and changes in fitness and resistance are monitored, routinely. Such tests are designed to measure differences in the reproductive potential of laboratory and field populations, and to investigate any association between biological fitness, factor of resistance (FOR) and control.

In common with the approach being taken by the Insecticide Resistance Action Committee (IRAC) in the current trials in Mexico to investigate insecticide use strategies for mosquito resistance management, (Penilla *et al,* these proceedings), we will refer to insecticides by chemical class, rather than as specific molecules.

MATERIALS AND METHODS

Resistant field strains

Multi-resistant strains, collected at field sites from around the world that had a history of intensive insecticide use, and represented "worst cases", were reared in the laboratory, under standard conditions (27±2°C, 50±10% relative humidity, 14 hours light/day) in the absence of further insecticide pressure. The objective was to determine whether, and how quickly, resistance declined under these conditions, and to discover whether there was some fitness cost associated with the high factors of resistance.

Trials sites

Baited jars or Roatel traps were used to collect live *Blattella* from each trials site before the planned treatments commenced. These insects were sent to the AgrEvo Environmental Health laboratories in Berkhamsted, UK, where they were maintained (as above). Further collections were made from each trials site at yearly intervals.

The trials sites were chosen to investigate the effect of instigating a practical, simple rotation programme where resistance was low to moderate, to one chemical class (the sites had a recent history of pyrethroid use), and very low to the other class (amidinohydrazone – which was only just becoming available to PCOs in that country) as an attempt to avoid control failure with a new product by the pre-emptive use of a resistance management strategy. In all cases the products were used at label rates.

Site 1. – treated with pyrethroid (liquid residual) only.
Site 2. – treated with amidinohydrazone (gel bait).
Site 3. – treated alternately with pyrethroid and amidinohydrazone, as above.

Baits tests showed no evidence of any tolerance/resistance to the amidinohydrazone in any of these field strains. The results of topical application tests using pyrethroids on the F1 generation are given in Table 1.

Table 1. Factors of resistance (FOR) to a pyrethroid of the *Blattella* from sites 1,2 and 3.

Strain	FOR (95% CL)
AEH	1
SITE 1	17 (13–21)
SITE 2	17 (13–27)
SITE 3	19 (12–30)

Treatment regimes

In all cases, the insecticides were applied by local Pest Control Operators (PCO) using their normal practise (or under guidance for the amidinohydrazone). Sticky traps were used to monitor the *Blattella* populations – with 10 traps per site, left in position for 2 nights at monthly intervals. Insecticides were only used at a site if the total trap catch exceeded 50 cockroaches. The decision to alternate use of actives at the rotation sites, rather than to set time periods (eg. 3 or 6 months) for the use of each product, or seasonal/annual rotations was taken to ensure that the populations were exposed to the same number of treatments of each insecticide – a pragmatic rather than scientifically preferred option.

Resistance tests

Male cockroaches were treated with 0.5μl of insecticide, dissolved in butanone, and applied, topically, to the ventral thorax. These tests were performed on the F1 generation from each collection at each site (Table 1) to provide a baseline for later studies. Replicated batches of 10 males were treated with 4 or 5 doses to span the dose response range. After test the insects were left for 6 days with food and water before mortality was assessed. Probit analysis (Maximum Likelihood Program, Lawes Agricultural Trust) was used to estimate the LD$_{50}$, statistic from which the FOR was determined, relative to the reference susceptible strain. Further tests were performed on males from the different populations after various periods of time (generations) in culture, in the absence of insecticide pressure.

Treatment efficacy at the trials sites

The monthly sticky trap catches (total caught in the 10 traps) were used to monitor the effectiveness of individual treatments at each site in terms of absolute numbers trapped, and percent reduction (each treatment occurred within one week of an assessment, and about 3 weeks before the next assessment).

Demographic studies and simulations

Design

Six female, F1 generation adults were selected from each strain (prior to melanisation of the cuticle) and paired with pre-melanised males in individual 500 ml clear plastic pots (DRG, Glacier 600). Each pot was supplied with a water source and a coil of paper as a harborage. A standard laboratory susceptible and more recently a field collected susceptible *B. germanica* strain were similarly set up as controls.

Observations were made at least twice-weekly, during which time food and water was replenished to ensure nutritional needs were met at all times.

Parents were removed from their offspring prior to the latter reaching the second instar and set up in fresh containers until death/cessation of oothecal production.

The following observations were recorded:

Adults

(1) The number of egg capsules (oothecae) produced per female
(2) The length of time between oothecal maturity
(3) Number of offspring produced per female
(4) Parental reproductive life span

Progeny

(5) Length of time spent as immature stages
(6) Death rate (% survival from nymph to adult)

Analysis

Two parameters could be used to determine whether there was a biological fitness cost associated with resistance:

a. Gross fertility rate (total number of offspring expected per female)
b. Total female population after a specified period of time.

Neither parameter was observed directly from the 6 individuals monitored, but both can be derived from the recordings. However, some idea of variability is required in order to compare across strains. The method chosen was to simulate what might have happened if 500 individuals per strain had been monitored, using the mean and standard deviations (from the actual data) of the observed variables and the functional relationship between these and the parameters of interest described above. The simulation method used here is called the Monte Carlo simulation

The method can be described as having 4 steps:
a. Define the distributions of each input variable – termed assumptions
b. Define the parameters of interest (output variables/forecast) as functions of the input variables
c. Randomly select a value from each input variable's distribution and calculate the value of each output variable – store it
d. Repeat "c" 500 times, thereby compiling a distribution of possible values for each output variable

The derived distributions of both variables were then compared across strains using the non-parametric Kolmogorov-Smirnoff test. This test assesses the similarity between the underlying distributions of 2 samples by comparing their cumulative distribution functions. Parametric tests such as the t-test are not appropriate for variable "Size of Female Population... ", as the distributions for all 3 strains were markedly skew. Although the distributions of gross fertility rate were more nearly normal and a parametric test would have been possible, the non-parametric test was used for reasons of consistency.

Assumptions

The mean and standard deviation for each variable were based on the observed data from the 6 females per strain. Where the lower limit of the distributions was less than 14 days for "Time taken to produce ootheca", the lower limit was forced to be 14. In addition:

- the nymphal development time is independent of ootheca number
- proportion of female survivors from total nymph production is independent of ootheca number
- no correlation between length of time for successive ootheca production
- no correlation between the number of nymphs and length of time to produce an ootheca
- no adult death rate
- 100% of female population produces 7 oothecae

RESULTS

The changes in the FORs to representative pyrethroids and organophosphates (OPs) in the 4 *Blattella* strains with highest resistance are shown in Figures 1A and 1B, respectively, and declined in time, to a greater or lesser extent, in every strain. In all cases, the FOR to the pyrethroid was much higher than to the OP, in the FI males (although treatment efficacy, based on percent reductions, was similar when the two classes were actually used at those sites). The FOR to pyrethroids declined from ×30–80 to ×10–20 during the course of 12 generations, compared with a similar scale of reduction in OP resistance (from ×10–13, to ×3–5) for 3 of the strains.

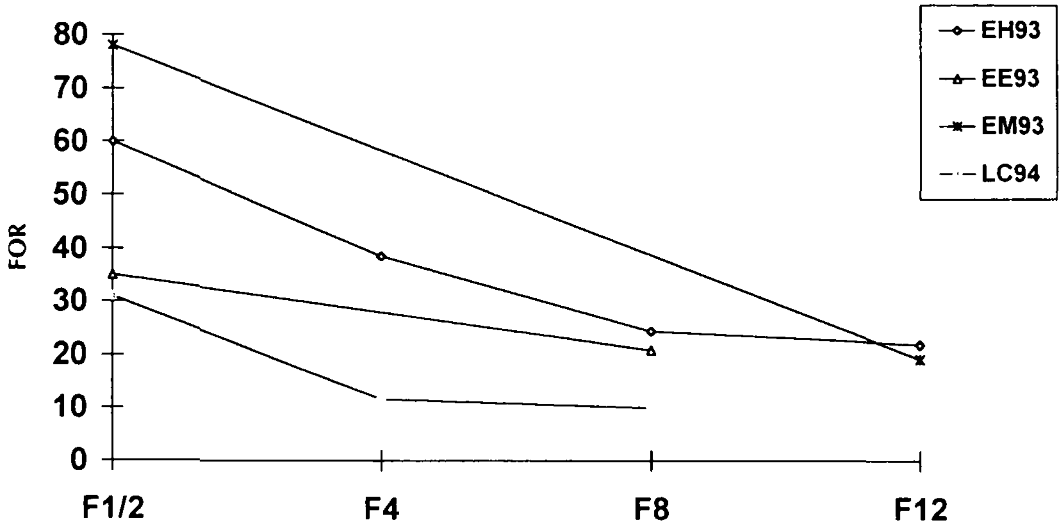

Figure 1a. Relative factors of resistance (FOR) to pyrethroids for field strains of *Blattella germanica* after limited (F1.2), and prolonged (F4–F12) periods of culture.

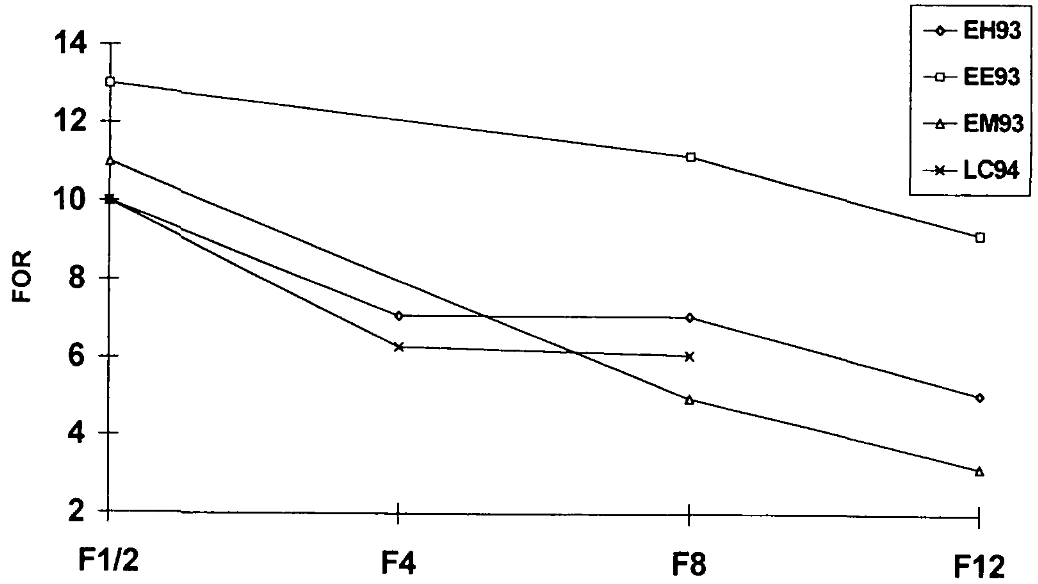

Figure 1b. Relative factors of resistance (FOR) to organophosphates for field collected strains of *Blattella germanica* after limited (F1.2), and prolonged (F4–F12) periods of culture.

Table 1 shows that there was a similar FOR to the pyrethroid for the strains from the 3 trials sites (×17–19; Table 1). When the FOR was determined after maintaining these strains without insecticide pressure, a decline was recorded for all the strains (Figure 2), as marked as in the more resistant populations described above.

At the field sites, the difficulties of attempting a proactive resistance management strategy, when use of the insecticides is efficacious, were amply demonstrated. Figure 3 shows the total number of *Blattella* trapped throughout the trials period at Site 1 and it was evident that a single application of the amidinohydrazone virtually eliminated the infestation within a month. Indeed, the number of cockroaches trapped remained in single figures for 18 months, and it would be impossible to know whether a future infestation that reached the treatment threshold was derived from cockroaches that had experienced exposure to the treatment, or were immigrants. The pyrethroid-

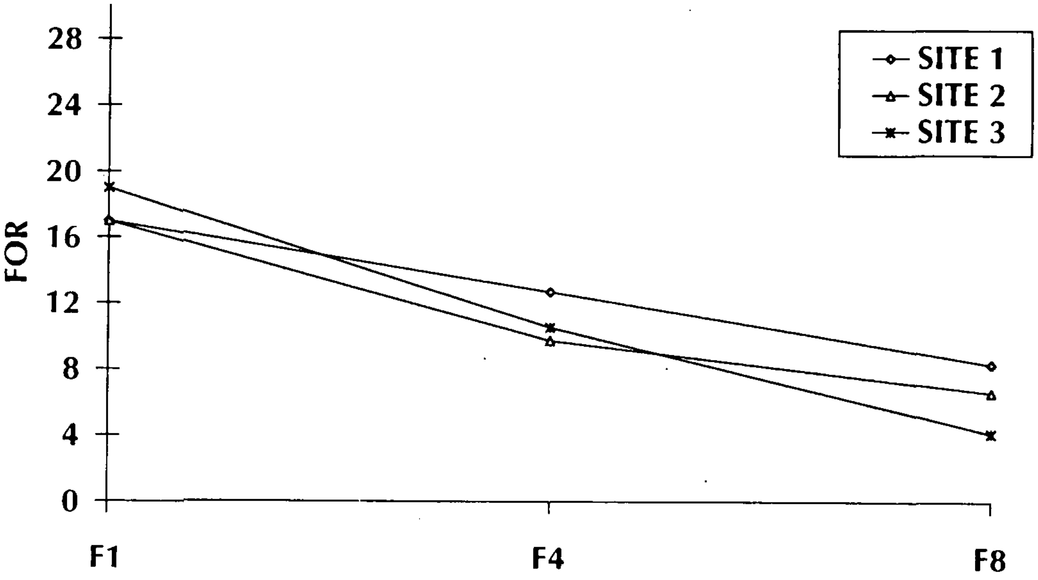

Figure 2. Relative factors of resistance (FOR) to pyrethroids for field strains of *Blattella germanica* after limited (F1) and prolonged (F4–F8) periods of culture.

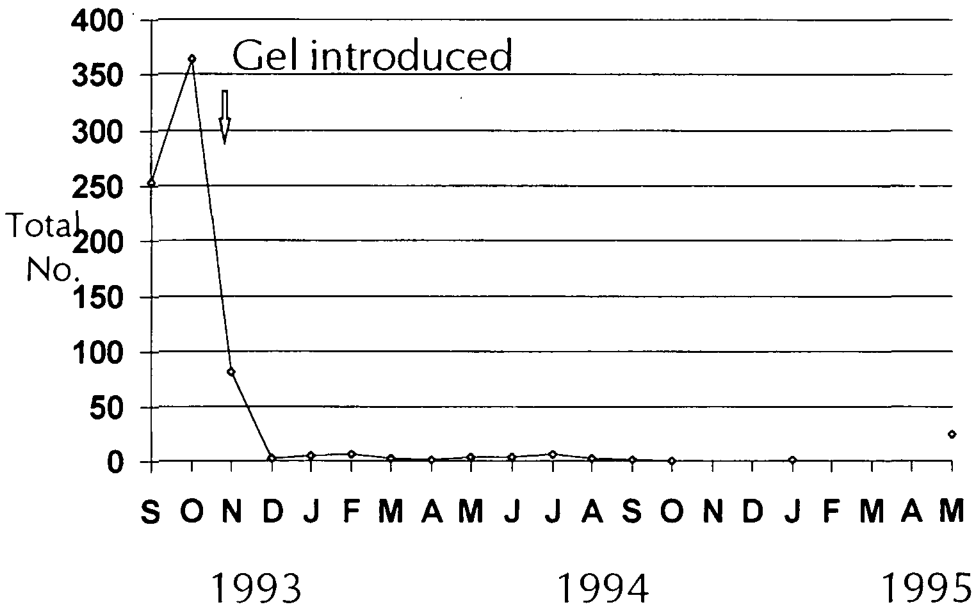

Figure 3. Total number of cockroaches caught in the 10 sticky traps at Site 1.

only site (Site 2), was characterised by poor and variable control, based on percent reductions (Figure 4). Reductions of up to 75% occurred on some occasions, while on others, the post-treatment catch was actually higher than the number recorded pre-treatment. In mid-1995 it was clear that further use of pyrethroids was no longer warranted, so a sample of cockroaches was trapped for further study (at Berkhamsted) and treatment was switched to the introduction of the amidinohydrazone. At the "rotation" site (Site 3), the initial pyrethroid treatment was extremely effective, and the introduction of the amidinohydrazone, while premature (cockroach numbers were below the treatment threshold), preceded eradication of the infestation, since no cockroaches were trapped between June 1994 and April 1995 (Figure 5).

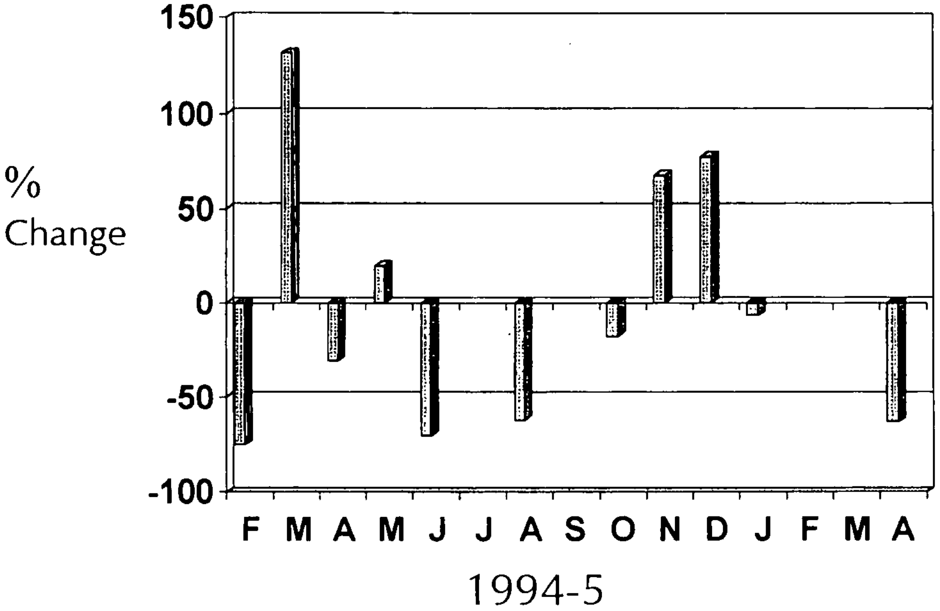

Figure 4. Percent change in cockroaches trapped after each pyrethroid treatment at Site 2.

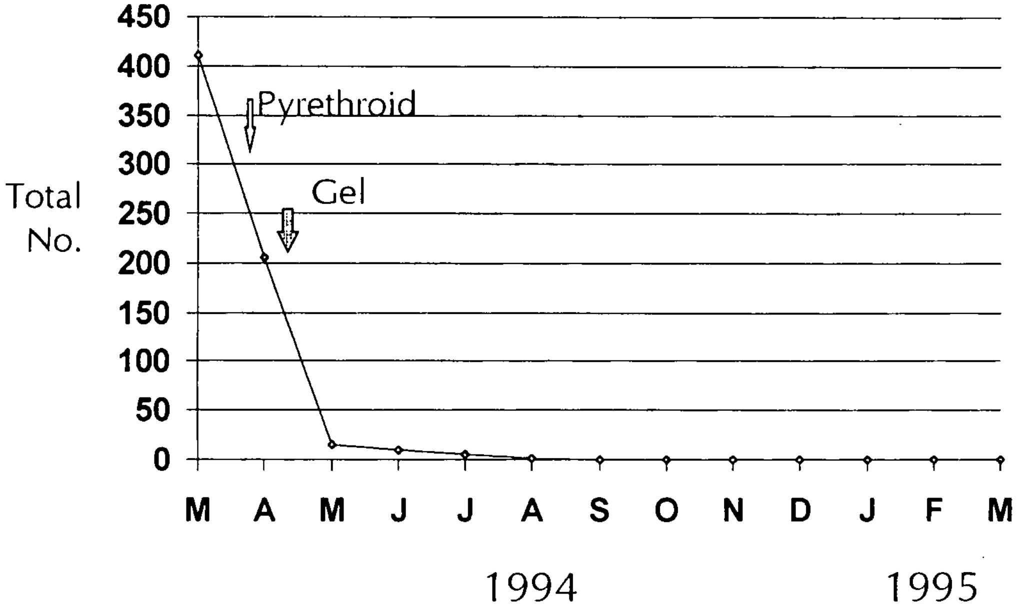

Figure 5. Total number of cockroaches caught in the 10 sticky traps at Site 3.

Reproductive parameters

The reproductive potential (Andrewartha and Birch, 1954) of each strain is summarised in two parameters – the gross fertility rate (the total number of female offspring that can be expected from one female) and the projected size of the female population after *n* days. Both these parameters are derived from a function of the variables collected.

The summary statistics of the gross fertility rate (GFR), ie. the number of offspring that can be expected from one female, are the mean and the 95% confidence limits of the size of the female population. The GFR for the susceptible strain (AEH) was 111 (109–113) compared with 96 (94–98) and 94 (92–96) for sites 1 and 2, respectively . Development time (mean time spent as a

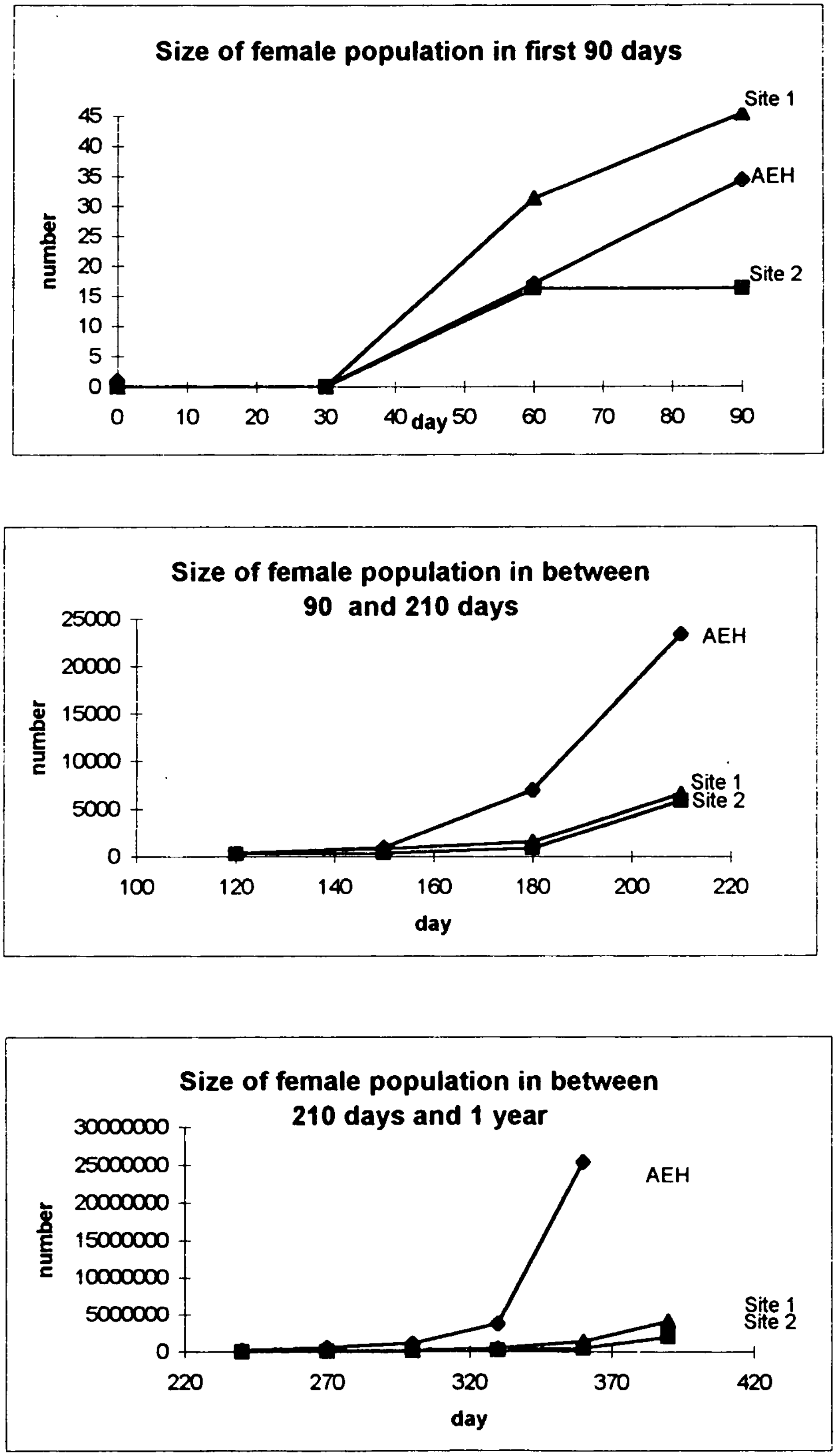

Figure 6. The projected female population growth of standard susceptible (AEH) and 2 pyrethroid resistant field strains (Sites 1 & 2) of *Blattella germanica*.

nymph) also differed between strains with the susceptible strain development of 34 days (range, 31–41) compared with 50 (36–62) and 52 (48–55) days for the *Blattella* from sites 1 and 2, respectively. Nymph survival rates were 83–92% for the 3 strains, and 42–46% of those surviving to adult were female. These statistics are broadly representative of all the resistant strains we have collected, and assessed, to date.

The projected female population growth of AEH and trials site strains of *B. germanica* in the absence of controls is described graphically in Figures 6, as the size of the population in the first 90 days, between 120 and 210 days and between 240 days and 1 year, respectively. After 1 year the estimated female population was 19.8 (95% confidence limits: 9.8, 39.2) million for the susceptible AEH strain, 0.7 (0.2, 2.7) million for the strain from site 1 and 1.7 (0.8, 3.9) million for the strain from site 2.

DISCUSSION

The data resulting from this study suggest a severe biological cost resulting in a reduced reproductive potential was associated with resistance that had evolved as a result of previous exposure to insecticides. Field collected strains of *B. germanica* resistant to pyrethroids and OPs tended to lose their tolerance when insecticide pressure was removed (Figure 1A, B, Figure 2). The demographic studies showed that field strains had a lower reproductive potential than the laboratory susceptible strain – amounting to a 10–fold reduction in female offspring over the course of 12 months for the strains from trials sites 1 and 2. Although it is possible that the long term adaptation to diet and laboratory conditions by the susceptible laboratory strain contributed to the magnitude of this effect, a recent assessment of an insecticide-susceptible field strain (FOR <×3 to representative OP and pyrethroid) has revealed similar trends in demographic development to the AEH strain. This suggests such adaptation to lifestyle may be limited.

The idea that heritable traits developed in the field due to insecticide pressure(s) may be deleterious to the resistant population is not new. Resistant strains of insects are often reported to show disadvantages in life history characteristics (Roush and McKenzie, 1987). However many previous studies have provided little more than a snapshot of biological fitness in resistant field colonies. The present laboratory – based study, aided by simulation modelling, was intended to gain a greater understanding of the long term demographic effects of resistance, and the possible impact of and justification for, control strategies in the field. Field treatments which leave survivors will tend to result in the accumulation of genes conferring resistance, over many generations. The possible impact of a particular choice of control tactics, under such circumstances, may well be determined by the implications of "being resistant" i.e. the fitness cost(s). One approach for dealing with resistant insects has been to switch to a new insecticide (class) either in addition to or in place of the resisted compound, rather than switch to new control tactics or strategies. However, this does not address the root cause of resistance and may exacerbate it through cross-resistance or multi-resistance.

When the amidinohydrazone was introduced to sites 1 and 3, the infestations were effectively eliminated (Figures 3, 5). This level of control, if it were 100%, could not result in any selection for resistance. If however, a few individuals remained, then some selection might be possible (unless they were immigrants). With the population at such a low level, there would be no need for frequent re-application, and it could be predicted that resistance would take many years to reach a level where control was compromised. Under such circumstances, there may be little to gain from implementing a "resistance management strategy" but, if subsequent treatments were less successful, then the use of alternative control methods should be advocated.

Intuitively, it takes energy and resources to "be resistant". Therefore, these resources are not used elsewhere, resulting in a fitness cost. The results of our investigations have demonstrated that such costs exist in resistant *Blattella*. Therefore, the essential pre-requisite for a management strategy based upon some form of rotation appears to be present. However, the field data demonstrated the difficulties of proving that such a tactic can be beneficial, since the non-resisted compound virtually eliminated the population, and extremely good control was associated with the pyrethroid treatment at site 3 (Figure 5) where the FOR was ×17 (Table 1). Without an infestation, it would be impossible to establish the value of any long term control tactic! At site 2, the FOR to pyrethroids was the same as at site 3, but control was much less effective. This highlights the difficulty of translating an FOR value into the likelihood of control failure, and emphasises the need to take a more holistic approach to control and resistance. For example, a fully susceptible population may be poorly controlled where the surfaces are dusty or greasy, or porous – or where baits are not positioned optimally and alternative food sources are available. Alternatively, 60–70% control was still being obtained by pyrethroids where the laboratory tests indicated an FOR of ×50!

The experience gained from these trials suggest there is some justification for a form of insecticide rotation as a practical resistance management strategy. Thus far, we have little data on the underlying resistance mechanisms, and no unequivocal field data to support the use of such a tactic, in practise, and prolong the useful life of chemical classes, as a result. Indeed, it is likely that trials sites would need to be monitored, and the use of insecticides controlled, over many years to demonstrate real advantages, particularly when resistance factors are low, or new, non-resisted chemistry is becoming available. However, the price of doing nothing at all is likely to be a continuation of the treadmill.

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