Project Report No. 584

Combating insecticide resistance in major UK pests

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CONTENTS

1. Abstract

Despite substantial progress with developing non-chemical methods of crop protection, pesticides remain essential for effective suppression of pests, pathogens and weeds in many cropping systems. Reliance on pesticides introduces a number of risks, including the appearance of resistance in target organisms. The overall aim of this project was to maintain chemical control of economically important invertebrate pests of agriculture and horticulture, by identifying effective insecticide resistance management strategies for target site resistance and developing an objective method for resistance risk assessment.

Work package 1utilised a novel mathematical model to simulate the evolution of target-site resistance in crop pests with contrasting life-histories. Optimal management tactics to delay the development of target-site resistance were explored for groups of pest species with contrasting life histories. This produced two key findings:

Firstly, simulations demonstrated that in most scenarios tested, a higher dose of insecticide leads to faster selection for resistance resulting from a single target-site mutation.

Secondly, simulations were performed to identify the optimal combination of two insecticides with different modes of action (MoA), to which resistance from two target-site mutations (one for each MoA) was developing. These demonstrated that when two insecticides were applied together at their label dose in a mixture, resistance developed considerably faster than when the two insecticides were alternated. However, if the dose of each insecticide was reduced so that the mixture provided the same control of the insect population as a single label dose of either product alone, then mixtures were often the most effective resistance management tactic. Only when the resistance resulted in substantial fitness costs in the insect species did alternating two insecticides at their label dose lead to slower resistance development than reduced-dose mixtures.

These results are in agreement with findings from modelling and experimental studies on fungicide resistance, but need experimental validation. As neither metabolic nor multi-site resistance were considered it is not known whether their inclusion would affect the results. The conclusions on resistance management need to be interpreted also to take account of the practical requirement for robust control.

Work package 2 investigated the influence of biological, agronomic and insecticide-related traits on resistance risk. A data set of over 100 historical cases of resistance (comprised of target site and metabolic resistance cases) was used to test which traits were associated with faster or slower development of resistance. Multivariate statistical methods were used to develop a resistance risk assessment model, which consisted of five traits (crop area, crop type, number of crop hosts, mode of reproduction and taxonomic order) and accounted for 45.9% of the variation in the speed at which resistance occurred. The model can be used to guide resistance risk assessment for novel pest/crop combinations since all the key traits are relatively easy to quantify without knowledge of prior

resistance history. Although, considerable uncertainty remains, the model provides an objective means of ranking pest-crop combinations from high to low risk, allowing proportionate resistance management strategies to be put in place.

2. Introduction

The development of insecticide resistance by insect and mite pests remains a constant threat to the sustainability and competitiveness of crop production. With a growing realisation that no pest/crop/insecticide combination is inherently immune from resistance, work is needed to strengthen our ability to predict the likely speed at which resistance will arise, and to appraise the likely effectiveness of tactics potentially available to mitigate resistance risks. Through retrospective analyses of historical cases of resistance, and mathematical modelling of resistance evolution under different insecticide use regimes, this project aimed to provide practicable tools and recommendations for anticipating and combating resistance.

The project comprised three work packages. Work package 1 assessed the effectiveness of insecticide resistance management strategies, to inform the implementation of resistance risk 'modifiers' through regulation and guidance. Work package 2 developed improved insecticide resistance risk assessment methods, to assist in determining the need for resistance management measures. Work package 3 disseminated results to relevant end users. Objectives, methods, results and the implications of the findings are given below for each work package. Full details of the work will be available in peer reviewed papers published from the project.

3. Work Package 1 - Compare the net benefit of different insecticide resistance management strategies for insect pests with contrasting traits (life-cycles, genetics and damage mechanisms)

3.1. Introduction

Resistance in agricultural pests to insecticides is a serious problem, with resistance to one or more insecticidal modes of action (MoA) having developed in most agricultural pests (Tabashnik *et al.*, 2014). Hence, it is essential to use insecticides intelligently in order to maximise the period of time over which they are effective at controlling the pest, by slowing down the rate at which resistance develops. Different strategies may include using more or less insecticide for a single application, or using more than one insecticide (by alternation or mixture).

Despite extensive research, uncertainty remains over what management strategies best slow resistance development. This uncertainty may result from differences between the many insect-insecticide systems, each with differing traits that have been studied. Management scenarios that

are appropriate for one insect pest species controlled by a particular insecticide may not be appropriate for a different insect pest controlled by a different insecticide, either due to the insect life history, the insecticidal MoA, or some other characteristic of the system. This work package attempted to provide some clarity, exploring management scenarios for a wide range of insect-insecticide scenarios, and, in particular, to identify the drivers that result in some management strategies being optimal for some systems and not for others.

Ideally, management strategies would be tested experimentally for their ability to slow the development of resistance. However, such experiments are expensive, limited in the diversity of systems they can test and practically constrained in the number of pest generations over which resistance evolution can be measured. For these reasons modelling has become an important component of resistance management research, with process-based modelling being the most common research tool (e.g. Comins, 1977; Tabashnik and Croft, 1982). Process-based models simulate the development of resistance in an insect population, and can then be used to determine which resistance management strategies are optimal for that insect population. The parameters and processes may be changed so that the model represents a range of different insects and insecticides, allowing different management strategies to be tested for a range of pests. Using a generic process-based model, that is able to simulate many different insect life histories and insecticide application programmes, this work package investigated a range of resistance management strategies and identified optimal strategies for controlling different groups of pests with target-site resistance to one or more single-site insecticides.

We considered target-site resistance in this work, where a resistance mutation at a single locus confers a high level of resistance towards an insecticide, because this type of resistance is well characterised and results in commercially important levels of resistance. Metabolic resistance, which is thought to be the primary mechanism of resistance in many pest species, was not considered in this work.

Objectives

1. Develop and test models for insecticide resistance selection and management.
2. Compare the effectiveness of anti-resistance strategies.
3. Define groups of pests for which similar anti-resistance strategies are most applicable.

3.2. Methods

Strategies for managing resistance were explored using a mathematical computer model that simulates the density of an insect population over several years, with initially a low frequency of resistance within the population made up of SS, SR and RR genotypes in a diploid pest (with a haplodiploid pest we also have the haploid S and R genotypes). When an insecticide is applied, the susceptible (SS) genotypes are killed more than the resistant genotypes (described in full below),

and the frequency of the resistance strains within the population increases. In addition, the model incorporates various insecticide application strategies, including the use of different dosages and timings of each insecticide, and the use of multiple single-site insecticides either in sequential use, alternation or mixture. The model therefore allows different application strategies to be compared with respect to the speed at which resistance builds up in a treated insect population.

3.2.1. Insect population model

The model simulates, over multiple years, the density of an insect population per unit area (e.g. per crop plant), and the frequency of resistance within that population to one or more insecticide MoA. The insect population is subdivided into insect stages (eggs, larvae, pupae and adults where appropriate), the instars within each stage, and the resistance genotypes (e.g. SS, SR, RR – see Fig. 1). At the start of a simulation the population is mostly susceptible (SS), while small proportions of the population are heterozygous (SR) and resistant (RR). At the start of each year insects appear, potentially from an overwintering population (see below), at the same density each year before developing according to the specified insects' life history. The model is generalizable so that it may be adjusted to represent specific insect pests, and can describe both diploid and haplodiploid insects (for a haplodiploid insect population we divide the population in to males and females: females are diploid, males are haploid), sexual and asexual insects, and hemi- or holometabolous insects (where hemimetabolous insects have only a larval and adult stage, whereas holometabolous insects have all four stages), among other traits. The traits of the insect pest, such as the birth rate, lifespan of each stage, number of instars, generations per year, etc. may be adjusted so that the model represents specific insect pests.

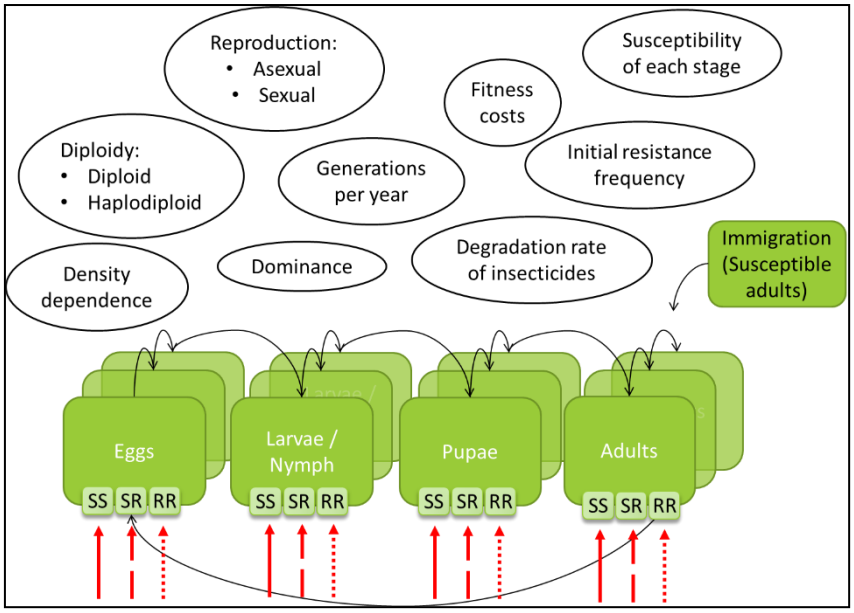

Figure 1. Schematic of the model structure and options. The model tracks the density of insects in multiple stages. The number of stages may be varied, as may the number of instars per stage.

Insecticide differentially affects the resistance genotypes within each instar. Several customisable traits exist in the model that allows the model to represent many different insect pest species.

Resistance to each insecticide in the model is encoded at a single locus (we refer, in future, to different loci as locus A, B, C, etc.) by a mutant allele (R) replacing the wildtype allele (S). In diploid insects there are three possible genotypes at each locus: homozygote susceptible (SS), heterozygote (SR) and homozygote resistant (RR) in all the life stages of the insect. Addition of a second insecticide adds another resistance locus and the total number of possible genotypes increases to 9 (3^2). For haplodiploid insects (in which males are haploid and females are diploid) there are only two possible male genotypes at each resistance locus: susceptible (S) or resistant (R). At the start of a simulation the majority of the population is susceptible to each insecticide, with small proportions of the resistant genotypes present at a low background level.

Insecticide kills susceptible individuals more than resistant individuals, and the mortality of heterozygote individuals is determined by the dominance of a particular gene: if recessive, then the mortality of heterozygote individuals will be similar to a susceptible homozygote; if dominant, the mortality of heterozygote individuals will be similar to a resistant homozygote. The resistance level of each genotype is specified by the LC_{50} and slope of the linear relationship between the logit of the mortality within a given time period and the log of the dose of insecticide applied, as found in experimental studies. It is assumed that a label dose of insecticide causes a 90% reduction in the population density of a fully susceptible population when applied to a crop (to represent complete control seldom being achieved in practice).

Additional factors are included in the model, which we describe briefly here (full details of the model are shown in Appendix 1):

- Overwintering emergence
    - Many insects in temperate climates emerge gradually into a crop from their overwintering location. An overwintering population is therefore implemented in the model from which these insects emerge each year. The resistance frequency of the overwintering population is determined by the frequency at the end of the previous year.
- Immigration from external sources
    - Many insect populations are not closed. Immigration into a treated crop from external sources is therefore included in the model. It is assumed that immigrants are adults, as this is typically the migratory phase of insects, and the rate at which the immigrants enter the population of interest is proportional to the density of adult insects in an untreated field. It is assumed that the insects from external sources have not been treated by the same insecticide MoA, and therefore the frequency of resistance in immigrants is constant and the same as the (low) initial frequency of resistance for the simulation. This represents the strongest possible case of untreated refuge.
- Fitness costs

- Resistance frequently confers a fitness cost. This is modelled as a decrease in the birth rate for homozygote resistant genotypes (whether diploid or haploid), while the birth rate of heterozygote individuals is determined by the same dominance as the resistance gene. Therefore a fitness cost of 20% would result in a 20% decrease in the birth rate for RR individuals.

- Incomplete insecticide coverage
  - Insecticide foliar applications do not provide total coverage of a crop, with areas such as the underside of leaves or within flowers being underexposed or unexposed to insecticide. To model this a secondary insect habitat is included in the model that is not contacted by any insecticide, and insects move freely between these treated and 'refuge' areas.
- Cross-resistance
  - When two or more insecticides are present, resistance to one insecticide may confer full or partial resistance to other insecticides, which may drastically affect the optimal use of the multiple insecticide MoA.

The basic model (hereafter referred to as the "simple" model) describes a non-specific insect population that is asexual, multivoltine, hemimetabolous (consisting of only larvae and adults) and diploid with a larval overwintering stage. The dominance of the resistance gene is exactly intermediate. The parameters of the simple model may be found in Appendix 2.

The model has been used to explore two key questions in resistance management:

1) If a single insecticide is available, is it optimal for resistance management to apply a full label dose of insecticide or to use a reduced dose?
2) If two insecticides are available, how should they be optimally combined in order to slow the development of resistance?

These questions were addressed using three approaches:

1) Analyse the simple biological model, and study how adding complexity to the model affects the results.
2) Consider the question for each of three realistically parameterised case studies, which contrast in their traits. The three insects and insecticides used were *Myzus persicae (*peach-potato aphid) controlled by lambda-cyhalothrin, *Meligethes aeneus* (pollen beetle) controlled by lambda-cyhalothrin, and *Frankliniella occidentalis* (western flower thrips) controlled by spinosad. *M. persicae* is asexual and hemimetabolous, *M. aeneus* is holometabolous and undergoes sexual reproduction, and *F. occidentalis*, is a haplodiploid species. The parameters for these insects and insecticides may be found in Appendix 2.
3) The third method involves a global parameter search. Each parameter (for the insect and insecticide) within the model is randomly chosen (see Appendix 3 for details), creating a

unique virtual insect-insecticide scenario. The optimal management tactics can then be tested for their generality.

3.3. Results

3.3.1. Process-based modelling

We first demonstrate that the simulation model is functional, before addressing the questions outlined in the Methods section. To demonstrate model functionality, the densities over time of each of the case study species are displayed in Figure 2.

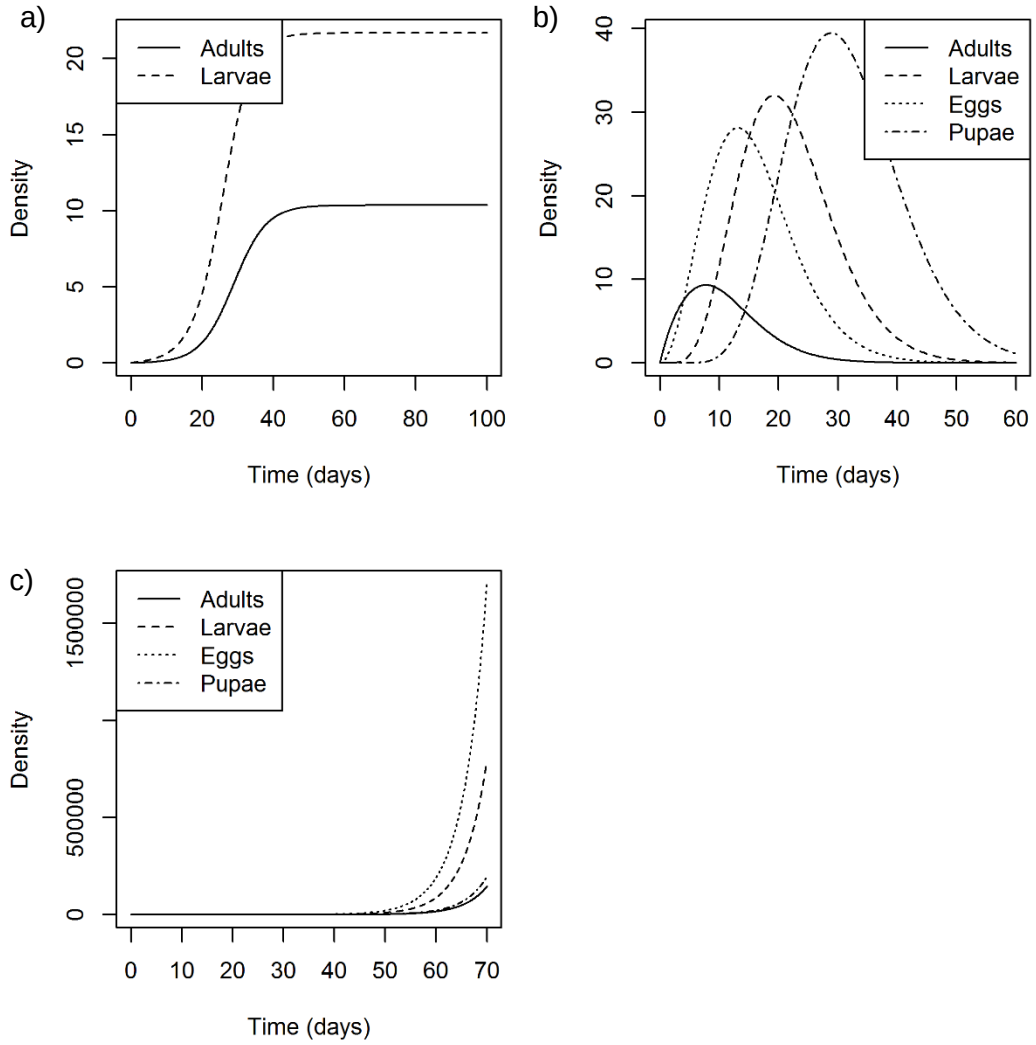

Figure 2. Population growth curves produced by the simulation model for a) *M. persicae*, b) *M. aeneus*, c) *F. occidentalis*.

The effect of an insecticide is demonstrated in Figure 3, in which a population of an asexual hemimetabolous species is challenged with two doses of insecticide; the label dose or half the label dose, or is left untreated. In Figure 3b the resulting resistance frequency over ten years is shown, and it is clear that, for this insect-insecticide scenario, the label dose leads to faster development of resistance.

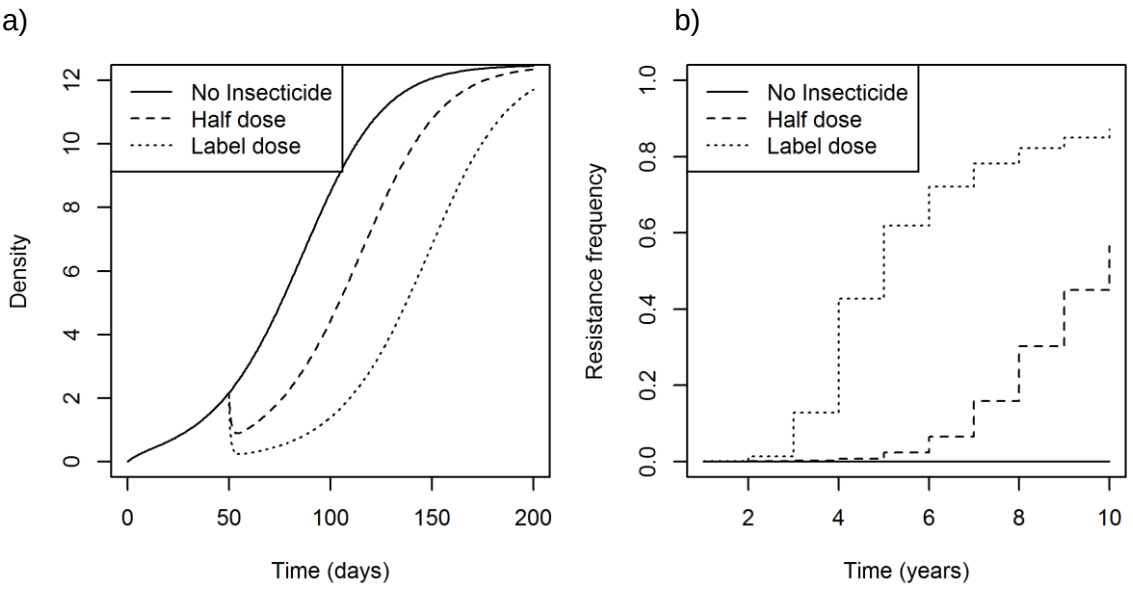

Figure 3. (a) Population growth when a hemimetabolous asexual insect pest is subjected to a full label dose, half dose or no dose of insecticide, and (b) the resultant increase in the frequency of the resistance allele over 10 years.

3.3.1.1 Single insecticide

The effect of dose is first explored in a model that has the simplest insect assumptions possible, on which a local (monofactorial) sensitivity analysis is performed, in order to explore which model parameters affect whether resistance develops faster under a high dose or a low dose. Then the effect of dose is studied in the three specific insect pest systems mentioned earlier.

Figure 4 demonstrates how the immigration level interacts with the dose of insecticide applied to the simple insect population with the addition of immigration and / or sexual reproduction. Each figure represents many simulations, with each simulation having a different immigration rate and dose of insecticide applied. The dose is set so that a single spray causes the mortality given on the x-axis, while the immigration is the percentage of the density of adults in an untreated population that enters the population of interest each day. In each figure the contour lines show the frequency of resistance after five years of insecticide application, while the red area indicates where the resistance frequency has not increased after five years. Figure 4a) relates to an asexual insect population, and Figure 4b) relates to a sexual insect population. It is evident that in the asexual population, reducing the dose of insecticide applied will decrease the frequency of target-site resistance after five years compared to higher doses, and, due to selection being counterbalanced by the immigration of unselected individuals, also make it likely that the resistance frequency will not increase. However, where the dose gave 90% mortality, the immigration levels need to be very high (>10% of an untreated susceptible population entering the

population each day) for resistance to not increase. In the sexual population, reducing the dose of insecticide applied also decreases the frequency of resistance after five years in most scenarios. However, there are some scenarios where reducing the dose of insecticide applied in the sexual population would result in losing suppression of resistance and increasing the resistance frequency after five years. This requires the dose of insecticide applied to be greater than 95% and for immigration rates to be high, e.g. reducing the dose from that causing 99% mortality to that causing 90% mortality and a 2% immigration rate.

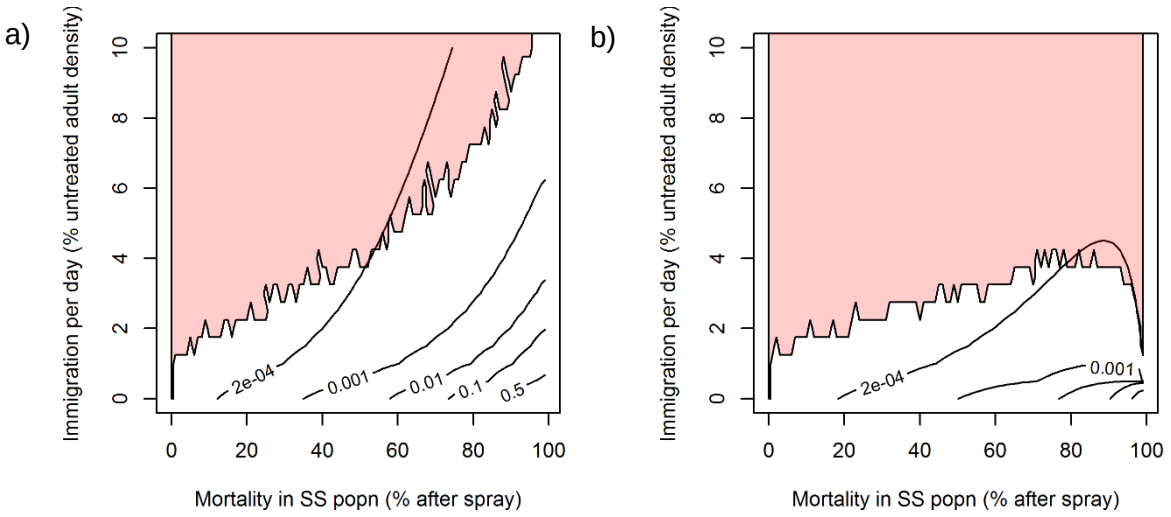

Figure 4. A trade-off between selection and dilution with immigration from an untreated population in a) the simple model, and b) the simple model with sexual recombination and a recessive resistance allele. Contour lines in each figure show the resistance allele frequency after five years of insecticide application. Shaded area shows where the resistance allele frequency is not increasing after five years of application.

Simulations demonstrated that an increasing dose always resulted in a more rapid build-up of target-site resistance if the model included all of the following:

- absence of immigration
- instantaneous emergence from an overwintering population
- all insect stages affected by the insecticide
- complete coverage of the crop with insecticide, i.e. no refuge areas

If one or more of the above factors was not true (i.e. there were susceptible immigrants entering the treated population, or the treated population emerged gradually from an overwintering population, or one or more life stages were unaffected by the insecticide, or there was incomplete coverage of the crop by the insecticide), then it was possible (although unlikely) that a high dose could provide reduced selection for resistance than a lower dose. This can be seen in Figure 4b, where, with an immigration rate of 1% of the density of an uncontrolled population into the treated population, the simulation resulted in resistance suppression when the insecticide efficacy was

99%, but resulted in increasing resistance frequency when the insecticide efficacy was reduced slightly to 95%.

In Table 1 each factor is added in turn to the simple insect model (hemimetabolous, asexual, diploid, no immigration, no emergence, all stages susceptible to the insecticide, no fitness cost), and two doses (high and low) applied. 'Yes' illustrates that the addition of a factor to the model enables a high dose to result in a lower resistance frequency than a low dose (in the simple insect model this is never the case). 'Yes' does not, however, imply that the addition of this factor always causes a high dose to reduce selection; this is typically an extreme occurrence.

All of the factors labelled 'Yes' in Table 1, whose inclusion results in the possibility of a high dose being more optimal for resistance management than a low dose, enable some portion of the insect population to be unaffected by an insecticide spray and therefore have a lower resistance frequency than the treated population after a spray. This results in a mechanism whereby a high insecticide dose can slow the build-up of target-site resistance: a higher insecticide dose reduces the density of the treated population to such an extent that any less resistant individuals that subsequently enter the treated population have a greater diluting effect than they would have if fewer of the treated population had died. The dilution effect therefore partially counteracts the effect of selection in the treated population; when the dose is high enough, dilution is greater than the selection effect.

Table 1. The table indicates whether inclusion of a factor into the simple model may result in target-site resistance building up slower when a high dose is applied compared to a low dose.

Factor	**Presence may result in high dose being optimal?**
Sexual reproduction	No
Haplodiploidy	No
Holometabolism (full life cycle)	No
One or more life cycle stages unaffected by insecticide	Yes
Gradual emergence from overwinter	Yes
Fitness cost of resistance	No
Univoltinism	No
Immigration	Yes
Incomplete coverage of insecticide	Yes

Case studies

Each species identified as a case study above was subjected to three doses of insecticide: 100%, 50% or 10% of a label dose, for five years. In each case the label dose resulted in the fastest increase in target-site resistance (Fig. 5). However the immigration rate of each insect was not

well explored. A parameter sweep was therefore conducted as in Figure 4 for each species (Fig. 6). For each species, resistance increased as the dose was increased (to increase the mortality of a single application) at all immigration rates. Additionally, for all three species it is unlikely that resistance will be suppressed unless the dose of insecticide was lowered to levels at which effective control was lost. Lowering the dose will, therefore, never lead to resistance building up when it had been suppressed at a higher dose.

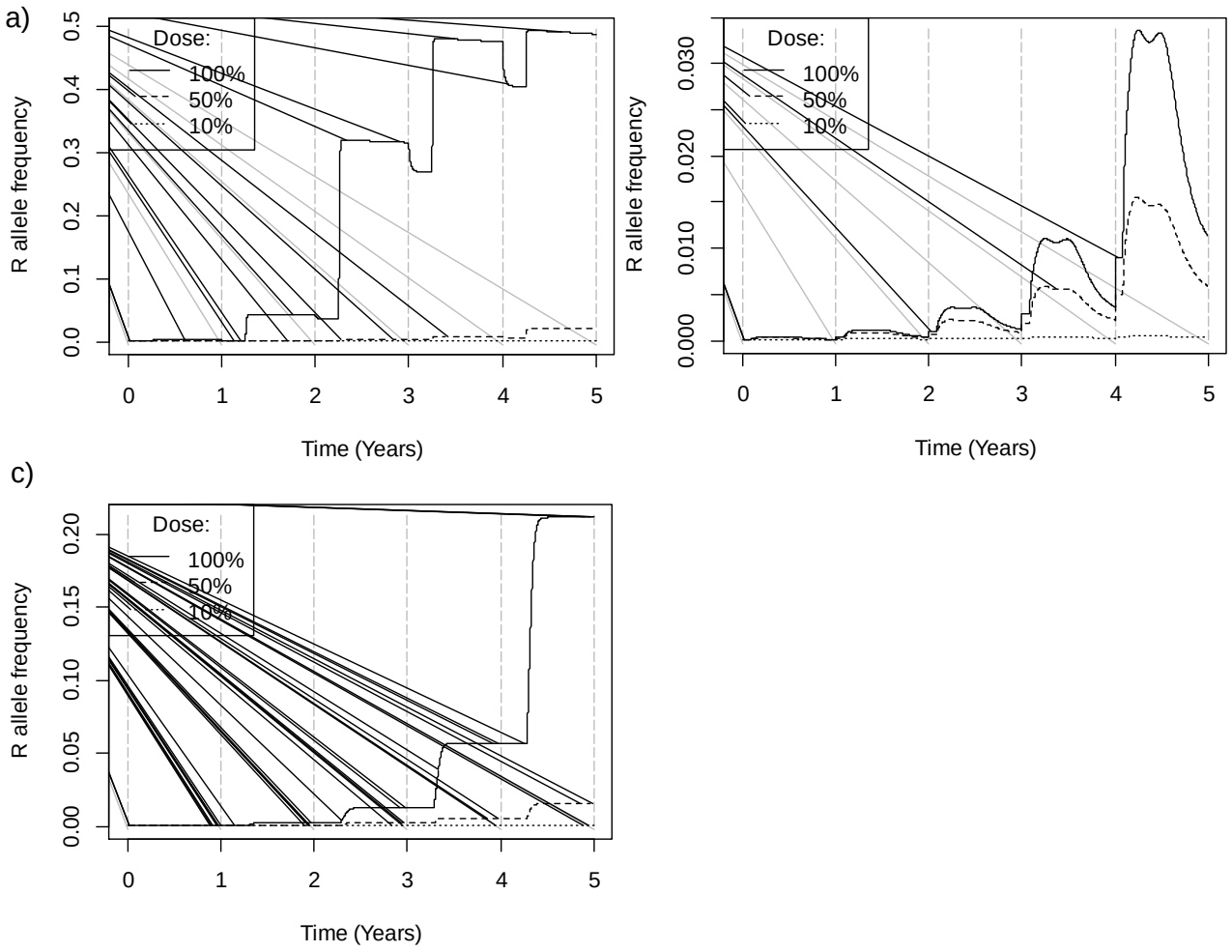

Figure 5. The effect of dose on the build-up of target-site resistance in three case study species: a) *M. persicae*, b) *M. aeneus*, c) *F. occidentalis*.

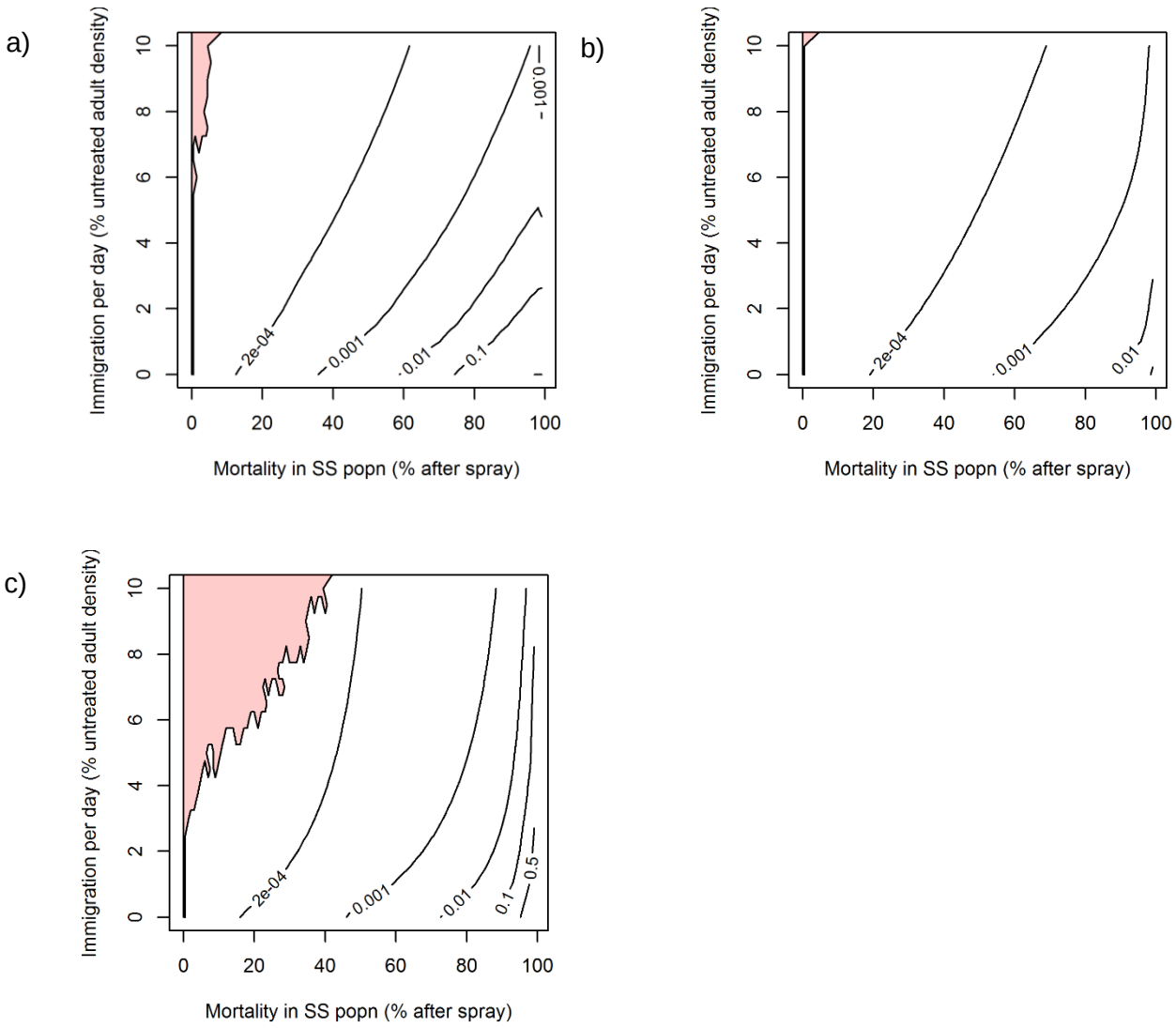

Figure 6. Selection in populations of three case study species: a) *M. persicae*, b) *M. aeneus*, c) *F. occidentalis*, under different levels of dose (set to give a mortality shown on the x-axis) and immigration. Contour lines show the resistance frequency after five years of applications. The shaded area shows when the resistance frequency does not increase: selection is balanced by dilution (see text for details).

1.3.1.2 Multiple insecticides

We consider here whether, if multiple insecticides are available, resistance is slowed the most when the insecticides are applied together in a mixture, or separately in an alternation. We first consider an alternation or mixture of insecticides applied at their label dose, before comparing an alternation of two insecticides at label dose with a mixture of two insecticides at a reduced dose that results in the same mortality as a single label dose.

Label dose mixture v label dose alternation

The scenario is first analysed, as before, with the simple insect model (being a hemimetabolous, diploid, asexual insect, with no fitness cost, no immigration, no gradual emergence from an

overwintering population, and no cross-resistance). Under these assumptions alternation is a better tactic than applying a label dose of each insecticide in a mixture (Figure 7). Resistance to each insecticide builds up independently of the other insecticide, and therefore applying both insecticides together in a mixture simply leads to resistance developing in half the time (Fig. 7a), compared to if the insecticides were applied on alternate years (Fig. 7b).

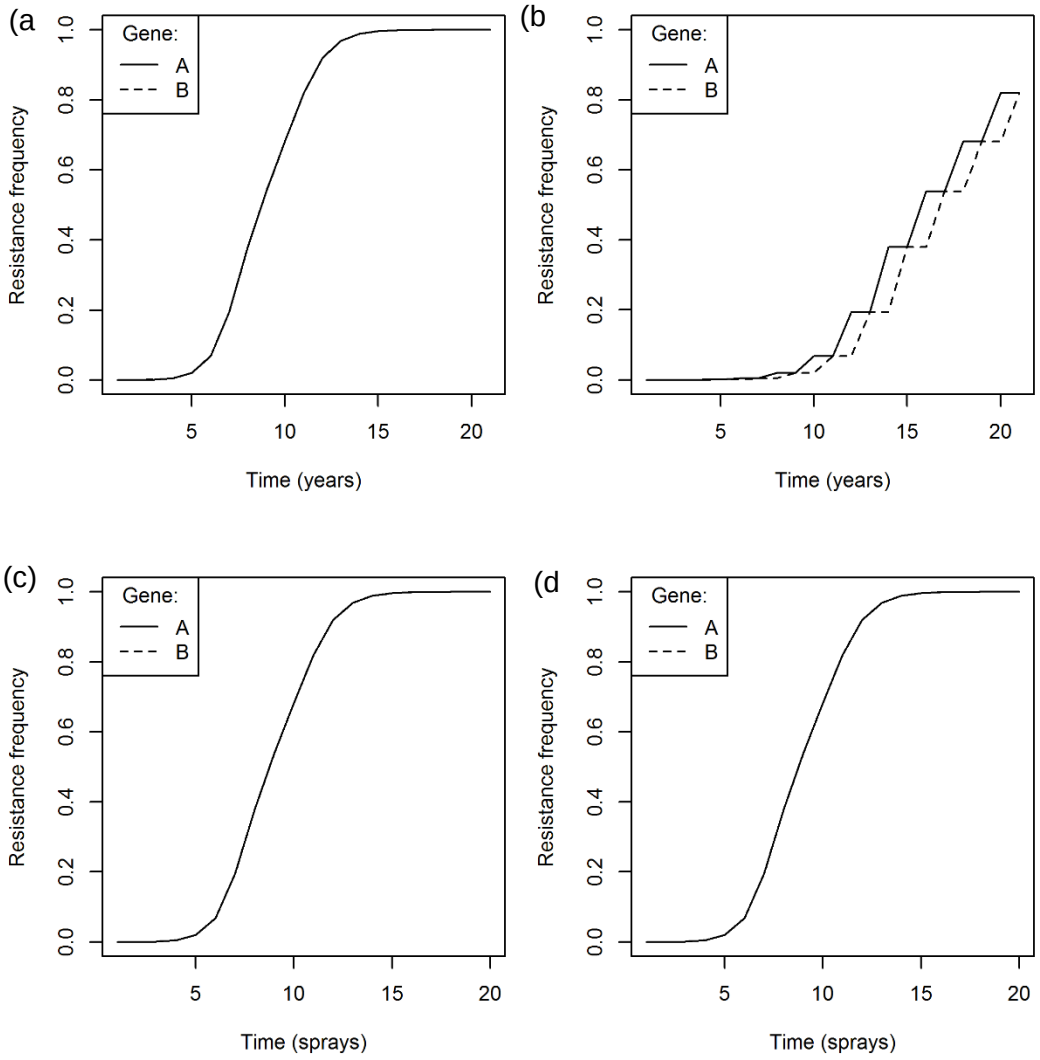

Figure 7. The frequency of each resistance allele (at genes A and B) increases each year (a and b) when a label dose of each insecticide is applied as (a) a mixture, and (b) an alternation. In a mixture the frequency of both genes increases at the same rate (gene A and B respond identically, and so the lines are superimposed). When plotted against the number of sprays (c and d) of the insecticide against which it confers resistance, the resistance genes increase at the same rate when applied either as a mixture (c), or as an alternation (d) (gene A and B respond identically, and so the lines are superimposed).

As resistance to the two insecticides increases at exactly the same speed according to the number of sprays (Fig. 7c and 7d), we can use this system to examine how different extensions to the

simple model (e.g. sexual reproduction) affect whether mixtures or alternations are better for slowing down the increase of resistance. Table 2 shows the resistance frequency of gene A after five applications of insecticide 1, applied either in a mixture of insecticide 1 and 2 at label dose (the frequency is then recorded after the 5th year), or in an alternation of insecticides 1 and 2 (the frequency is then recorded after the 9th year). In all cases where some portion of the population becomes unaffected by the insecticide (e.g. if one of the life stages is unaffected, there is immigration of susceptible individuals, or incomplete coverage of the insecticide on the crop) mixtures provide better resistance management than alternations. When there is a fitness cost, however, alternating the insecticides is a better strategy.

However, a mixture of two insecticides at their label dose leads to over-application of insecticide, if they both target the same insect pest. We next therefore consider resistance management in an alternative scenario; where the dose of the two components of a mixture is reduced so that the mixture provides the same efficacy as alternation.

Table 2. The table shows the resistance frequency of gene A after five applications of insecticide 1, either in a label-dose mixture, or in an alternation. The resistance frequency is shown for the simple model, and with the addition of each extension.

Factor	**Resistance frequency (Gene A) after 5 applications**		**Mixture or Alternation optimal?**
	Mixture	**Alternation**	
Simple model	0.491	0.491	-
Extensions:			
Sexual reproduction	0.623	0.623	-
Haplodiploidy	0.735	0.746	M
Holometabolism (full life cycle)	0.606	0.611	M
One or more life cycle stages unaffected by insecticide	0.0002	0.036	M
Gradual emergence from overwinter	0.608	0.620	M
Fitness cost of resistance	0.400	0.169	A
Univoltinism	0.0013	0.0017	M
Immigration	0.004	0.074	M
Incomplete coverage of insecticide	0.594	0.602	M
Cross-resistance	0.489	0.543	M

Reduced dose mixture v label dose alternation

Again we analyse this scenario with the simple insect model as before, and compare the speed at which resistance develops when the population is controlled either by alternating two identical insecticides applied at their label dose (assumed to give 90% mortality in a susceptible population) or as a mixture of each insecticide at a lower dose, which, combined, give a 90% mortality. Applying as an alternation or a mixture leads to a similar rate of resistance development, as shown in Figure 8 where the resistance frequency of gene A at year 6 was 0.133 when selected by the alternation strategy (Fig. 8a), whereas it was 0.124 when selected by the reduced-dose mixture strategy (Fig. 8b).

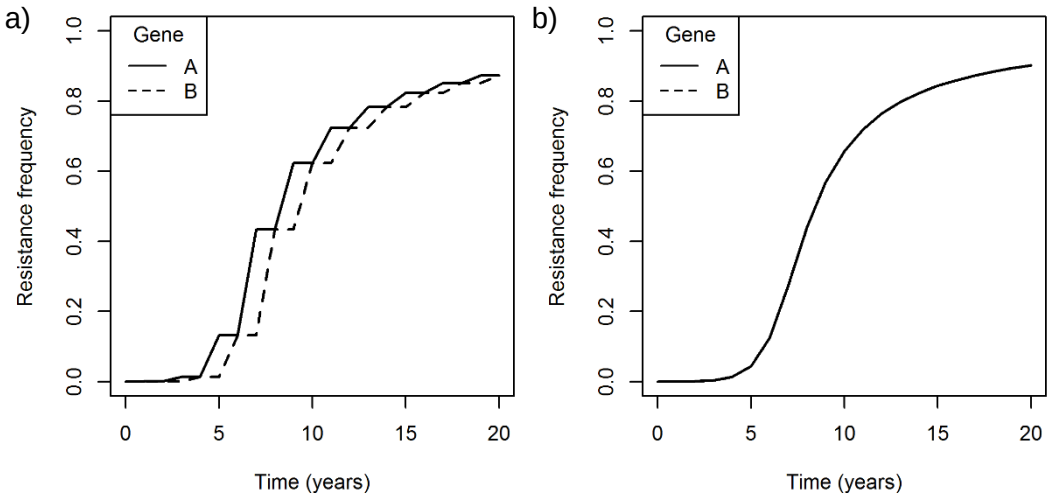

Figure 8. Comparison of a) an alternation of two label doses and b) a reduced-dose mixture in the simple insect model on the speed with which the frequency of resistance alleles of both genes (A and B) increase (in Figure 8b the lines overlap as both genes increase at the same speed). The yearly applications, whether in alternation or mixture, have the same efficacy and lead to resistance developing at the same rate.

Case studies

We next consider whether reduced-dose mixtures or alternations would be optimal with each case study insect. We assume, for simplicity, that each of the two insecticides have the same LC_{50}, with no cross-resistance. For *M. persicae* there was little difference between using a mixture or an alternation (Fig. 9a), the resistance frequency of both genes increased at a similar rate whether the insecticides were applied as mixtures or in alternation. However, for both *M. aeneus* (Fig. 9b) and *F. occidentalis* (Fig. 9c) resistance increased significantly slower when the insecticides were applied in a mixture.

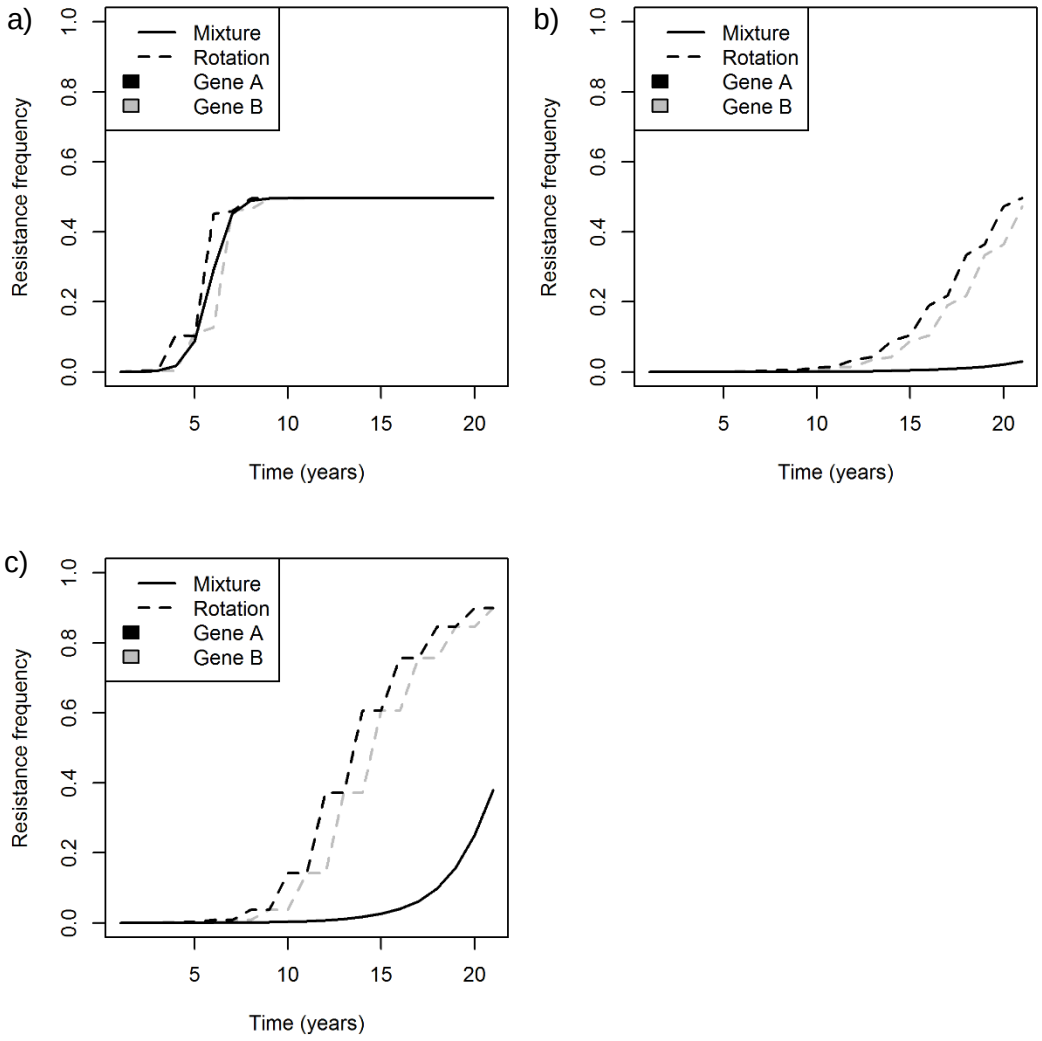

Figure 9. Comparing mixtures and alternations for three case study species: a) *M. persicae*; b) *M. aeneus*; c) *F. occidentalis*. In all cases the resistance frequency of genes A and B in a mixture increase at the same rate.

Global parameter search

A global parameter search was run to compare reduced-dose mixtures with label-dose alternations. Each generated scenario was then run twice; once with the insecticides being applied in a mixture, and once with the insecticides being applied in alternation. The number of years until both resistance genes were greater than 10% frequency was then measured under each application strategy. Figure 10 shows a histogram of all those cases where there was a difference in the number of years, with positive numbers being the extra years gained by applying the insecticides as a mixture and not an alternation, and negative numbers being the years lost by applying the insecticides as a mixture and not an alternation.

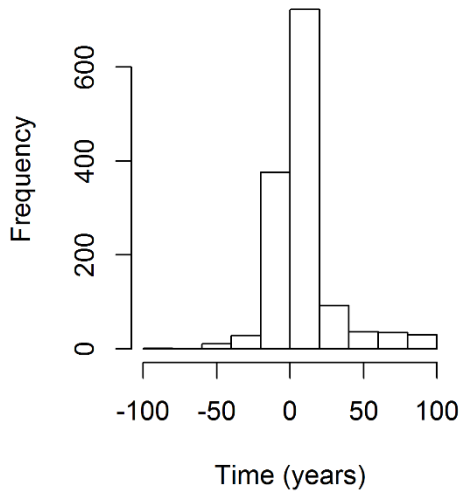

Figure 10. Difference in time until both genes exceeded 10% resistance frequency in a mixture – in an alternation.

3.4. Discussion

The process-based modelling approach addressed two questions. Firstly, the model was used to identify the optimal dose of a foliar-applied insecticide to reduce the development of resistance. Previous modelling work (Georghiou and Taylor, 1977; Georghiou, 1994) has suggested that a high dose of insecticide can suppress the development of target-site resistance to a better extent than a low dose of insecticide. Our simulations, using a more detailed model, suggest however, that in nearly all scenarios considered, reducing the dose reduces the speed at which resistance builds up. Whether target-site resistance is predicted to increase to substantial levels or not, reducing the dose of insecticide applied nearly always leads to a slower resistance evolution. While resistance could be suppressed using a high dose of insecticide in our modelling, it seems unlikely to be a biologically plausible scenario, as it requires high influx of untreated individuals to dilute the treated population, and a very effective insecticide. The influx of untreated individuals will then counteract the effect of selection; if the dose was lower, and the population density of the treated population was not as small, the diluting effect of the susceptible individuals entering the treated population would be lower. Information regarding immigration rates of insects was not easily available, but the immigration rates required seem high and in practice individuals entering a treated population are likely to have been exposed (or their forebears are likely to have been exposed) to the MoA previously, thus reducing the dilution effect. Also, insecticide treatment may not routinely achieve 99% effectiveness in the field. On balance it seems unlikely that the use of a high dose of insecticide will suppress resistance indefinitely. From the modelling work done in this project, the best resistance management strategy in the situation where a single insecticide is being applied against an insect pest that has developed target-site resistance is expected to be to lower the dose of insecticide applied, where this is possible without compromising effectiveness, yield or quality of the crop.

With two or more insecticides available with different modes of action, the best combination method for the two insecticides is less clear. Current guidance (FAO, 2012; IRAC, 2012) states that, when two or more insecticides are to be used in a mixture, they should be mixed at their full label doses. When targeted at the same species, however, this would result in the redundant application of insecticides (since the mortality provided by two simultaneous label doses would be substantially greater than that for a label dose of either component alone) and target-site resistance would increase more rapidly than if the doses of the components are adjusted. However, since the dose of each component of a mixture can be altered, a mixture could be made that has the same efficacy as a single label dose of either component. When this was simulated in the simple model, resistance built up at roughly the same speed as if each insecticide was used separately in alternate years. Alternations and mixtures are, in this scenario, equivalent. However, most realistic insect-insecticide scenarios are more complicated than this scenario. Upon addition of other complexities into the model, most scenarios resulted in insecticide mixtures being more effective at retarding the development of target-site resistance than rotating the insecticides. One factor, the presence of a fitness cost, led to alternations performing better. Whether to combine multiple insecticides in a mixture or alternate them will therefore depend on whether there are large fitness costs; if so, alternation may be more optimal. Otherwise a mixture is likely to be more effective at reducing the development of target-site resistance.

Most life-cycle characteristics of the insect did not materially affect whether either a high dose was appropriate or whether mixtures performed better than alternation (results not shown). The main characteristic that determined whether a high dose was optimal for resistance management was the relative influx of untreated individuals into the population being treated with the insecticide. Similarly, whether mixtures or alternations function better for combining two insecticides appears to be mediated largely by factors other than life cycle parameters; mostly by immigration rates, fitness costs, and cross-resistance, of which immigration rate is the only variable that may be known before resistance is detected. Fitness costs are generally not known until resistance is at a high proportion in the population, but may be the key component determining whether alternation will be a better strategy than mixtures.

We considered target-site resistance in this work, where a single resistance mutation confers a high (100 to 10000 fold resistance factor (see Appendix 2 for more info)) level of resistance towards an insecticide. While target-site resistance is well characterised and results in commercially important levels of resistance, metabolic resistance is thought to be the primary mechanism of resistance in several pest species. Metabolic resistance may be mediated by a single locus, or be polygenic, or based on the copy number of a gene. Neither polygenic resistance nor gene duplication have been analysed in detail in insecticide resistance models. Whether they cause the conclusions of this report to change is not known. While the dilution of a

treated population with unselected individuals would still be expected to be higher at higher insecticide doses, the different genetic assumptions could lead to selection acting quite differently.

The insects used as case studies were initially selected as they had very different life cycles, *M. persicae* having an incomplete life cycle and being asexual, *M. aeneus* being a sexual species, and *F. occidentalis* being haplodiploid. It is clear however that these traits are not key drivers of whether high doses are beneficial when considering the dose of an individual insecticide, or whether reduced-dose mixtures perform better than alternations when more than one insecticide is available. The rates of immigration of unselected individuals into the treated population and the presence of fitness costs appear, from this study, to be the most important factors to understand. For each case study, however, no fitness costs were found for the target-sites used, and immigration rates may be very hard to accurately determine. Nevertheless, those species that are relatively immobile are more likely to benefit from reducing the dose of a single insecticide (where this is practically feasible). The knowledge of movement rates may help to identify when lowering the dose could be effective, although it is likely that an insect would have to be very mobile for high doses to be an effective resistance management practice. The presence of fitness costs, however, will not be able to be determined until resistance has already developed. However, when resistance has been identified with a high fitness cost, and there are areas where the resistance is not yet present, rotations of different insecticides may be more optimal than the use of reduced-dose mixtures. Where fitness costs are not high, however, mixtures would be expected to slow resistance development.

The results in this model are based on the assumed processes that were coded in the model. While the model appears to function as expected, the model results need to be validated in experimental simulations to ensure the conclusions are credible for real insect populations. It was originally intended to provide some validation of the model using published data, however an extensive literature search found that the available data was not sufficient for model validation.

We emphasise that the results presented in this report are focused on reducing the development of insecticide resistance. We are therefore studying resistance management and not pest management. In a practical management situation there will be additional constraints to those we have studied. For example there will be a minimum dose below which the dose cannot be lowered without compromising yield. The results from this paper must therefore be considered in the wider context of pest management.

4. Work Package 2 - Develop a method to assess insecticide resistance risk based on objective and measurable criteria

4.1. Introduction

The development of insecticide resistance is an evolutionary process, governed by interactions between the biological characteristics (traits) of the pest, its host plants, and the way particular insecticides are deployed (Denholm et al., 1998). The risk of a new insecticide losing control efficacy due to the evolution of resistance differs widely (Roush and McKenzie, 1987). An objective means of assessing resistance risk would therefore be valuable for informing resistance management strategies. In the EU, resistance risk assessments form part of the registration and renewal process for plant protection products. Such assessments also form part of the implementation guidance (EPPO standard PP 1/271(2)) for the Comparative Assessment and Substitution process under EU regulation EC 1107/2009. This regulation determines whether a plant protection product can be substituted for another product that has failed to meet certain hazard criteria, where the substituted product is deemed safer and is used for the same purpose. The aim of including a resistance risk assessment in this process is to ensure that sufficient modes of action are retained when there is a high risk of resistance.

Resistance risk assessments in the EU follow guidance in EPPO standard PP 1/213(4), which involves the evaluation of inherent risk by considering factors defining the biology of the target pest, the cropping system under consideration, and the proposed pattern of insecticide use. However, these guidelines incorporate some subjective judgements and require prior knowledge which may not be available for new chemistry or newly prevalent pests. Hence, there is a need for a more objective, evidence-based approach to assessing resistance risk assessment for insecticides.

Objectives

1. Construct a trait-based database of previous cases of resistance.
2. Model the effect of biological, agronomic and operational traits on observed times taken for resistance to appear.

4.2. Methods

Work outlined below uses methods developed for assessing resistance risk in fungicides (Grimmer et al., 2015); findings from which are now informing and influencing practical decision making, through EPPO guidance. This work package used published data and other resources to populate a database of cases where resistance has developed which, alongside information on relevant biological, agronomic and operational traits, was statistically analysed to develop a model relating combinations of easily defined traits to the speed at which resistance is predicted to develop.

4.2.1. Data set of cases of insecticide resistance

Published literature on insecticide resistance was investigated in order to identify all the cases of resistance occurring for a specific arthropod (i.e. insect or mite) pest to a specific insecticide MoA group (as defined by the Insecticide Resistance Action Committee, IRAC) (Sparks and Nauen, 2015). Consideration was given to resistance cases occurring in countries belonging to the European Plant Protection Organisation (EPPO), as this is the organisation that produces the guidance by which risk is usually assessed in Europe (EPPO standard PP 1/213(4)). The majority of cases were extracted from the Arthropod Pesticide Resistance Database (APRD), an online database (http://www.pesticideresistance.org/index.php) of cases of insecticide resistance globally hosted by Michigan State University and funded through grants from the US Department of Agriculture, CSREES Pest Management Alternatives Program, the Insecticide Resistance Action Committee (IRAC), and the Generating Research and Extension to meet Economic and Environmental Needs (GREEEN) Project. In all cases the primary source of the report was consulted to verify the database entry, and if verified to obtain other information relevant to our study. Where multiple cases of resistance of a pest against a group of insecticides were reported in different countries, only the first report of resistance was included since it was considered that subsequent reports could result from pest dispersal from the country that first reported resistance, rather than *de novo* resistance development. Hence, only unique cases were analysed (i.e. one case per pest species by MoA combination).

4.2.2. Determining time until the first detection of resistance

The risk variable to be predicted by the risk assessment was time until the first detection of resistance (FDR time). This is defined as the time in years from the year of first exposure of a particular pest species to an insecticide of the MoA group under consideration until the year in which resistance was first reported in that pest. The year of first exposure was obtained preferentially from primary, peer-reviewed literature or a Chemicals Regulation Directorate (CRD) database on first approvals for active substances in the UK. Where such data could not be found from these sources (e.g. for countries other than the UK), expert judgement or the equivalent year in the CRD database (for non-UK cases) was used. When using the CRD database, where an active substance was not registered for a particular pest on a specific crop but that pest was considered to commonly occur on that crop, then exposure was assumed. This allowed incidental exposure to a MoA through the treatment of another species on the same crop to be considered and ensured that a realistic year of first exposure was determined. No assumptions or estimates were made in relation to the amount of exposure.

4.2.3. Generating a data set of traits for predicting FDR time

A list of candidate traits for insecticides, pests and agronomic systems was constructed, each candidate trait had a mechanistic rationale for being a potential determinant of FDR time. Trait

data for each resistance case were obtained preferentially from the primary, peer-reviewed literature. Where such data could not be found, secondary sources such as books and published reports were used. As a last resort, expert judgement was relied upon. The traits were described as either variates (consisting of numeric values with a continuous distribution) or factors (consisting of discrete categories of numerical or descriptive information). Factor categories containing less than five cases were amalgamated with other categories of four or less cases to create relevant 'other' categories (e.g. 'Other - Central Europe' and 'Other - Mediterranean' countries), otherwise cases were amalgamated simply into 'Other' categories. In total there were 17 candidate explanatory variates and 27 factors, with traits related to the pest, the agronomic system or the insecticide treatment (Table 3).

Table 3. Candidate traits of pests, insecticides and agronomic systems tested for significant association with FDR time (years to first detection of resistance). F = factor, V = variate. Where a trait is followed by both F and V it was analysed as both a factor and variate. *Separated into two traits; one relating to the crop on which resistance was detected and the other to all crops on which the pest is a problem.

Arthropod pest traits	**Insecticide/Acaricide**	**Agronomic system traits**
Phylum (F)	Active ingredient (F)	Agronomic intensity (V)
Class (F)	H-bond donor potential (F + V)	Amount of active used per year (V)
Order (F)	H-bond acceptor potential (V)	Annual/Perennial* (F)
Family (F)	H-bond potential (V)	Application method (F)
Genus (F)	Chemical group (F)	Area treated (V)
Species (F)	IRAC MoA (F)	Country (F)
Dispersal mode (F)	Molecular complexity (V)	Crop type (F)
Generations per year (F)	Molecular weight (V)	Crop area (V)
Lifetime fecundity (F)	Solubility (V)	Global status (F)
Metamorphosis (F)	Solubility ratio (V)	Insecticide use intensity (area) (V)
Number of crop hosts (F)	Target protein length (V)	Insecticide use intensity (weight) (V)
Reproductive rate (F)		Number of applications per year (V)
Reproductive strategy (F)		Production system* (F)
Risk of human assisted spread (F)		Tonnage (stored crops only) (V)
Susceptible stage exposed (F)		Tonnage treated (stored crops only) (V)

The definitions for each trait are as follows:

Active ingredient: BSI/ISO accepted common name for an individual active ingredient. Based on the IRAC MoA Classification Scheme (Sparks and Nauen, 2015).

Agronomic intensity: Land area of crop* in specific country** as a proportion of the total land area of country.

Amount of use: Amount of active ingredient applied to crop* each year**.

Annual/Perennial: Whether crop is annual, perennial or stored**. Separated into two separate traits; one relating to the crop on which resistance was detected and the other to all crops on which the pest is a problem.

Application method: Primary application method of the active ingredient.

Area treated: Area of crop* treated with foliar applications of the active ingredient**. Area of seed treatments was investigated but no data was available for relevant resistance cases.

Chemical group: Insecticide grouping based on chemical consideration.

Country: Country in which samples used to confirm resistance were collected.

Crop type: The crop* grouped into crop types based on the agronomy of their production. For example, protected edibles, arable vegetables, and cereals and oilseed rape. These groupings of crops may differ in intensity of control, pest types, scale of production and presence of untreated refuges.

Dispersal mode: The primary method of pest dispersal.

Generations per year: Number of life cycles of the pest completed per year**.

Global status: First incidence of resistance; Europe, outside of Europe or both.

H-bond acceptor potential: Number of hydrogen acceptors in the active ingredient compound structure.

H-bond donor potential: Number of hydrogen donors in the active ingredient compound structure.

H-bond potential: Number of hydrogen donors (both donor and acceptor) in the active ingredient compound structure.

Insecticide use intensity (area): Area of crop* treated with the active ingredient as a proportion of the total area of the crop**.

Insecticide use intensity (weight): Amount (kg) of crop* treated with the active ingredient as a proportion of the total crop** (hectares for cultivated crop or tonnes for stored crops).

IRAC MoA: Grouped according to chemical relatedness of structures and MoA. Based on the IRAC MoA Classification Scheme (Sparks and Nauen, 2015).

Lifetime fecundity: Mean total number of offspring produced by an individual over its life-time**.

Metamorphosis: The form of development immature stages undergo; partial (hemimetabolous) or complete (holometabolous).

Molecular complexity: Estimate of the complexity of the active ingredient in terms of elements contained and structural features such as symmetry.

Molecular weight: The sum of all atomic weights of the constituent atoms in the compound

Number of applications per year: Number of foliar applications of the active ingredient applied to crop* each year**.

Number of crop hosts: Number of primary, common crop species on which the pest is commonly treated**.

Phylum-Species: Classification according to taxonomic group.

Production system: Whether crop is grown outdoors, in a protected situation (e.g. glasshouse) or is a stored crop**. Separated into two separate traits; one relating to the crop on which resistance was detected and the other to all crops on which the pest is a problem.

Reproductive rate: Mean number of offspring produced by an adult per day**.

Risk of human assisted spread: Likelihood of the pest being spread by human activities.

Solubility: Solubility of the active ingredient in water.

Solubility ratio: A partition coefficient or distribution coefficient of the active ingredient that is a measure of differential solubility of a compound in two solvents (octanol/water).

Susceptible stage exposed: The life-stage of the pest that is exposed to the active ingredient.

Target protein length: Mean protein length of insecticide target protein. If unavailable for the pest species the value for the closest taxonomic relative was used.

Tonnage: Tonnage of crop* stored annually** (stored crops only).

Tonnage treated: Tonnage of crop* treated with specific insecticide annually** (stored crops only).

*Refers to the crop on which resistance was first detected.

**Refers to country in which resistance was first detected (see 'Country' trait definition).

The units, categories (where relevant) and the source of the values were recorded in the database, along with any assumptions made or calculations used and the perceived relevance of the trait to resistance development.

4.2.4. Development and validation of a model to explain variation in FDR time

Individual traits (Table 3) were first statistically analysed for their relationship with FDR time using regression analysis. Those that were significantly ($P < 0.05$) associated with FDR were then included in a step-wise multiple regression analysis to identify the key traits explaining the variation in FDR time. The step-wise regression method selects the trait explaining the greatest percent variation accounted for (% VAF) followed by the trait that explains the second greatest % VAF and so on. At each step, after the addition of the most significant term, the method evaluates the partial F-ratios of the terms in the new model to decide whether any term should now be dropped. It repeats the process until no terms should be eliminated, and then resumes the forward selection process. The process was constrained so that the final model did not predict for more than 1000 factor combinations. This was done to ensure the model was of practical use and to limit extrapolation of the data to trait combinations not represented in the database. Residuals from the

final model were visualised to check for any evidence of a skewed distribution and non-constant or systematic variance. Point estimates with 95% confidence intervals were generated for means of traits within the final model, to indicate the levels of uncertainty surrounding model predictions of FDR time.

In order to validate the new model, all the possible explanatory variates and factors were randomised to the 125 observations of FDR time 1000 times, to give 1000 new versions of these variates and factors for modelling. In each case, the best model was found by invoking the same forward selection routine using the % VAF for inclusion of model terms, to output the Akaike information criteria (AIC) (Akaike, 1974) for each model. The proportion of models out of the 1000 that attain an AIC less than the final model indicate how powerful the final model is compared with the models of the randomised data.

4.3. Results

4.3.1. Data set of cases of insecticide resistance

The 125 verified unique cases of insecticide resistance comprised a wide range of different insecticide groups, crops and pests, with resistance being detected in 20 countries belonging to EPPO. Details of these cases are given in Appendix 4. The frequency distribution of FDR times (Fig. 11) showed FDR values to be positively skewed, so square root transformed FDR (sqrt FDR) time was used as the response variate in model development. The number of cases per four year period peaked at 5-8 years following insecticide introduction then gradually decreased with time.

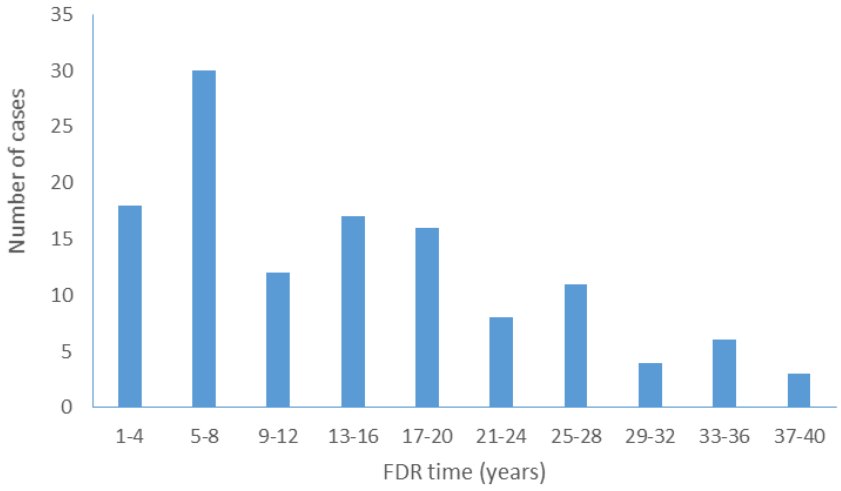

Figure 11. Frequency distribution of FDR time for 125 unique cases of resistance in pests in countries belonging to EPPO.

4.3.2. Development of a resistance risk assessment model

17 of the 44 trait variables and factors showed a significant ($P < 0.05$) individual association with sqrt FDR time (Table 4). This suggested that identifying a subset of traits that together explain a substantial proportion of the observed variation in FDR time would be possible. The factor 'crop type' had the strongest correlation and on its own explained 33% of the variation in sqrt FDR time. The categories used for this factor and their influence on FDR are shown in Figure 12. Similar graphs for all other traits in Table 4 are presented in Appendix 5 showing the categories in each trait and their relationship with sqrt FDR time.

Table 4. Traits significantly (F-test) associated with sqrt FDR time (years to first detection of resistance). RH = crop on which resistance was detected, AH = all crops on which the pest occurs.

Trait	*P*-value	F	F df	Residual df	% VAF
Crop type	<0.001	7.56	9	112	32.8
Log crop area	<0.001	19.87	1	103	15.4
Genus	0.004	2.43	15	109	14.8
Species	0.005	2.42	15	109	14.6
Production system RH	<0.001	8.92	2	122	11.3
Family	0.009	2.50	10	114	10.8
Order	0.002	3.98	5	119	10.7
Reproductive strategy	0.002	5.34	3	121	9.5
Active ingredient	0.029	1.98	13	111	9.3
Susceptible stage exposed	0.002	5.17	3	121	9.2
Production system AH	0.011	3.91	3	121	6.6
Risk of human assisted spread	0.006	5.25	2	122	6.4
Log agronomic intensity	0.008	7.23	1	103	5.7
Class (taxonomic)	0.009	7.13	1	123	4.7
Generations per year	0.021	4.01	2	122	4.6
Metamorphosis	0.009	6.99	1	123	4.6
Number of crop hosts	0.023	5.33	1	123	3.4

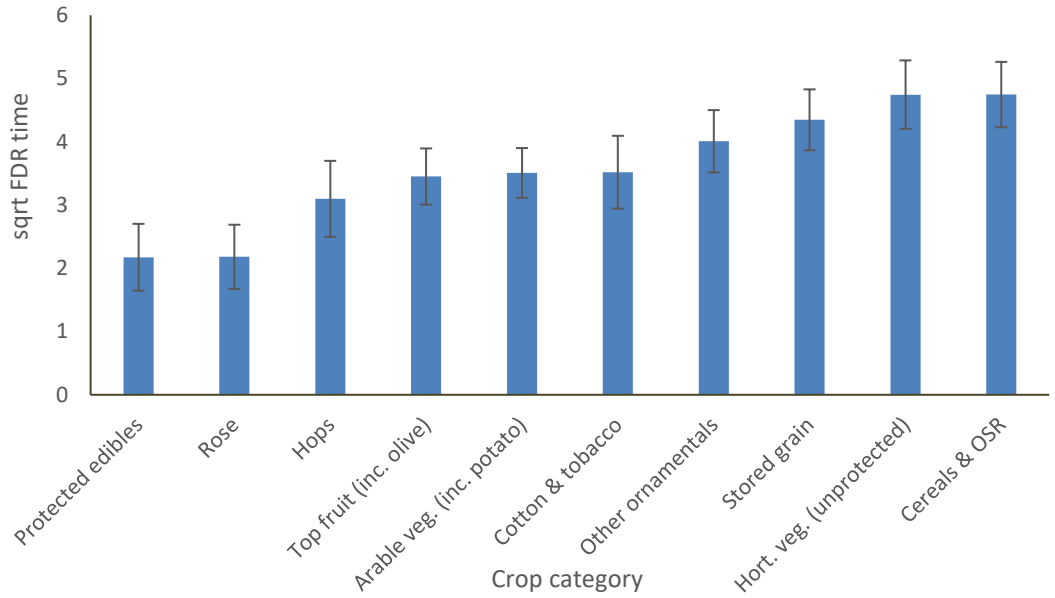

Figure 12. Relationship between the crop type on which resistance had been detected and FDR time. Bars indicate the standard error of the estimate.

The taxonomic categories, 'family', 'genus' and 'species', were excluded from further analysis as they contained too many poorly-populated categories. Stepwise multiple regression analysis of the remaining 14 traits against sqrt FDR time identified the following final model, with traits listed in the order selected by the routine:

Sqrt(FDR) = Constant + β*log Crop area + Crop type + Number of crop hosts + Reproductive strategy + Order

where each trait assumes a numerical value depending on the assigned category. Model parameter estimates are presented in Appendix 6.

The variables and the factors included for the model are defined as:

Crop type: The crop type on which resistance was first detected. This factor had eight categories; 'arable vegetables (including potatoes)', 'cereals & oilseed rape', 'cotton & tobacco', 'horticultural vegetables (unprotected)', 'protected edibles', 'rose', 'other ornamentals' and 'outdoor fruit'.

Number of crop hosts: The number of important crops on which the pest is commonly treated (either directly, through treatments specifically for the pest, or indirectly, through treatments for another pest). This factor had two categories; '1-9 crop hosts' or '>9 crop hosts'.

Reproductive strategy: The reproductive strategy employed by the pest. This factor had four categories; 'holocyclic' (aphids that alternate parthenogenesis with sexual reproduction, e.g. *Nasonovia ribisnigri*), obligate parthenogenesis (aphids that reproduce solely or predominantly asexually e.g. *Sitobion avenae* and *Myzus persicae* in the UK), 'sexually reproducing diploid

organisms' (e.g. moths, beetles and flies) and 'sexually reproducing haplodiploid organisms' (e.g. whiteflies, thrips and many mites).

Order: The taxonomic order of the pest. This factor had six categories; 'Acari' (comprising Prostigmata and Astigmata), 'Coleoptera', 'Diptera', 'Hemiptera', 'Lepidoptera' and 'Thysanoptera'.

None of the potential interactions between the model terms were significant. The accumulated analysis of variance, showing the terms in the model, is shown in Table 5. The step-wise regression method did continue to add further traits to the model but these had minimal additional effects on predictive power and were rejected as they led to more than 1000 category combinations. A plot of the residuals from the final model indicated a random scatter resembling a normal distribution.

Table 5. Accumulated analysis of variance (ANOVA) for terms in the final insecticide resistance risk assessment model. The model was derived by testing for association of traits with FDR time (years to first detection of resistance). This table shows the order in which traits were included as model terms and the extent to which they explained the observed variation in FDR time[a].

Change	df	ss	ms	Vr	F pr
+ Log crop area	1	31.37	31.37	31.06	<0.001
+ Crop type	7	55.68	7.95	7.88	<0.001
+ No. of crop hosts	1	3.98	3.98	3.94	0.05
+ Reproductive strategy	3	4.78	1.59	1.58	0.201
+ Order	5	10.33	2.07	2.05	0.08
Residual	87	87.86	1.01		
Total	104	193.99	1.87		

[a]df: degrees of freedom; ss: sums of squares; ms: mean square, vr: variance ratio; F pr: F-probability (P-value).

The final model, with observed and predicted values shown in Figure 13, accounted for 45.9% of the variation in sqrt FDR time ($P < 0.001$, F-test), with a standard error of observations estimated to be 1.00. Of the possible 125 observations of FDR time, this model used 105. 20 cases involving storage pests (cases 1, 2, 23, 27, 28, 37, 42, 52, 53, 55, 58-62, 81-84 and 89, Appendix A) were omitted as defining crop area values was not possible for these cases.

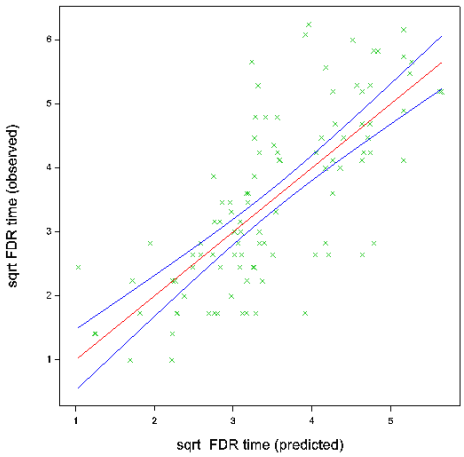

Figure 13. Comparison of observed sqrt FDR times with the values predicted in the insecticide resistance risk assessment model developed in this project (FDR time being years until first detection of resistance). Straight line = fitted equation for the relationship between the observed and predicted values. Curved lines = 95% confidence interval for fitted line.

The degree of uncertainty surrounding model predictions can be determined by generating the 95% confidence interval associated with each point estimate. Figure 14 shows the back transformed point estimates and confidence intervals for four high, medium and low risk category combinations. The category combinations that existed in the database are indicated with an asterisk.

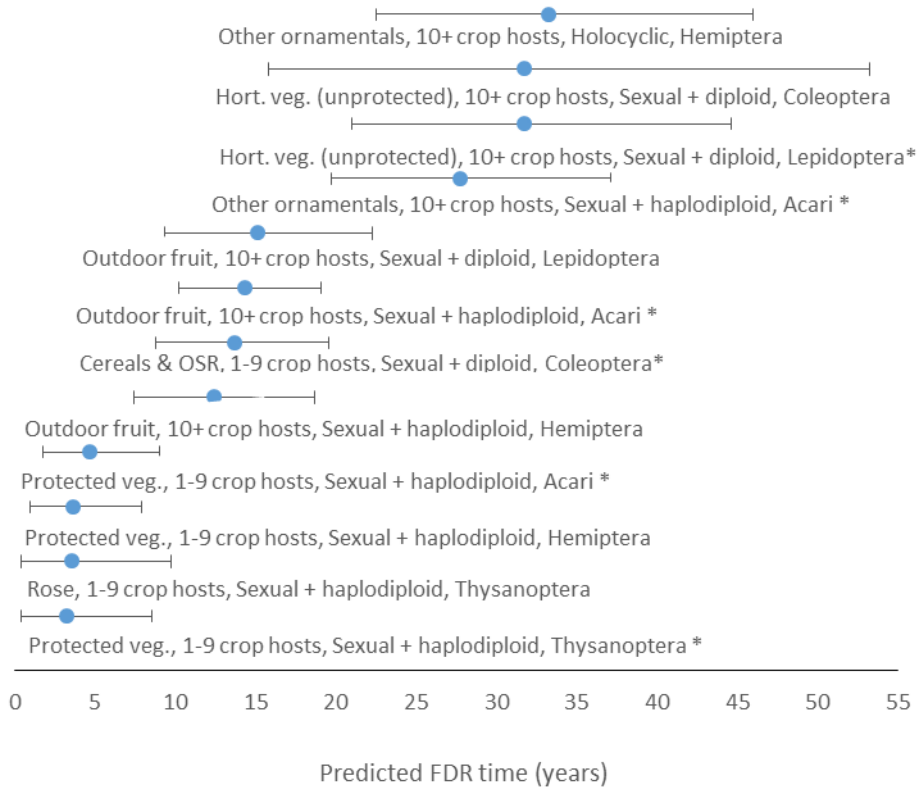

Figure 14. Estimates of FDR time (years until first detection of resistance) back transformed from the square-root scale for 12 category combinations (4 each of low, medium and high risk pest and agronomic system combinations). Bars indicate the 95% confidence interval. The estimate and confidence intervals are based on a mean log crop area in the database of 4.28 (back transformed value = 19053 ha). * = category combinations that existed in the database.

4.3.3. Validation of the new risk assessment model

The forward selection routine used to fit models to the 1000 randomised data sets was limited to the best five parameter model. This limitation was chosen to ensure a relevant comparison to the new five parameter risk assessment model described above. Only 11 out the 1000 models (1.1%) had an AIC less than the final model (AIC value = 506), illustrating how likely it was to obtain a model with a lower AIC using randomised data than the final model. Given that the forward selection routine only adds traits to the model if it significantly ($P < 0.05$) improves the model, it might be expected chance alone would allow a certain percentage of models to provide a better fit of the randomised data using AIC (in regard to the theory of type 1 error rates). The value obtained is well within this expectation, meaning that random chance is highly unlikely to produce an improved model (in terms of predictive power and number of observations) than the final model described above.

4.4. Discussion

Although ultimately driven by exposure of pests to pesticides, the development of insecticide resistance has long been known to be under the influence of a large suite of biological, genetic, agronomic and operational traits defining the pest, cropping system and the type of control regime applied (Roush, 1989; Denholm and Rowland, 1992). Effects of varying such traits have been explored using computer simulations and analytical models (e.g. Taylor and Georghiou, 1979; Tabashnik and Croft, 1982), providing valuable insight of their generic influence without reference to particular pest populations or case-histories. The current study is, to the best of our knowledge, the first time a trait-based approach has been used to identify the most important and consistent determinants of resistance in a wide range of observed outbreaks of insecticide resistance. The findings not only highlight a number of individual traits showing a significant association with speed at which resistance appeared, but have also identified a combination of traits with the greatest power to describe FDR times.

The USA-based Arthropod Pest Resistance Database (APRD) is a global compendium of all known reports of insecticide resistance up to the present day. These reports have varying degrees of scientific verification. We applied a number of filters to obtain a tractable number of case-histories of proven provenance. Firstly, the geographical scope of the study was restricted to Europe and neighbouring countries subscribing to EPPO. Secondly, we eliminated reports in the APRD that were unsupported by a sufficiently detailed research publication – ideally in a peer-reviewed journal although some cases reported in conference or workshop proceedings were also admitted. Thirdly, only the first validated report of resistance of a particular pest to a particular MoA was included since subsequent reports from the same or different countries could result from gene flow from a point of origin, and reports for different molecules within a MoA group could reflect cross-resistance conferred by the same gene or mechanism. Fourthly, 20 cases relating to pests of post-harvest produce were excluded during the analysis since one of traits of emerging importance (crop area) could not be quantified in a comparable manner with field or glasshouse crops. The final shortlist of 105 case-histories spanned reports of resistance between 1930 and 2009, and covered 20 counties, 48 pest species and 23 IRAC MoA groups (comprising 66 active ingredients).

Retrospective assessment of the times taken for resistance to develop is fraught with difficulty. The original sources of each resistance report were scrutinised to determine the date when a field sample was collected (as opposed to the date of publication). Dates of first exposure to a specified MoA will incorporate some error, since the year of approval may not equate with the year of first use. Despite these concerns, FDR time as defined in this paper is probably the most objective and readily quantifiable metric that can also be broadly applied across a range of resistance reports (Grimmer et al., 2014).

Estimated FDR times for the case-histories in our database ranged from one to 39 years. The frequency distribution of FDR times showed that most cases occurred just under a decade after first exposure before gradually tailing off. The peak in FDR times is likely to follow from the increase in market share of a new insecticide group following commercialisation, resulting in an increase in use and selection pressure. The effects of this evolutionary pressure are then seen when resistance is detected subsequently. The reduction in the number of new cases over time is understandable as most of the limited number of pests that are regularly treated (and for which control failures would be noticed) are likely to develop resistance at some point, resulting in a finite number of detected resistance cases. Some MoAs also have a limited life-span before falling into disuse, sometimes for reasons unrelated to resistance.

The final model, accounting for 45.9% of the variation in FDR times, comprised five traits relating to the pest and the agronomic system. Some of these traits have an intuitive rationale for their direction of influence on resistance evolution. The interpretation of other traits is less clear. They are discussed below, in the order in which they were included as terms in the final model.

Log crop area: FDR time was positively correlated with log crop area. This is likely to reflect the higher insecticide use intensities, conditions more conducive to population growth and more limited access to refuges (non-treated areas) associated with crops grown across smaller areas. Broad acre pests are more likely to go untreated (due to the nature of their damage and spray application practicalities) and have greater access to refuges (be it untreated crops or non-crop hosts). With mixing pest sub-populations this could result in a reduced competitive advantage of the resistant strain over the wild type.

Crop type: Short FDR times were strongly associated with (i) crops where low damage tolerance results in increased levels of control (e.g. fruit and ornamentals) and therefore increased selection pressure; (ii) crops grown in environments more conducive to rapid pest development and reproduction (e.g. greenhouses and polytunnels), thus promoting repeat applications of insecticide and allowing resistance to be selected more rapidly; and (iii) crops which have fewer restrictions on insecticide use (e.g. non-edible crops such as tobacco). Longer FDR times were associated with crops where refuges are readily accessible (e.g. broad acre crops).

Number of crop hosts: Shorter FDR times were associated with highly polyphagous pests (>9 crop hosts). This is likely to be due to increased exposure to insecticides across a range of crops, at the same or different times of the year.

Reproductive strategy: Short FDR times were associated with haplodiploid pests that reproduce sexually. A resistance mutation would be expressed in haploid males irrespective of dominance, resulting in a greater proportion of resistant males mating with diploid females. The high reproductive rate of many haplodiploid pests (e.g. whiteflies and spider-mites) means that this selection for the resistant allele is likely to be further increased. Intermediate FDR times were

associated with holocyclic and obligately parthenogenetic aphids, which reproduce clonally for at least part of their life-cycle so that resistant clones are readily selected for. Longer FDR times were associated with sexually reproducing diploid pests. This may be because, depending on the dominance of the resistance allele, the competitive advantage of a resistance mutation may only be realised once it reaches a frequency at which homozygous resistant individuals form an appreciable component of the population.

Order: Shorter FDR times were associated with Thysanoptera and Acari; intermediate FDR times with Lepidoptera, Hemiptera and Diptera; and longer FDR times with Coleoptera. This is likely due to the contrasting life-histories (e.g. reproductive rates and strategies, and forms of metamorphosis) between the taxonomic groups and the intensity of control applied to crops on which they tend to be pests.

There were a number of other traits that were significantly associated with FDR time when analysed individually but did not feature in the final model. Some of these explained a greater amount of variation in FDR time than those in the final model; however their effect was reduced during stepwise regression due to strong correlations with other traits, rendering them redundant.

The active ingredient, amalgamated into groups (see Figure 8 in Appendix 5) based on the IRAC MoA Classification Scheme (Sparks and Nauen, 2015), was the only insecticide trait found to be significantly associated with FDR time. A similar trait-based approach to the development of fungicide resistance found a number of fungicide-related traits to be important (Grimmer et al., 2015) and it would be surprising if such characteristics had no influence on the development of insecticide resistance. That they feature so weakly in this work may be due to the likely range of resistance mechanisms that occur in the database (in the fungicide work target-site resistance probably accounted for the majority of cases), as it is probable that different insecticide traits have an influence on the evolution of different resistance mechanisms. As information on the resistance mechanism of historical resistance cases was unavailable for the majority of resistance cases in the database, a resistance mechanism trait was not included in this work. It is also likely that some traits in the final model (particularly log crop area and crop type) act as proxies for insecticide use traits such as the amount of insecticide applied and number of applications. Reliable data relating to insecticide use patterns (quantities applied, numbers of applications, area treated, use intensity, etc.) were difficult and sometimes impossible to locate, leading to missing values and low statistical power in the regression analyses. The predictive power of the fitted model is satisfactory considering the complexity of the system being modelled. With improved information on insecticide use patterns (quantities applied, numbers of applications, area treated, use intensity, etc.) and estimates of pest traits, it is possible that predictive power could be increased further.

There are a number of potential sources of bias in the published literature that this study depended on. There is a bias towards countries where research teams have the expertise to monitor and analyse resistance problems. There is probably a bias towards more recent resistance cases as the

awareness of resistance and its implications has spread. There may be a bias towards more 'dramatic' cases that are newsworthy, and therefore more likely to attract funding. These points should be considered when interpreting the risk assessment method, although they apply similarly to any other method of assessing risk based on experience of previous resistance cases.

The model we propose provides a foundation for objectively and quantifiably determining resistance risk and can be used for novel pests or insecticide MoA for which there is little information on resistance mechanisms that might evolve. All traits in the final model should be relatively simple to quantify given some knowledge of pest biology. In the case of a species newly introduced to a region, information should be available from research at the place of origin. As is clear from the 95% confidence intervals around predicted FDR times, there is a large degree of uncertainty surrounding the predictions of risk that reflects inherent noise and the stochastic, or still unexplained, processes governing the emergence and selection of resistant populations. There will never be a tool enabling the number of years for resistance to evolve to be predicted with complete accuracy. Our aim in this study has been to develop an objective means of ranking pest-crop combinations from high to low risk, allowing proportionate and effective resistance management strategies to be put in place. This can complement and strengthen the current EU risk assessment guidelines (EPPO standard PP 1/213(4)).

Future work

Details of future cases of insecticide resistance can be used to check the accuracy of the model's forecasts, and adding these to the database as they arise should, after a period of time, enable a re-analysis and modification of the model to increase further its predictive power. Following formal publication of this study in the peer-reviewed literature, the database containing existing trait values and accompanying notation will be placed in the public domain for scrutiny and use by others. The mechanisms for access and updating the database will be agreed following discussions with CRD, EPPO, IRAG-UK and IRAC. It may be beneficial to widen the scope of the work to include resistance cases occurring outside of Europe (particularly North America and Australia) to determine whether the same traits remain of predictive value.

5. Work Package 3 - Transfer new knowledge of anti-resistance strategies and risk assessment to relevant end-users.

5.1. Introduction

Effectively communicating progress with principal stakeholders is important to ensure that the approaches used are appropriate and that findings can be discussed at an early stage. Hence, there was active exchange of information with all the stakeholders, to keep the project focussed on delivering relevant information, and keep all the stakeholders informed about progress.

Objectives

1. Translate results into messages for AHDB levy payers, and guidance to underpin regulatory decisions on resistance management by CRD in liaison with IRAG.
2. Subject findings to peer review and publish in international journals.

5.2. Knowledge transfer activities

IRAG-UK is an organisation that provides information on resistance status and resistance management strategies to UK farmers and growers, advisers and regulatory authorities. As its members include a number of important stakeholders, it was felt that the organisation constituted the ideal stakeholder group for the project. The knowledge transfer activities undertaken as part of the project are shown in Table 6.

Table 6. Knowledge transfer activities

Date	Activity
12/11/2013	31st meeting IRAG (1st stakeholder meeting)
18/03/2014	32nd IRAG meeting (2nd stakeholder meeting)
Winter 2014	HGCA Agronomy Workshops (x4)
14/04/2014	Bayer oilseed rape conference
10/02/2015	BBRO winter conference (Poster)
April 2015	Research in Action article, Arable Farming magazine
Spring 2015	Article, ADAS Technical Update
August 2015	Article, Crop Production magazine
16/09/2015	EPPO Panel on Resistance meeting
12/11/2015	Australian Agrichemical Resistance Meeting
14-16/09/2015	Resistance 2015 International conference (x2 posters)
06/04/2016	36th IRAG meeting (3rd stakeholder meeting)

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7. Appendices

7.1. Appendix 1 - Simulation model details

Insect model

As stated in the main text, the model simulates the density of an insect population, within which the population is sub-divided into stages (eggs, larvae, pupae, adults), instars and resistance genotypes. The mean duration of each stage (egg, larvae, pupa, adult) is μ_S days, where $1/\mu_S$ is therefore the rate of transition from one stage to the next. Adult insects give birth to offspring at rate $\beta\left(1-\frac{L+A}{K}\right)^{+}A$, such that the birth rate is zero when the total density of insects reaches a threshold, K; this is constrained so that the number of births cannot be negative.

The system of equations for a model of an insect species comprising just adults and larvae (for example an aphid species) is summarized as:

$$\frac{dL_{GG}}{dt}=\beta\left(1-\frac{L_{TT}+A_{TT}}{K}\right)^{+}A_{TT}p_{GG}-\frac{1}{\mu_L}L_{GG}$$

$$\frac{dA_{GG}}{dt}=\frac{1}{\mu_L}L_{GG}-\frac{1}{\mu_A}A_{GG}$$

where subscript GG denotes the genotype under consideration (either SS, SR, or RR), and subscript T denotes the sum of all genotypes, while p_{GG} denotes the proportion of all offspring that result in genotype GG.

The number of generations per year of this insect population can be adjusted by altering the mean lifespans of each stage of the insect, with a shorter lifespan giving more generations per season. In order to model only one generation per year, the transition between the stages may be severed, either by setting $\beta=0$, so that adults do not give birth to offspring, or by setting $\mu_S=\infty$, so that a particular stage does not develop into the next state in the life cycle. In each case the resistance frequency of the insects at the start of the next year will be determined by the frequency in the stage that overwinters.

Reproduction

The model incorporates both asexual and sexual reproduction, and the implementations of both are described below. For sexual reproduction we additionally consider sexual reproduction of a haplodiploid insect population. In each case we aim to determine the proportion of all offspring that are of each genotype, p_{GG}. In an asexual (i) population, the proportions of each genotype of new larvae are at the same proportion as the adults. In a sexual (ii) population, the genotypes of the new larvae are determined according to random mating between all adults and, in a haplodiploid (iii) population the genotypes are determined from random mating of haploid males and diploid

females. p_{GG} is therefore a function of the density of the genotypes of all adults in the population, and differs depending on the reproduction strategy.

(i) Asexual population:

$$p_{GG} = \frac{A_{GG}}{A_{TT}}$$

where A_{GG} is the density of adults of genotype GG, and $A_{TT} = A_{SS} + A_{SR} + A_{RR}$.

(ii) Sexual population

For a sexual population we assume recombination as determined by Mendelian inheritance. The proportion of each genotype in new offspring is given by:

$$p_{SS} = \frac{A_{SS} \cdot A_{SS} + A_{RS} \cdot A_{SS} + 0.25 \cdot A_{RS} \cdot A_{RS}}{A_{TT} A_{TT}}$$

$$p_{SR} = \frac{2 \cdot A_{RR} \cdot A_{SS} + A_{RR} \cdot A_{SR} + A_{SS} \cdot A_{SR} + 0.5 \cdot A_{SR} \cdot A_{SR}}{A_{TT} A_{TT}}$$

$$p_{RR} = \frac{A_{RR} \cdot A_{RR} + A_{RR} \cdot A_{SR} + 0.25 \cdot A_{SR} \cdot A_{SR}}{A_{TT} A_{TT}}$$

(iii) Haplodiploid sexual reproduction

In a haplodiploid population, males result from splitting of unfertilized female eggs. In the following A_T denotes the total density of male haploids, and A_{TT} denotes the total density of the female diploids:

$$p_S = \frac{A_{SS} + 0.5 \cdot A_{SR}}{A_{TT}}$$

$$p_R = \frac{0.5 \cdot A_{SR} + A_{RR}}{A_{TT}}$$

Females result from random recombination between the haploid males and diploid females:

$$p_{SS} = \frac{A_S \cdot A_{SS} + 0.5 \cdot A_S \cdot A_{SR}}{A_T \cdot A_{TT}}$$

$$p_{SR} = \frac{0.5 \cdot A_S \cdot A_{SR} + A_S \cdot A_{RR} + A_R \cdot A_{SS} + 0.5 \cdot A_R \cdot A_{SR}}{A_T \cdot A_{TT}}$$

$$p_{RR} = \frac{0.5 \cdot A_R \cdot A_{SR} + A_R \cdot A_{RR}}{A_T \cdot A_{TT}}$$

The effect of the insecticide

Each locus confers resistance to one or more insecticides. We model the effect of the insecticide on each genotype as a linear relationship between the logit of the mortality for each resistance genotype within a specified time period and the log of the insecticide dose applied, which is similar in form but easier to manipulate than the traditional probit-dose curves of experimental insecticide

literature. Experimental probit-dose curves have demonstrated that the probit of insect mortality over a given time period is linearly related to the log of the applied insecticide dose. Translating the logit-dose line into a mortality rate based upon the dose (D) of the insecticide is relatively straight-forward, and results in the per capita mortality resulting from all insecticides being given by $g(D) = -\log(1-\omega_T)$, where ω_T is the mortality within a time period from an insecticide. For each insecticide, i, the mortality for a single genotype is given by $\omega_i = 1/(1+10^{a_i}D_i^{b_i})$, where a and b specify the intercept and gradient of one of the logit-dose lines. The mortality from each insecticide is then combined into a total mortality by multiplying the proportion that survive each insecticide $\omega_T = 1 - \prod_{i=1}^{N\_insecticides}(1-\omega_i)$. In the following simulations we assumed that the slope of the logit-dose line does not vary between genotypes, but the intercept of the logit mortality with log dose is lower for the resistant genotypes (Figure 1). The intercept of both the susceptible and resistant homozygotes genotypes are specified (see Table 1 for parameters), and the intercept of the heterozygote is determined by the dominance of the resistance allele; $a_{SR} = (1-\phi)\cdot a_{SS} + \phi \cdot a_{RR}$, such that for a dominant resistance gene ($\phi = 1$) a individual with a heterozygote resistance genotype has the same logit-dose line as an individual with the homozygous resistant genotype.

In order to standardise the following simulations, and to be relatable to agricultural practice, we assume that a full label dose of any insecticide ($D = 1$) causes a 90% reduction in the insect population, unless stated otherwise.

The insecticide dose decays exponentially at rate ξ.

$$\frac{dD}{dt} = -\xi D$$

Overwintering

The overwintering population contains the same number of genotypes as the within-field population (O_{GG}). At the end of each season the proportion of each genotype in the overwintering insect stage is recorded, and determines the frequency of the genotypes in the overwintering population. The total density of the overwintering insect stage is the same at the start of each season ($i0$), and the insects emerge from this overwintering stage into the within-field population over time at constant rate, η. The rate of emergence of each genotype of the overwintering population is therefore:

$$\frac{dO_{GG}}{dt} = -\eta O_{GG}$$

Depending on whether the insect overwinters as eggs, larvae, pupae or adults, this same term is an influx into the appropriate stage. If an overwintering phase is not needed the rate at which the insect emerges is infinite ($\eta = \infty$), meaning that insects essentially emerge instantaneously from their overwintering location.

Immigration from external sources

We assume that there is immigration from insects that are not undergoing resistance selection, so the immigrant individuals are always predominantly sensitive. The resistance frequency of these individuals (θ_{GG}) is fixed at the same (low) resistance frequency as used to initiate the simulation. This assumption maximises the impact of immigration on resistance evolution, so provides a useful test case. We also assume that immigrants are adults, as this is typically the migratory phase of insects. The rate at which adults immigrate into the population is specified as a proportion of an untreated population, and therefore add to the adult genotype equations a constant immigration rate (ι) throughout the season. The rate of change of adults of genotype GG therefore becomes: $\frac{dA_{GG}}{dt} = \mu L_{GG} - \omega A_{GG} + \iota\theta_{GG}$.

Fitness cost

Resistance frequently confers a fitness cost. Within the model a fitness cost is included as a decrease in the birth rate of the insect, dependent on the resistance genotype. The birth rate of each genotype is therefore $\beta_\zeta = (1-\zeta)\beta$, where ζ is the proportional decrease in the birth rate as a result of being resistant at a single locus. We assume that each resistance loci confers a similar fitness cost, and that if an insect has a both resistance genes there is a multiplicative reduction in the birth rate.

Incomplete insecticide coverage

With incomplete coverage there may be some portion of an insect population that does not get exposed to the insecticide. An additional insect population is therefore modelled that is not exposed to the insecticide, with movement between the two populations at a certain rate (for this study the movement rate, $\eta = 1.0$).

7.2. Appendix 2 - Parameters for case study species

Simple model

The simple model is a non-specific insect population, which consists of the most basic formulation of this model. The insect is assumed to be asexual, multivoltine, hemimetabolous (consisting of only larvae and adults) and diploid. Both the larvae and adults were assumed to have a mean lifespan of 20 and 10 days respectively, a mean birth rate of 0.5 larvae per day per female, and no natural mortality for either stage. The insect overwinters as the larval stage. The gradient of the logit-dose line is 1.5, and the intercepts are 0.5 for the SS genotype, and -4 for the RR genotype, giving a resistance factor of 1000. Dominance is assumed to be exactly intermediate, with the SR genotype having an intercept of -1.75. The simple model was simulated over 200 days and a single spray was applied on day 50.

Myzus persicae

The peach potato aphid is a multivoltine pest of many arable and greenhouse crops. The insect is viviparous, and so only larvae and adults are modelled, with each having a mean lifespan of 12 days and 30 days respectively, and the adults having a birth rate of 1.3 larvae per day per female (given in HYPPZ database (Anon), assuming an average temperature of 20 degrees), and natural mortalities of 0.2 for both adults and larvae. The insects overwinter as larvae, and are assumed to emerge from this population in an average of 10 days. Mortality for larvae and adults are based on kdr ('knock-down resistance') to the pyrethroid *lambda*-cyhalothrin. The gradient (b) of the logit-dose line is assumed to be the same for each genotype, 2.0, while the intercept (a) is 2.0 for the SS genotype and -2.0 for the RR genotype (a resistance factor of 100), with allele dominance of 0.45. The season over which *M. persicae* is active was assumed to be 200 days, and a single spray was applied on day 50. No fitness costs were found for target-site resistance mechanisms and so none were assumed. Metabolic resistance, for example esterase activity against pyrethroid insecticides, is present in the peach-potato aphid, but is not being modelled in this study.

Meligethes aeneus

The pollen beetle is a common pest of oilseed rape in Europe. It is a univoltine, sexually reproducing beetle. The beetle overwinters in its adult stage outside the crop, before migrating into the crop in early spring, where the adults feed on unopened buds before the flowers have opened, damaging yields if present in large numbers. The adults lay eggs inside unopened buds where the larvae feed causing additional damage. Development rates are dependent on temperature, and therefore approximate values are used for a representative environment. The lifespan of each stage is: Egg = 5 days; Larvae = 20 days; Pupae = 10 days; Adults = 12 days. Females were assumed to lay 4 eggs per day (Ferguson et al., 2015), giving a birth rate of 2 eggs per day per beetle. Daily per-capita mortality rates were calculated from the percentage survival of each stage

in (Nielsen and Axelsen, 1988) and converted to mortality rates, giving 0.01 and 0.025 per egg and larva respectively. Mortality of pupae was determined from (Büchi, 2002) giving a mortality rate of 0.01 per pupa. Data on the natural mortality of adults was not found, and so was set as 0.01. While kdr-resistance to pyrethroids is known in pollen beetles LC_{50}s are not given, however 100% kdr-resistance does confer full resistance to *lambda*-cyhalothrin (Nauen et al., 2012). We therefore assumed a gradient of 2.0, and that resistance confers a 1000 fold resistance ratio. The season over which *M. aeneus* was modelled was 60 days, with a single spray on day 5, as on oilseed rape early sprays are important to protect an early crop from damage to unopened buds. No information regarding fitness costs was found for *M. aeneus*, and so no fitness cost was used in simulations.

Frankliniella occidentalis

Western flower thrips is a polyphagous multivoltine haplodiploid insect affecting many greenhouse crop species. The males are haploid and are produced from unfertilized eggs, and the females are diploid resulting from fertilized eggs. We parameterized the model based upon the insect feeding on cucumber (*Cucumis sativus*) at 20 degrees C, from (Gaum et al., 1994). The lifespan of each stage is therefore: 6.7, 9.8, 5.2 and 25.2 days on average for the eggs, larvae, pupae and adults respectively. The birth rate was altered to be independent of density, as this insect can grow exponentially to very high levels, and was set to be 8.6 of which 50% became male, and 50% became female. Only one target-site resistance mechanism is currently confirmed in *F. occidentalis*, that of resistance to spinosad (Bielza et al., 2007). However an LC_{50} for the resistant strain was not given, only that the resistance ratio was, in the most extreme case, > 13500 (Bielza et al., 2007). Therefore we assumed a resistance factor of 10^5 in our simulation and a logit-dose gradient of 1.0. The insecticide is similarly efficacious for adults and larvae (Jones et al., 2005). Each season was modelled for 70 days, the maximum length possible before the computational limit of the density of the insect in the absence of insecticide application was reached. A single spray was applied on day 20. No fitness cost was found associated with target-site resistance, indeed the opposite has been found, where target-site resistance to spinosad led to increased fecundity (Bielza et al., 2008). No fitness costs were therefore assumed.

7.3. Appendix 3 - Parameters for global analysis

Table A3.1 displays the parameter ranges varied in the global parameter search, in section 4.1.2. Unless otherwise stated, each parameter was drawn from a uniform distribution between the upper and lower limit.

Parameter	Lower limit	Upper limit
Insect birth rate (per female)	2	20
Natural mortality rate	0	0.1
Lifespan of each (modelled) stage (days)	1	20
Dominance of each gene	0.0	1.0
Insecticide decay rate	0.02	1.0
Immigration proportion (log10)	10^{-6}	10^{-1}
Initial gene frequency (power-law distribution)	0.0	1.0
Cross-resistance	0%	100%
Proportion of population not contacted by insecticide	0%	50%
Movement rate of insecticides	0.0	10.0
Fitness cost	0%	20%
Rate of emergence from overwintering population (log 10)	10^{-2}	10.0
Resistance ratio (log 10)	10	10000
Categorical variables	**Options**	
Reproduction	Sexual / Asexual	
Stage(s) susceptible	Eggs / Larvae / Pupae / Adults (depending on life cycle)	

Parameter	Lower limit	Upper limit
Overwintering stage	Eggs / Larvae / Pupae / Adults (depending on life cycle)	
Diploidy	Diploid / Haplodiploid	
Life cycle	Hemimetabolous / Holometabolous	

7.4. Appendix 4 – Resistance cases used in risk assessment database

Cases of unique insecticide resistance occurring in countries subscribing to EPPO used to identify traits that explain the observed variation in FDR time (the period in years between the introduction of a new IRAC group to control a pest and the development of resistance in that pathogen against one or more active substances within that IRAC group).

Cases	IRAC group	IRAC	Order	Species	Crop	Country	FDR time
1	Alkyl halides	8A	Coleoptera	*Tribolium castaneum*	Stored grain	Global inc UK	34
2		8A	Coleoptera	*Tribolium confusum*	Stored grain	Global inc Europe (UK, FI, CY, GR, GE, ES)	34
3	Amitraz	19	Hemiptera	*Cacopsylla pyri*	Pear	France	22
4		19	Prostigmata	*Panonychus ulmi*	Apple	Turkey	23
5	Amitraz	19	Prostigmata	*Tetranychus urticae*	Ornamentals	Belgium	27
6	Avermectins/Milbermycins	6	Prostigmata	*Tetranychus urticae*	Rose	Netherlands	8
7	Benzoylureas	15	Lepidoptera	*Cydia pomonella*	Apple, pear	France	17
8	Buprofezin	16	Hemiptera	*Bemisia tabaci*	Rose	Netherlands	4
9		16	Hemiptera	*Trialeurodes vaporariorum*	Tomato	Belgium	5
10	Carbamates	1A	Coleoptera	*Leptinotarsa decemlineata*	Potato	Serbia	15
11		1A	Coleoptera	*Meligethes aeneus*	Oilseed rape	Poland	17
12		1A	Hemiptera	*Aleurothrixus floccosus*	Citrus	Spain	17
13		1A	Hemiptera	*Aphis gossypii*	Chrysanthemum	UK	7
14		1A	Hemiptera	*Aphis nasturtii*	Sweet pepper	Czechoslovakia	8
15		1A	Hemiptera	*Ceroplastes floridensis*	citrus	Israel	8
16		1A	Hemiptera	*Myzus persicae*	Tobacco	Greece	20

Cases	IRAC group	IRAC	Order	Species	Crop	Country	FDR time
17		1A	Hemiptera	*Nasonovia ribisnigri*	Lettuce	France	24
18		1A	Hemiptera	*Phorodon humuli*	Hops	UK	4
19		1A	Prostigmata	*Tetranychus urticae*	Rose	Finland	2
20		1A	Thysanoptera	*Frankliniella occidentalis*	Rose	Denmark	5
21		10A	Prostigmata	*Panonychus ulmi*	Aubergine	France	6
22		10A	Prostigmata	*Tetranychus urticae*	Ornamentals	Belgium	17
23		2A	Astigmata	*Acarus siro*	Cheese	UK	15
24		2A	Coleoptera	*Ceutorhynchus assimilis*	Oilseed rape	Poland	27
25		2A	Coleoptera	*Leptinotarsa decemlineata*	Potato	Poland	18
26		2A	Coleoptera	*Meligethes aeneus*	Oilseed rape	Poland	27
27		2A	Coleoptera	*Sitophilus granarius*	Stored grain	Greece	28
28		2A	Coleoptera	*Sitophilus oryzae*	Stored grain	UK	28
29		2A	Diptera	*Delia antiqua*	Onion	Finland	7
30		2A	Diptera	*Delia radicum*	Brussels sprouts	UK	7
31		2A	Diptera	*Merodon equestris*	Daffodil	UK	11
32		2A	Hemiptera	*Bemisia tabaci*	Cotton	Israel	32
33		2A	Hemiptera	*Myzus persicae*	Chrysanthemum	UK	9
34		2A	Hemiptera	*Nasonovia ribisnigri*	Lettuce	France	38
35		2A	Hemiptera	*Phorodon humuli*	Hops	UK	11
36		2A	Lepidoptera	*Earias insulana*	Cotton	Israel	3

Cases	IRAC group	IRAC	Order	Species	Crop	Country	FDR time
37		2A	Thysanoptera	*Frankliniella occidentalis*	Rose	Switzerland	1
38	DDT/Methoxychlor	3B	Coleoptera	*Leptinotarsa decemlineata*	Oilseed rape	Poland	20
39		3B	Coleoptera	*Meligethes aeneus*	Oilseed rape	Poland	18
40		3B	Hemiptera	*Myzus persicae*	Chrysanthemum	UK	18
41		3B	Hemiptera	*Trialeurodes vaporariorum*	Glasshouse crops	UK	16
42		3B	Lepidoptera	*Tortrix viridana*	Oak	Spain	19
43	Diamides	28	Lepidoptera	*Tuta absoluta*	Tomato	Italy	6
44	Fenoxycarb	7B	Lepidoptera	*Cydia pomonella*	Apple	Czechoslovakia	12
45	METI pesticides	21A	Prostigmata	*Panonychus ulmi*	Apple	France	5
46		21A	Prostigmata	*Tetranychus urticae*	Hops	UK	7
47	Neonicotinoids	4A	Hemiptera	*Aphis gossypii*	Peach	Portugal	10
48		4A	Hemiptera	*Bemisia tabaci*	Cotton	Spain	3
49		4A	Hemiptera	*Myzus persicae*	peach	France	16
50		4A	Hemiptera	*Trialeurodes vaporariorum*	Glasshouse crops	Netherlands	8
51		4A	Lepidoptera	*Cydia pomonella*	Apple	Spain	6
52	Organophosphates	1B	Astigmata	*Acarus farris*	Cheese	UK	8
53		1B	Astigmata	*Acarus siro*	Stored grain	UK	23
54		1B	Coleoptera	*Ceutorhynchus assimilis*	Oilseed rape	Poland	22
55		1B	Coleoptera	*Cryptolestes ferrugineus*	Stored grain	UK	16
56		1B	Coleoptera	*Leptinotarsa decemlineata*	Potato	Serbia	20

Cases	IRAC group	IRAC	Order	Species	Crop	Country	FDR time
57		1B	Coleoptera	*Meligethes aeneus*	Oilseed rape	Poland	7
58		1B	Coleoptera	*Oryzaephilus surinamensis*	Stored grain	UK	27
59		1B	Coleoptera	*Sitophilus granarius*	Stored grain	UK	16
60		1B	Coleoptera	*Sitophilus zeamais*	Stored grain	Global inc UK	13
61		1B	Coleoptera	*Tribolium confusum*	Stored grain	Cyprus	13
62		1B	Coleoptera	*Trogoderma granarium*	Stored grain	Tunisia	7
63		1B	Diptera	*Bactrocera oleae*	Olive	Greece	37
64		1B	Diptera	*Ceratitis capitata*	Peach	Spain	39
65		1B	Hemiptera	*Aphis fabae*	Sugar beet	Czechoslovakia	20
66		1B	Hemiptera	*Aphis gossypii*	Ornamentals	Netherlands	34
67		1B	Hemiptera	*Bemisia tabaci*	Cotton	Turkey	31
68		1B	Hemiptera	*Cacopsylla pyri*	Pear	France	28
69		1B	Hemiptera	*Dysaphis pyri*	Cherry	Switzerland	3
70		1B	Hemiptera	*Dysaphis plantaginea*	Apple	Switzerland	3
71		1B	Hemiptera	*Myzus persicae*	chrysanthemum	UK	8
72		1B	Hemiptera	*Nasonovia ribisnigri*	Lettuce	France	33
73		1B	Hemiptera	*Phorodon humuli*	Hops	UK	9
74		1B	Hemiptera	*Trialeurodes vaporariorum*	Glasshouse crops	UK	16
75		1B	Lepidoptera	*Spodoptera littoralis*	Vegetables/ potato/ lucerne	Cyprus	12

Cases	IRAC group	IRAC	Order	Species	Crop	Country	FDR time
76		1B	Prostigmata	*Panonychus ulmi*	Top fruit	UK	13
77		1B	Prostigmata	*Tetranychus cinnabarinus*	Apple	Israel	9
78		1B	Prostigmata	*Tetranychus urticae*	Glasshouse crops	Netherlands	3
79		1B	Thysanoptera	*Frankliniella occidentalis*	Rose	Denmark	2
80		12B	Prostigmata	*Tetranychus urticae*	Lemon verbena	UK	28
81	Phosphine	24A	Coleoptera	*Rhyzopertha dominica*	Stored grain	Greece (global)	16
82		24A	Coleoptera	*Sitophilus granarius*	Stored grain	UK, Poland, Spain, Cyprus and Turkey	16
83		24A	Coleoptera	*Sitophilus oryzae*	Stored grain	Global inc Europe (UK, ES, PO)	16
84		24A	Coleoptera	*Tribolium confusum*	Stored grain	Global inc Europe (UK, ES, GR, CY, FI, GE).	16
85	Pyrethroids/Pyrethrins	3A	Coleoptera	*Ceutorhynchus obstrictus*	Oilseed rape	Germany	32
86		3A	Coleoptera	*Leptinotarsa decemlineata*	Potato	Hungary	10
87		3A	Coleoptera	*Meligethes aeneus*	Oilseed rape	Denmark	22
88		3A	Coleoptera	*Psylliodes chryscephala*	Oilseed rape	Germany	30
89		3A	Coleoptera	*Sitophilus granarius*	Stored grain	UK	12
90		3A	Diptera	*Bactrocera oleae*	Olive	Greece	3
91		3A	Hemiptera	*Aleyrodes proletella*	Kale	UK	36
92		3A	Hemiptera	*Aphis gossypii*	Ornamentals	Netherlands	27
93		3A	Hemiptera	*Bemisia tabaci*	Cotton	Turkey	8
94		3A	Hemiptera	*Cacopsylla pyri*	Pear	France	18
95		3A	Hemiptera	*Myzus persicae*	Sugar beet	UK	3

Cases	IRAC group	IRAC	Order	Species	Crop	Country	FDR time
96		3A	Hemiptera	*Nasonovia ribisnigri*	Lettuce	France	17
97		3A	Hemiptera	*Phorodon humuli*	Hops	Germany	23
98		3A	Hemiptera	*Sitobion avenae*	Cereals	UK	34
99		3A	Hemiptera	*Trialeurodes vaporariorum*	Tomato	UK	1
100		3A	Lepidoptera	*Cydia pomonella*	Apple	France	19
101		3A	Lepidoptera	*Helicoverpa armigera*	Artichoke	Spain	27
102		3A	Lepidoptera	*Spodoptera exigua*	Rose	Netherlands	23
103		3A	Lepidoptera	*Spodoptera littoralis*	Cotton	Israel	7
104		3A	Lepidoptera	*Tuta absoluta*	Tomato	Portugal	1
105		3A	Prostigmata	*Tetranychus urticae*	Hops	UK	8
106		3A	Thysanoptera	*Frankliniella occidentalis*	Pepper	Spain	15
107		3A	Thysanoptera	*Thrips tabaci*	Leeks, salad onions	UK	28
108	Pyriproxyfen	7C	Hemiptera	*Bemisia tabaci*	Rose	Israel	2
109	Spinosyns	5	Thysanoptera	*Frankliniella occidentalis*	Sweet pepper	Spain	3
110	Sulfoximines	4C	Coleoptera	*Leptinotarsa decemlineata*	Potato	Poland	7
111	Tetradifon	12D	Prostigmata	*Panonychus ulmi*	Top fruit	UK	3
112		12D	Prostigmata	*Tetranychus cinnabarinus*	Apple	Israel	7
113		12D	Prostigmata	*Tetranychus urticae*	Glasshouse crops	Netherlands	6
114		23	Prostigmata	*Panonychus ulmi*	Apple	Germany	6
115		23	Prostigmata	*Tetranychus urticae*	Rose	Netherlands	6

Cases	IRAC group	IRAC	Order	Species	Crop	Country	FDR time
116	Unknown/Unlisted	UN	Lepidoptera	*Cydia pomonella*	Apple	Germany	12
117		UN	Prostigmata	*Panonychus ulmi*	Apple	UK	5
118		UN	Prostigmata	*Panonychus ulmi*	Apple	Turkey	18
119		UN	Prostigmata	*Panonychus ulmi*	Top fruit	UK	13
120		UN	Prostigmata	*Panonychus ulmi*	Apple	UK	10
121		UN	Prostigmata	*Tetranychus cinnabarinus*	Apple	Israel	7
122		UN	Prostigmata	*Tetranychus urticae*	Rose	Netherlands	7
123		UN	Prostigmata	*Tetranychus urticae*	Ornamentals	Belgium	13
124		UN	Prostigmata	*Tetranychus urticae*	Rose	UK	5
125		UN	Prostigmata	*Tetranychus urticae*	Rose	UK	3

7.5. Appendix 5 – Single trait analyses

Direction of effect for all traits significantly associated with sqrt FDR time. Traits presented in order of decreasing percent VAF.

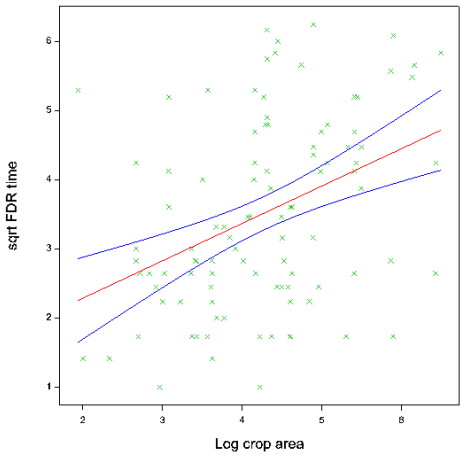

Figure 1. Relationship between log crop area and sqrt FDR time (VAF = 15.4%). Red line = fitted equation for the relationship between the observed and predicted values. Blue line = 95% confidence interval for fitted line.

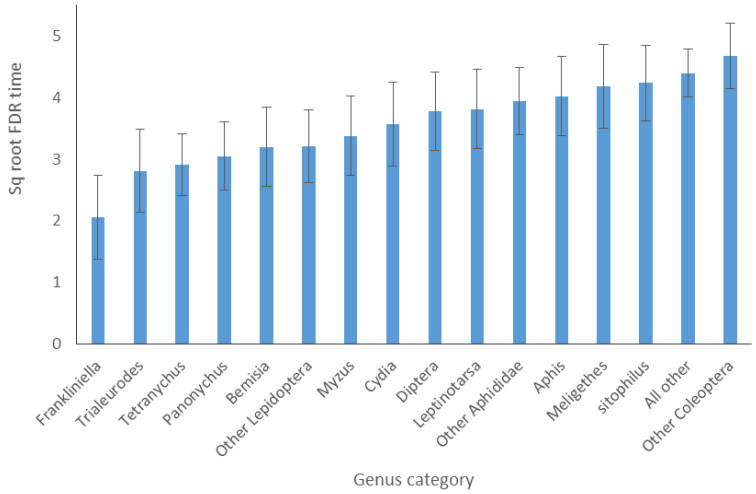

Figure 2. Relationship between genus and sqrt FDR time (VAF = 14.8%). Bars indicate the standard error of the estimate.

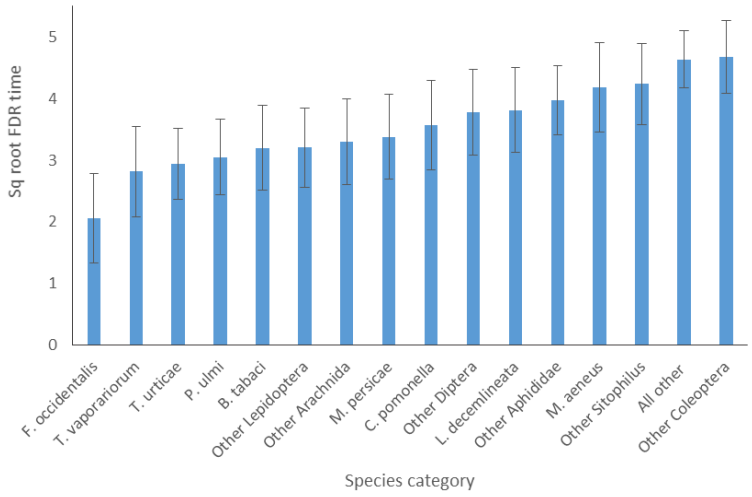

Figure 3. Relationship between species and sqrt FDR time (VAF = 14.6%). Bars indicate the standard error of the estimate.

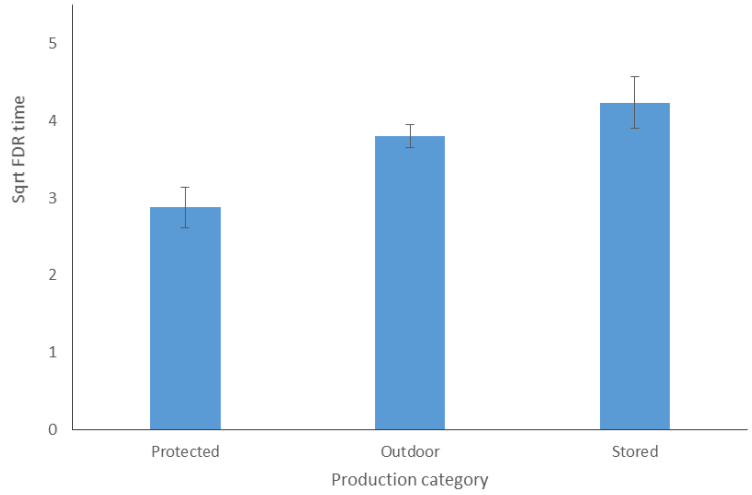

Figure 4. Relationship between the production system under of the crop on which the resistance was detected and sqrt FDR time (VAF = 11.3%). Bars indicate the standard error of the estimate.

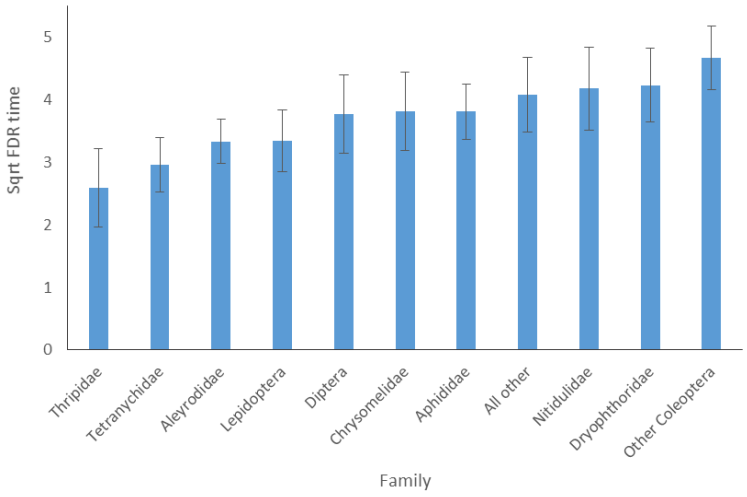

Figure 5. Relationship between family and sqrt FDR time (VAF = 10.8%). Bars indicate the standard error of the estimate.

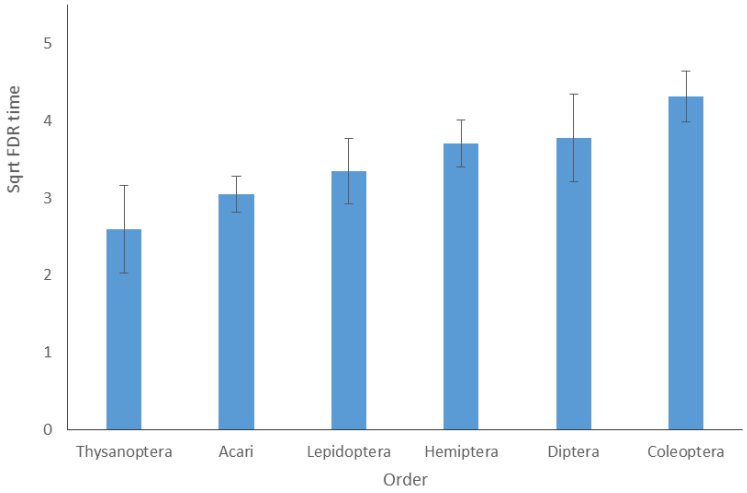

Figure 6. Relationship between taxonomic order and sqrt FDR time (VAF = 10.7%). Bars indicate the standard error of the estimate.

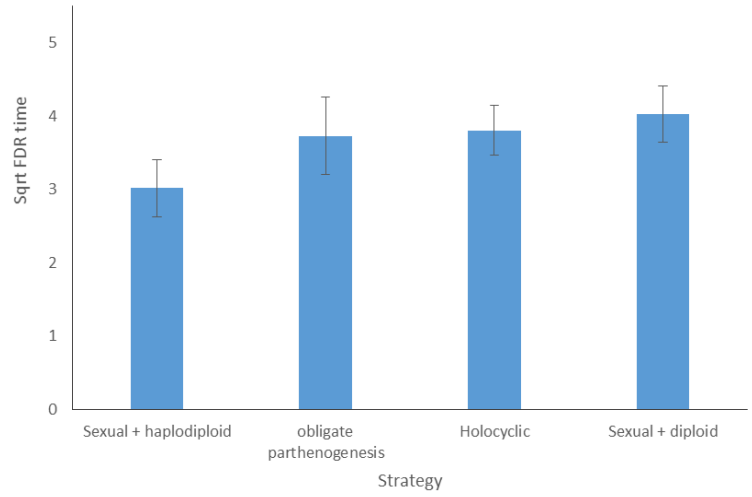

Figure 7. Relationship between the reproductive strategy of the pest and sqrt FDR time (VAF = 9.5%). Bars indicate the standard error of the estimate.

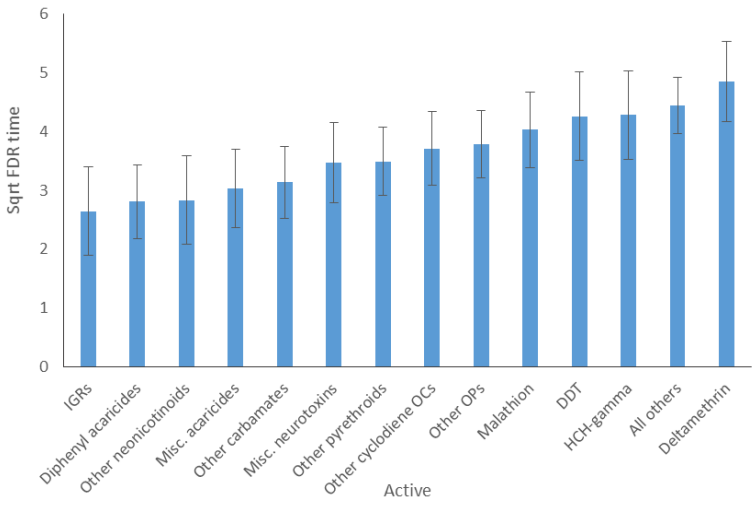

Figure 8. Relationship between the active substance and sqrt FDR time (VAF = 9.3%). Bars indicate the standard error of the estimate.

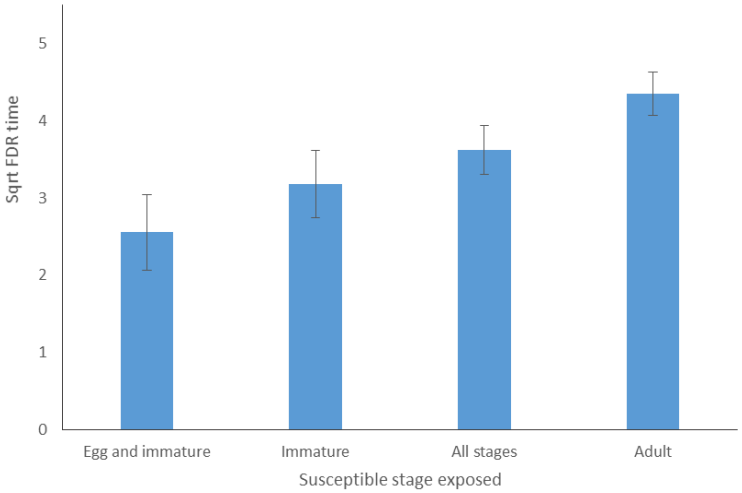

Figure 9. Relationship between the susceptible stage of pest exposed to the insecticide and sqrt FDR time (VAF = 9.2%). Bars indicate the standard error of the estimate.

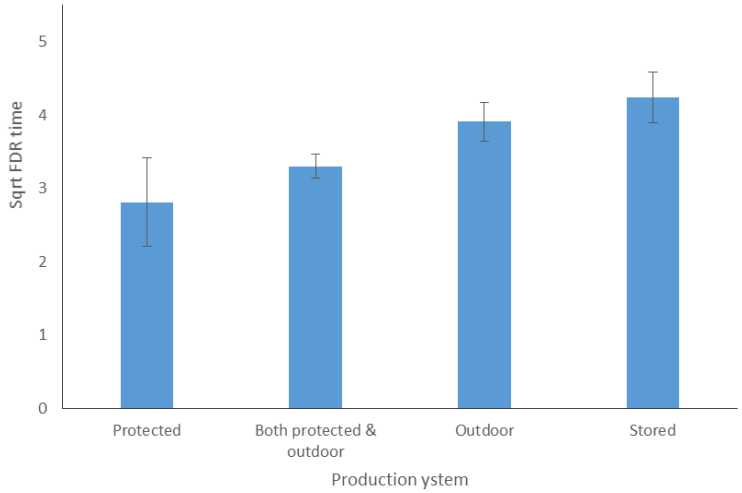

Figure 10. Relationship between the production system of all crops on which the pest is a problem and sqrt FDR time (VAF = 6.6%). Bars indicate the standard error of the estimate.

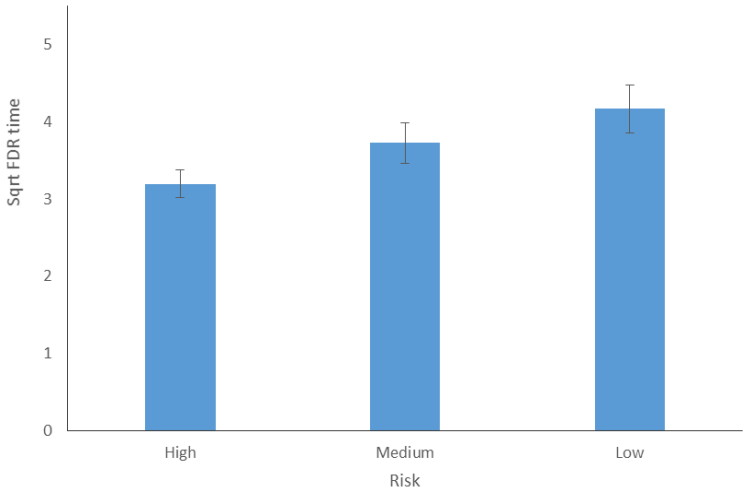

Figure 11. Relationship between the risk of human mediated spread of the pest and sqrt FDR time (VAF = 6.4%). Bars indicate the standard error of the estimate.

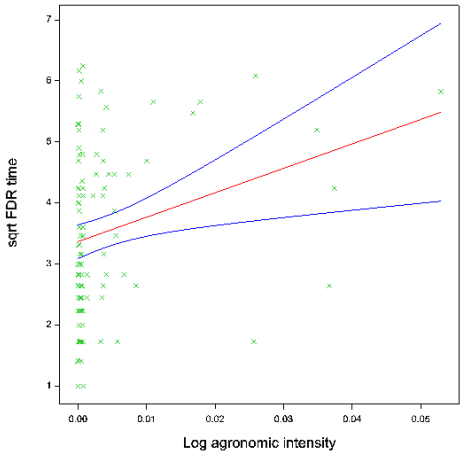

Figure 12. Relationship between log agronomic intensity and sqrt FDR time (VAF = 5.7%). Red line = fitted equation for the relationship between the observed and predicted values. Blue line = 95% confidence interval for fitted line.

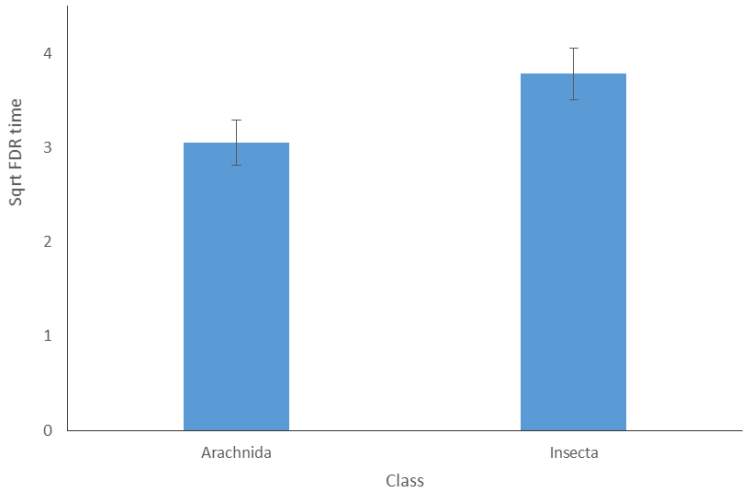

Figure 13. Relationship between class and sqrt FDR time (VAF = 4.7%). Bars indicate the standard error of the estimate.

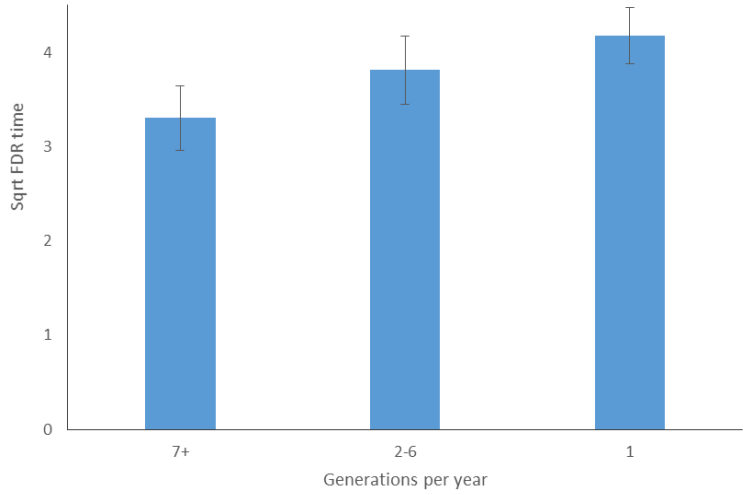

Figure 14. Relationship between the number of generations a pest completes annually and sqrt FDR time (VAF = 4.6%). Bars indicate the standard error of the estimate.

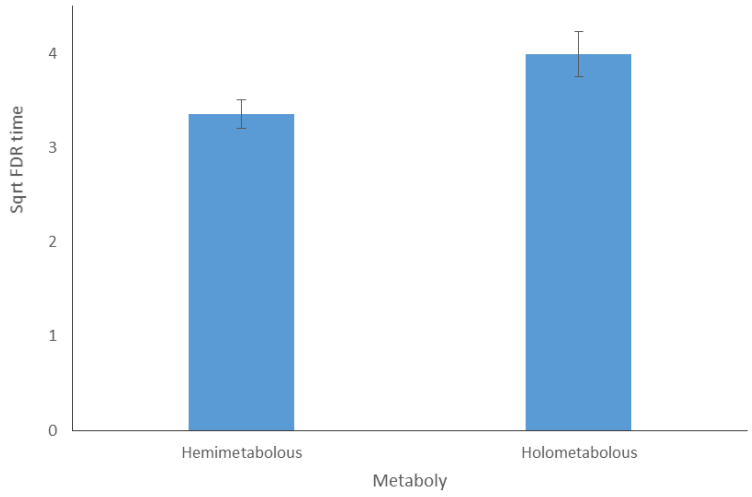

Figure 15. Relationship between the form of metamorphosis the pest undergoes and sqrt FDR time (VAF = 4.6%). Bars indicate the standard error of the estimate.

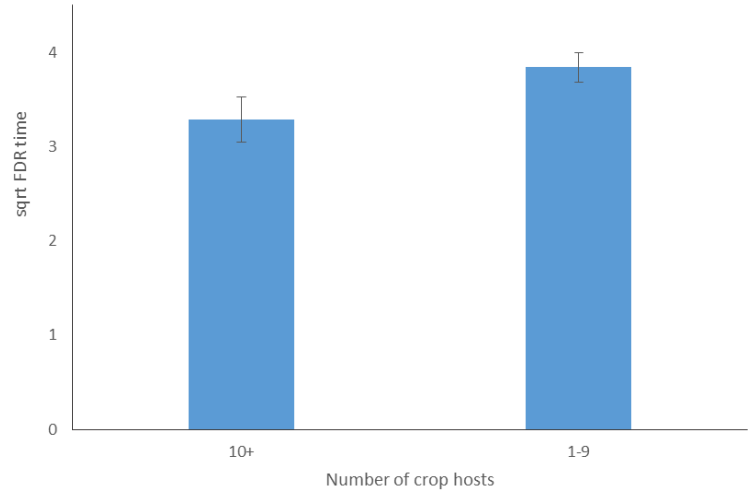

Figure 16. Relationship between the number of crop hosts of a pest and sqrt FDR time (VAF = 3.4%). Bars indicate the standard error of the estimate.

7.6. Appendix 6 – Parameter estimates of terms in the resistance risk assessment model

Trait	Parameter	Estimate	s.e.	t(87)	t pr.
Constant		-0.660	1.060	-0.620	0.535
Log crop area		0.833	0.159	5.250	<.001
Crop type	Arable vegetables	0.000	0.000	0.000	0.000
Crop type	Cereals & OSR	1.441	0.505	2.860	0.005
Crop type	Cotton & tobacco	0.084	0.691	0.120	0.903
Crop type	Horticultural vegetables (unprotected)	2.498	0.714	3.500	<.001
Crop type	Other ornamentals	2.238	0.680	3.290	0.001
Crop type	Outdoor fruit	0.757	0.620	1.220	0.226
Crop type	Protected vegetables	0.020	0.707	0.030	0.978
Crop type	Rose	0.110	0.709	0.160	0.877
Crop type	Stored grain	0.000	*	*	*
No. of crop hosts	1-9	0.000	0.000	0.000	0.000
No. of crop hosts	10+	0.884	0.310	2.850	0.005
Reproductive strategy	Holocyclic	0.000	0.000	0.000	0.000
Reproductive strategy	Obligate parthenogenesis	-1.089	0.506	-2.150	0.034
Reproductive strategy	Sexual + diploid	1.440	0.658	2.190	0.031
Reproductive strategy	Sexual + haplodiploid	-0.757	0.449	-1.690	0.096
Order	Acari	0.000	0.000	0.000	0.000
Order	Coleoptera	-2.088	0.991	-2.110	0.038
Order	Diptera	-2.531	0.859	-2.950	0.004
Order	Hemiptera	-0.260	0.383	-0.680	0.500

Trait	Parameter	Estimate	s.e.	t(87)	t pr.
Order	Lepidoptera	-2.093	0.808	-2.590	0.011
Order	Thysanoptera	-0.364	0.537	-0.680	0.500