paper2\_resistance\_mechanisms\_mixtures

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### version 8

# Insecticide effectiveness predicted to be most important factor determining whether insecticide mixtures can slow evolution of resistance.

Target : Malaria journal

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(This file is automagically generated by R from paper2\_resistance\_mechanisms\_mixtures.Rmd)

## ANDY TO DO

1. add a screenshot of the UI
2. add to intro including about about results in levick et al.
3. explain resistance-restoration and its relation to selection coefficient
4. expand discussion to include relevance to real world
5. in discussion address potential criticism that these results are just for a limited region of paramter space (a. point to Levick et al. that explored more of space, b. point to UI that allows readers to explore spce for themselves and identify whether there are places where this does not hold).
6. ? briefly refer to linkage disequilibrium and say we are investigating.
7. can I develop explanatory diagrams to describe the input parameters ?
8. mention other potential effects e.g. costs of resistance, sex linkage, ...
9. add to discussion about relevance of these mechanisms in the field e.g. exposure can be reduced if either insecticide has a repellant effect, which can be set in formulation. (Birget & Koella 2015 mention this not for resistance but for ITNs with repellancy requiring higher coverage for elimination)
10. mention in discussion that repellency would lead to decrease in exposure
11. is there a refernce to say that the effectiveness of insecticides can be modified at the design stage ?

## Abstract

TODO needs 350 words, currently 375 Background: the context and purpose of the study Results: the main findings Conclusions: a brief summary and potential implications

#### Background

Insecticide resistance has been identified as a major problem for the control of malarial mosquitoes with potentially huge consequences for malaria burden. To address this problem effort is being made to develop new insecticide tools including new active ingredients and combinations with existing ones. There are different potential strategies for using new insecticides and recomendations for how best to preserve their utility for controlling mosquitoes and malaria. There is however limited recent, accessible modelling work to support suggestions of which strategies may be best in different situations. Previous work suggests that preferred strategies will be situation specific. This points to a need for an accessible, mechanistic understanding of how the evolution of insecticide resistance is likely to respond to potential intervention strategies to help guide both management and policy. Such a mechanistic understanding would be more robust to uncertainty in parameter values in field situations.

Here we use a relatively simple model to develop a mechanistic understanding of how insecticide resistance is expected to increase under different intervention strategies, namely the use of insecticides in isolation, sequence and mixtures. The model flexibly represents two independent genes coding for resistance to two insecticides allowing us to represent the different intervention strategies. We look principally at the ability of the insecticides to kill susceptible mosquitoes, the proportion of mosquitoes that come into contact with the insecticide, and how much the resistance counteracts the effect of the insecticide.

#### Results

We show firstly that using a second insecticide, at the same time as a first, always slows evolution of resistance to the first. Secondly we show that the ability of an insecticide to kill the susceptible mosquitoes (effectiveness), is the most important factor influencing whether using insecticides in a sequence or a mixture is likely to lead to faster resistance to both of them. These results are explained in terms of the mechanisms that cause them. We discuss how these results can inform intervention strategies in the field.

#### Conclusions

We describe an R package and user-interface that allows further exploration of the development of resistance. The user-interface (<https://andysouth.shinyapps.io/MixSeqResist1>) allows replication of our results and exploration of what happens when inputs are changed outside of the ranges we have used.

## Keywords

insecticide resistance; public health; mosquitoes; vector-borne diseases; infectious diseases; malaria; population genetics

## Background

Insecticide resistance is a problem for malaria [1][2] other vector borne diseases [3] and agriculture [4].

TODO add more stuff here or after following para about protocols for resistance management.

Insecticide resistance becomes a problem when genes coding for resistance firstly arise in a population and secondly increase in frequency. We concern ourselves with this second process of how insecticide resistance increases in frequency within a population. There are suggestions [e.g. ref from ASTMH] that mutations are present in populations even prior to exposure to a novel insecticide thus leading to the potential for selection.

The changing frequency of insecticide resistance is a population genetic process that can be influenced by the parameters outlined in Table 1.

We use a 2-locus population genetic model of insecticide resistance [5] to illustrate the mechanisms by which these parameters are expected to influence the development of insectide resistance under alternative insecticide use strategies. Levick et al. (in press) describe the technical details of the model and explore large parameter ranges. We provide an accessible summary of the model and use selected parameter values to develop a mechanistic understanding of how inputs influence the development of resistance. This mechanistic understanding can provide a more robust framework for the debate on the relative merits of different insecticide use strategies.

**Table 1. Parameters inflencing the development of insecticide resistance**

|  |  |
| --- | --- |
| Parameter | Description |
| 1. Effectiveness | proportion of susceptible (SS) insects killed by exposure to insecticide |
| 2. Exposure | proportion of insects exposed to the insecticide |
| 3. Dominance | resistance of an insect with only 1 resistance allele (SR) |
| 4. Resistance restoration | ability of resistance (RR) to restore fitness when insect exposed to the insecticide |
| 5. Frequency | frequency of resistance alleles within the population |

## Methods

The simulation represents a population of randomly mixing individuals using standard population genetic approaches to avoid the need to follow every individual. One locus is represented for each insecticide. There are two potential alleles per locus. Each allele confers either resistance or susceptibility to the insecticide. The fitness of an individual depends on it's alleles, exposure to the insecticide and other inputs that can be set in the model. These inputs include the effectiveness of the insecticide, the dominance of the resistance allele and the ability of resistance to restore fitness in the presence of the insecticide (see Table 1).

The simulation proceeds through generations. In each generation selection is represented by multiplying genotype frequencies in the population by their relative fitness. This acts to make the fitter alleles more common over time. Thus a summary of the selection stage is :

selection coefficient = resistance restoration \* effectiveness

Locus fitness :  
SS = 1-effectiveness  
RS = 1-effectiveness + (dominance\*selection coeff.)  
RR = 1-effectiveness + selection coeff.

Allele fitness :  
SS1SS2 = SS \* SS  
SS1RS2 = SS \* RS  
… and for other combinations  
RR1RR2 = RR \* RR

Population fitness = Allele fitness \* exposure to insecticide

In each generation :  
Allele frequency[t+1] = Allele frequency[t] \* Population fitness

Sexual reproduction is represented first by creating gametes by splitting the adult genomes in two with recombination, and second by forming offspring genotypes by adding gametes.

So far this description just considers a single insecticide and associated resistance allele, with equal behaviour for males and females (Fig 1A). To allow males and females to be exposed differently separate genders are also represented (Fig 1B). In addition a second insecticide and resistance allele are included and fitness is calculated by multiplying these with those for the first insecticide described above (Fig 1C). Thus the simulation can represent populations exposed to two insecticides together in a mixture.

Here we principally look at model outputs of the change in allele frequencies over time (generations). We investigate three insecticide use strategies.

1. single insecticide
2. two insecticides used in sequence, replacing the first with a second once the proportion of resistance alleles reaches 50%
3. two insecticides used in a mixture (concurrently)

The model is implemented in R [6], the code is hosted on Github [7].

## Results

### Single insecticide

For single insecticide use, faster resistance spread resulted from higher values of any of the inputs outlined in Table 1, namely effectiveness, exposure, dominance, the resistance restoration coefficient, and starting frequencies of resistance (Figs 2-4). In the plots each input was varied in isolation with the remaining set to 0.5 (except for starting frequency of resistance which was set to 0.01 in all bar Fig 4).

### Two insecticides

When two insecticides are used the relative performance of a mixture strategy compared to sequential use changes dependent on input values. To help us understand the mechanisms influencing this relative performance we started with a single base scenario and investigated changing inputs individually. The base scenario was similar to that used for the single insecticide. Values for the first four inputs in Table 1 were set to 0.5 and the starting frequencies of resistance to 0.01. For this base scenario resistance arises slower for sequential than mixture (Fig 5A). This plot, and those that follow, compares mixture and sequence for a single combination of inputs. Resistance to both insecticides in the mixture follow the same path and reach the threshold at the same time (as would be expected given that they have identical input parameters). When used in sequence the curve (dashed) for each insecticide individually is steeper than in the mixture but, because they happen one after the other, it takes longer for both to reach the resistance threshold of 50%.

#### results: Insecticide exposure and effectiveness

Increasing exposure to the insecticides (the proportion of insects that come into contact) from 0.5 to 0.8 (Fig 5C) decreases the time-to-resistance for both the sequential and mixture strategy and so does not change their relative performance; time-to-resistance remains longer for the sequence. In contrast keeping the exposure constant at 0.5 and increasing effectiveness (the proportion of SS insects that are killed by contact) of one of the insecticides to 0.8 (Fig 5D) results in a longer time-to-resistance for the mixture relative to the sequence. Resistance for the more effective insecticide (in red) increases faster than the lesser. Resistance to the less effective insecticide in the mixture increases slowly initially and then speeds up after the more effecttive insecticide has reached the resistance threshold (~50 generations).

Thus a more effective insecticide increases time-to-resistance when used in a mixture (compare the solid lines in Fig 5A & B). This is opposite to what happens when used in sequence (compare the red dashed line in Fig 5A & B) and in isolation (Fig 2A), when more effective insecticides shortened times-to-resistance.

Figure 5D has the same inputs as Fig 5B except that the exposure is increased from 0.5 to 0.8. The increase in exposure reduces time-to-resistance more for the mixture relative to the sequence. The result is that the advantage of mixtures over sequence is removed by the increase in exposure, leading to approximately equal times-to-resistance for the two strategies (Fig 5D).

Figure 6 shows how increasing the effectiveness of either or both insecticides improves the performance of mixtures over sequences in terms of time-to-resistance. Figure 6A uses the same inputs as Fig 5B. From this scenario a higher effectiveness for insecticide2 (Fig 6C) or insecticide 1 (Fig 6B) or both (Fig 6D) all result in a greater positive difference in time-to-resistance for mixture over sequence.

#### results: Dominance and resistance restoration

Increasing dominance or resistance restoration decreases time-to-resistance for both sequences and mixtures (Fig 7) in the same way that it did for sole use (Fig 3). The result is that increasing either or both of dominance and resistance restoration (Figs 7B,C,D) doesn't change the relative time-to-resistance for mixtures and sequences from that in the base scenario (Fig 7A) where it takes resistance longer to develop for the sequence.

#### results: Starting frequencies of resistance

Changing the starting frequency of resistance had a similar effect on time to resistance for both sequences and mixtures and thus had little effect on their relative performance. For example taking the base scenario and reducing the starting frequency of one resistance allele did not change from sequence being favoured (compare Fig 8C to 8A). Similarly taking a scenario in which time-to-resistance is longer for a mixture (Fig 8B) and decreasing the starting frequency of that resistance allele did not change the fact that the mixture was favoured (Fig 8D).

#### results: reduced male exposure

*? maybe cut this section and fig 9* Reduced male exposure with respect to female (Fig 9) has a similar effect to changing overall exposure. Reducing male exposure (Figs 9C & D) increases times-to-resistance and favours mixtures over sequences.

## Discussion

The response of resistance to insecticide use in the model can be explained by a number of mechanisms that can help us develop a more robust understanding of how we would expect resistance to respond in the field.

When single insecticide use was represented (Figs 2-4) in all cases the initial increase in resistance was most rapid with straight lines on the log plots up to around 50% followed by a slowing rate of increase up to 100%. Higher values of the five inputs investigated all led to more rapid increases in resistance.

For single insecticide use resistance responded identically to changing exposure and effectiveness (compare Fig 2A to 2B). This makes sense as vector kill is effectively a product of exposure times effectiveness. For example exposing 50% of a population to an insecticide which is 75% effective would be expected to have the same result as exposing 75% of the population to one which is 50% effective. This observation points to the mechanism by which increasing both exposure and effectiveness lead to a faster increase in resistance. In both cases the increased deaths of susceptible vectors cause a higher selection pressure that can explain the faster increase.

The pattern of more rapid increase in resistance at higher dominance levels (Fig 3A) can be explained by increased survival of heterozygotes in the presence of the insecticide. Higher dominance of the resistant allele causes it to contribute more to the phenotype of the heterozygotes leading to higher survival. Thus selection pressure for the resistance allele will be increased because it confers more of an advantage when only present on one chromosome.

The faster development of resistance under higher resistance-restoration (Fig 3B) can be explained by it's effect on the survival of the resistant genotypes. Resistance-restoration restores the survival of resistant genotypes in the presence of the insecticide back towards what it would be in the absence of the insecticide, thus increasing the selection pressure of the resistance allele.

The effect of the starting frequency of resistance (Fig 4) is the most different from the other inputs. Simply, when starting from a higher frequency of resistance there is a smaller change to make to reach the higher resistance thresholds.

These mechanisms explaining the response of resistance to single insecticide use under different input values are summarised in Table 2. Exposure and effectiveness act by decreasing the survival of all genotypes, dominance acts by increasing survival of RS and resistance-restoration acts by increasing survival of RR & RS.

#### discussion: Two insecticides

In comparison with the use of a single insecticide, using two insecticides either in sequence or in mixtures differs in some ways and where it differs that can again help us to understand the mechanisms. Looking at our base scenario (Fig 5A) the curves for resistance to both insecticides in the mixture are shallower than those for sequential use and in this particular example are identical because they have set the same parameter values for each insecticide to be the same. The shallower curves for the mixture can be explained by each insecticide killing individuals that are resistant to the other insecticide. Thus each insecticide reduces the selection pressure for the increase in resistance to the other and could be said to 'protect' the other. However this protection is not necessarily enough to ensure that resistance arises more slowly in a mixture relative to a sequential strategy. As shown in our base scenario, resistance can arise more slowly for both insecticides in sequential use, despite being faster for each, because they occur one after the other.

#### discussion: Insecticide effectiveness

The main difference between mixtures and the other strategies is that in mixtures increasing the effectiveness of either insecticide slows the development of resistance to both (compare Fig 5B to 5A). In contrast in both the sequential (Fig 5B) and single insecticide (Fig 2A) scenarios the development of resistance was faster when effectiveness was higher. This can be explained by the increased effectiveness of one insecticide in a mixture increasing killing of vectors that are resistant to the other and thus decreasing selection pressure.

This effect can be seen by comparing Fig 5B, where the effectiveness of insecticide 1 has been increased, to Fig 5A. In the mixture, resistance to insecticide 1 (with the increased effectiveness) rises at a similar speed in both figures. However, the increased effectiveness of insecticide 1 causes resistance to insecticide 2 to increase more slowly initially in the mixture. Once insecticide 1 reaches resistance of around 50% at around 50 generations, the curve for insecticide 2 becomes steeper. Once the first insecticide has become ineffective due to resistance it stops killing individuals that are resistant to the second insecticide and thus stops limiting the rise in resistance to the second insecticide. A similar effect is visible when the effectiveness of both insecticides are increased (Fig 6C). The identical resistance curves for each insecticide in the mixture are shallower than at the lower effectiveness (Fig 5A) because more individuals resistant to each insecticide are being killed by the other insecticide. In this case the 'protection' given to both insecticides by the other declines at the same rate so we don't see the change in slope seen in Fig 5B.

Fig 5B also demonstrates that when two insecticides have a different effectiveness in a mixture, other parameters being equal, resistance will be expected to increase faster to the more effective insecticide. The more effective insecticide prompts both a) higher selection pressure for it's own resistance and b) greater 'protection' reducing the rise in resistance to the other insecticide.

#### discussion: Exposure

It seems initially counter-intuitive that whereas increasing effectiveness slows the development of resistance for mixtures, increasing exposure speeds it up. Increasing the exposure to both insecticides results in a decrease in time-to-resistance (from 90 in Fig 5A to 40 in Fig 5C) where increasing the effectiveness of both insecticides by the same amount results in a slight increase (from 90 in Fig 5A to 110 in Fig 5B). This contrasts with the identical effects that exposure and effectiveness have on a single insecticide (Figs 2 A,B) and on insecticides in sequence (the red dashed lines are the same in Figs 5B & 5C).

*todo : develop a concise explanation of this difference between effectiveness and exposure*

Mechanisms for the response of resistance in mixtures are summarised in Table 3. Table 4 summarises mechanisms explaining the difference between mixture and sequential strategies.

### Discussion summary points

1. In a mixture each insecticide reduces the rate of increase in resistance to the other by killing individuals that are resistant to the other.
2. In a mixture higher effectiveness of either insecticide kills more individuals resistant to the other and thus increases time-to-resistance for the other. Thus although increasing effectiveness of an insecticide decreases time-to-resistance when used alone, when used in a mixture time-to-resistance for both insecticides is increased.
3. For a mixture of 2 insecticides with differing effectiveness. Resistance to the insecticide with a greater effectiveness increases faster. The less effective insecticide is 'protected' by the more effective. Resistance to the less effective insecticide increases slowly until resistance to the more effective insecticide reaches a high level.
4. exposure ...

### TODO ADD TO DISCUSSION : link these refs to the results here

[8] "use of mixtures is always more effective in delaying the onset of resistance, often by many orders of magnitude" ~ assumed that SS always killed i.e. effectiveness=1 ~ exposure fixed at 0.9

[9]

insecticidal toxins genetically engineered into plants

additive rtaher than multiplicative fitness

effectiveness ~0.2-0.5 (might be expected to favour sequences by our results)

no clearly superior strategy

[10]

identified mortality of SS homozygotes (our “insecticide effectiveness” parameter) as critical (his Fig 3) and concluded (page 1784) : “As a result of incomplete coverage and residue decay, the mortality of susceptible homozygotes is rarely consistently high enough for pesticide mixtures to be effective”

[11] potential IRM strategies : ~ moderation : preserve susceptible genes by limiting selection pressure ~ saturation : high dose so that heterozygous resistants are killed ~ multiple attack : independently acting pressures neither of which is strong enough to lead to resistance

[12] *suggests incomplete dominance in the field*

[13] We show that indoor use of insecticides leads to less selection pressure than their use as larvicides. Reasons for relatively low selection pressure by adulticides (i) males are not affected by the ITNs (ii) insecticides are also repellents, keeping mosquitoes at bay from contacting the insecticide but also driving them to bite either people who do not use the insecticide or alternative hosts.

[14] The impact of pyrethroid resistance on the efficacy and effectiveness of bednets for malaria control in Africa. Transmission dynamics models indicate that even low levels of resistance would increase the incidence of malaria due to reduced mosquito mortality and lower overall community protection over the life-time of the net. *+ may have some useful stuff about expected degradation of nets over time*

[15] can anything be done to maintain effectiveness of nets ?

[16] Lessons from agriculture for malaria vector management ~ suggests that partially effective tools used in combination as a part of Integrated Vector Managament is a more sustainable solution in the light of evolution ~ this modelling approach could be used to investigate implications of such combined strategies

[Summary tables to refer to from discussion.]

**Table 2. Effect of inputs on resistance when insecticides used singly or in sequence**

|  |  |  |
| --- | --- | --- |
| Parameter to increase | effect on resistance | Mechanism |
| 1. Effectiveness | faster | increased deaths of susceptibles |
| 2. Exposure | faster | increased deaths of susceptibles |
| 3. Dominance | faster | increased survival of heterozygotes |
| 4. Resistance restoration | faster | increased survival of resistants |
| 5. Frequency | faster | less change needed to reach resistance threshold |

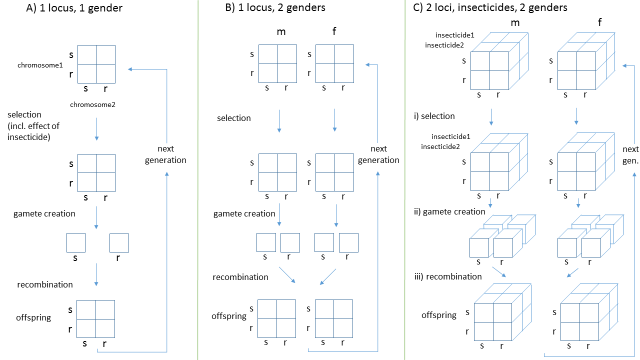
**Table 3. Effect of inputs on resistance when insecticides used in a mixture**

|  |  |  |
| --- | --- | --- |
| Parameter to increase | effect on resistance | Mechanism |
| 1. Effectiveness | **slower** | increased deaths of individuals resistant to the other insecticide |
| 2. Exposure | faster (but less than for single) | increased deaths of individuals susceptible to one insecticide increases selection pressure for that resistance. However at the same time selection pressure is reduced by higher deaths of resistant individuals caused by the other insecticide. |
| 3. Dominance | faster | increased survival of resistants |
| 4. Resistance restoration | faster | increased survival of resistants |
| 5. Frequency | faster | less change needed to reach resistance threshold |

**Table 4. Effect of inputs on the difference between mixture and sequential use**

|  |  |  |
| --- | --- | --- |
| Parameter to increase | increase favours mix or sequence | Mechanism |
| 1. Effectiveness | mixture | Higher effectiveness gives faster resistance for sequence and slower resistance in mixture |
| 2. Exposure | sequence | Higher exposure gives faster resistance for sequence and mixture but the greater effect on mixture favours sequence. |
| 3. Dominance | neither | Higher dominance gives faster resistance in both sequences and mixtures such that the difference between them is not changed. |
| 4. Resistance restoration | neither | As for dominance. Higher resistance restoration gives faster resistance in both sequences and mixtures such that the difference between them is not changed. |
| 5. Frequency | neither | As for dominance and resistance restoration. Higher starting frequencies give faster resistance in both sequences and mixtures such that the difference between them is not changed. |

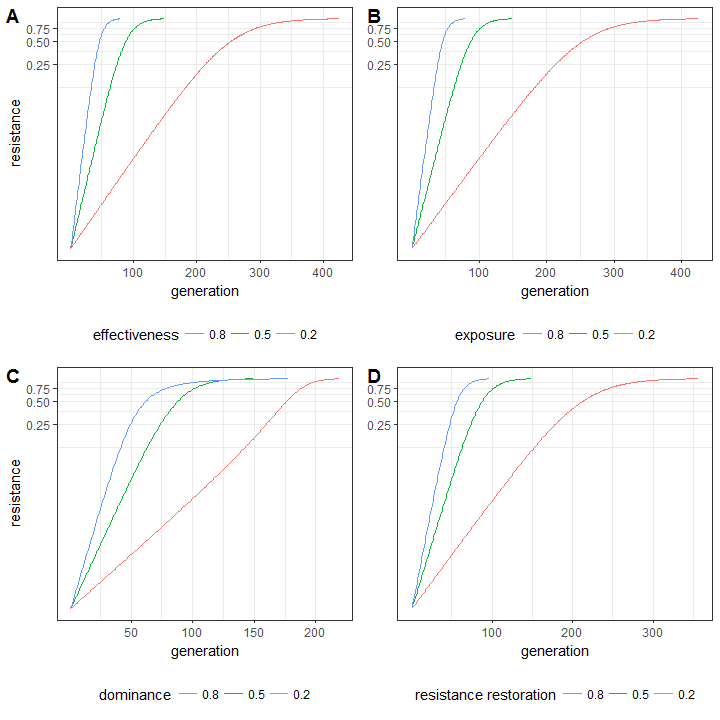
## Figures



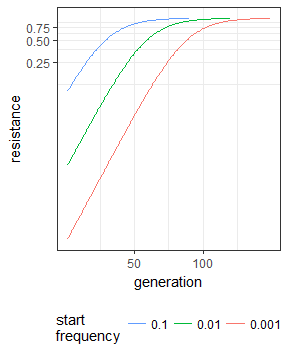
Building up the model structure.

##   
## Attaching package: 'cowplot'

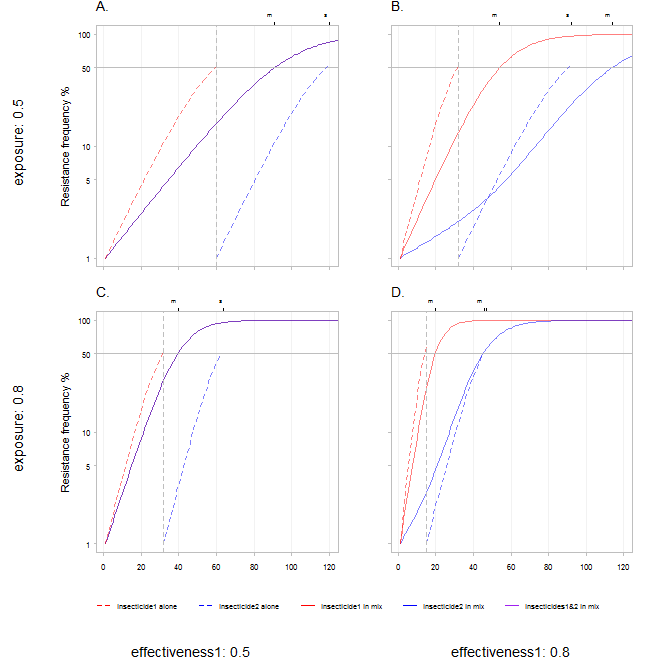
## The following object is masked from 'package:ggplot2':  
##   
## ggsave



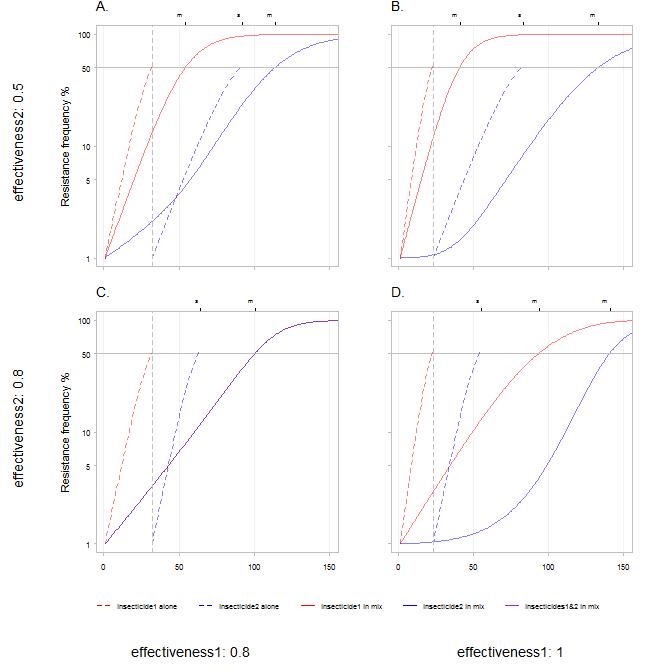
Effect of inputs on single insecticide use. A. Effectiveness, B. Exposure, C. Dominance, D. Resistance restoration



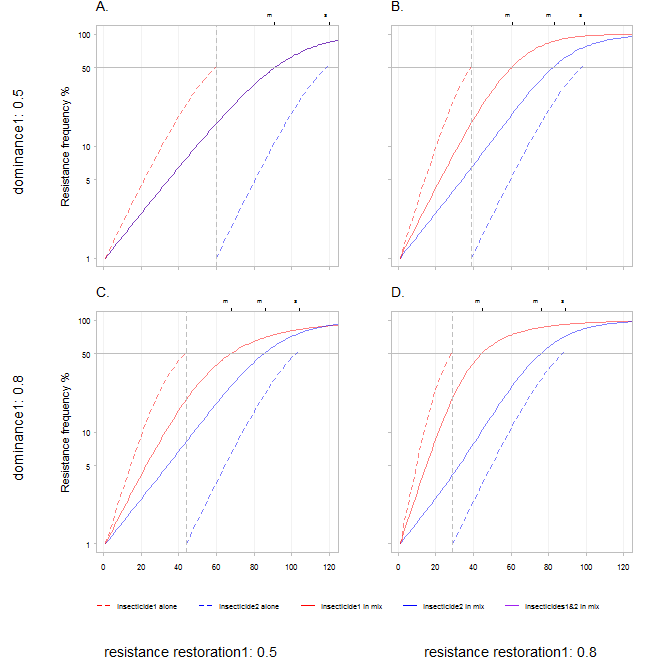
Effect of resistance starting frequency on single insecticide use.



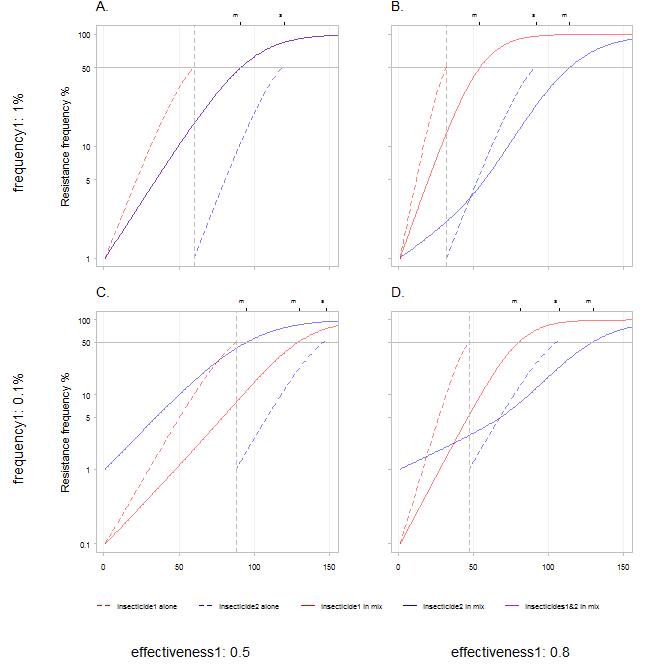
*KEY FIG OF PAPER* Influence of insecticide effectiveness and exposure on time-to-resistance for mixtures and sequences. A. All control inputs equal at 0.5 : time-to-resistance is longer for sequential use, B. Effectiveness of insecticide1 increased from 0.5 to 0.8 : time-to-resistance is longer for the mixture, C. Exposure increased to 0.8 : time-to-resistance is longer for sequential use, D. Effectiveness of insecticide1 and exposure increased to 0.8 : time-to-resistance equal for mixture and sequence



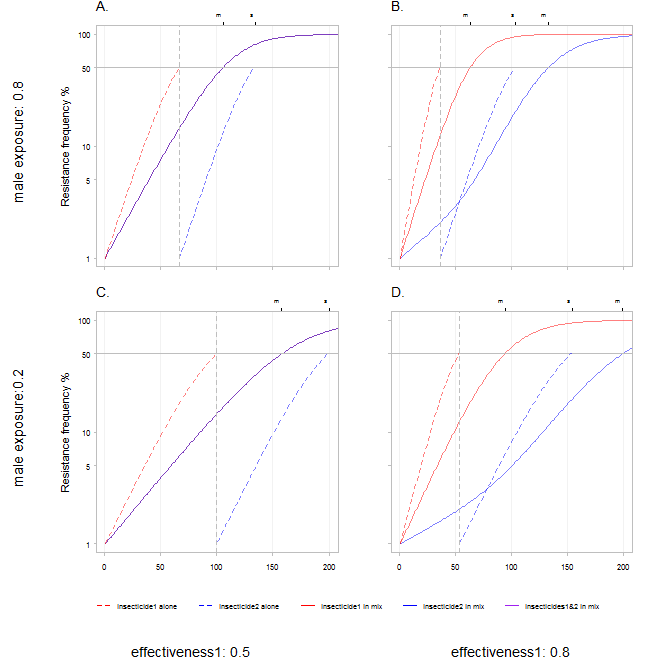
Higher effectivenesses for both insecticides, times-to-resistance longer for the mixture in all scenarios. A. Effectiveness of insecticide1 increased from 0.5 to 0.8, B. Effectiveness of insecticide1 increased from 0.5 to 1, C. Effectiveness of insecticides 1 and 2 increased to 0.8, D. Effectiveness of insecticide2 0.8 and of insecticide1 1



A. All control inputs equal at 0.5, B. Resistance restoration for insecticide1 increased from 0.5 to 0.8, C. Dominance for insecticide1 increased to 0.8, D. Resistance restoration and dominance for insecticide1 increased to 0.8



A. All control inputs equal at 0.5, starting frequencies of resistance at 0.01, B. Effectiveness for insecticide1 increased from 0.5 to 0.8, C. Starting frequency of resistance for insecticide1 decreased from 0.01 to 0.001, D. Effectiveness for insecticide1 increased from 0.5 to 0.8 and starting frequency for insecticide1 decreased from 0.001 to 0.0001



A. All control inputs equal at 0.5, male exposure 0.8, B. Effectiveness for insecticide1 increased from 0.5 to 0.8, C. Male exposure decreased from 0.8 to 0.2, D. Effectiveness for insecticide1 increased from 0.5 to 0.8 and Male exposure decreased from 0.8 to 0.2

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