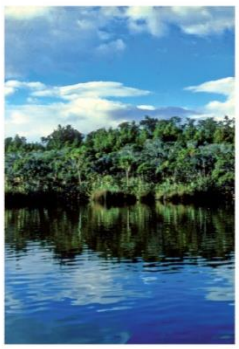

The Trojan Female Technique: Feasibility studies for invertebrate pest control

Landcare Research
Manaaki Whenua

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Summary

Project

A new approach to pest fertility control has been proposed, called the 'Trojan Female Technique' (TFT), involving the release of females carrying naturally occurring mitochondrial DNA haplotypes that reduce the fertility of their male offspring. Proof-of-concept development work for the TFT was awarded 'Smart Ideas Phase 1' funding in 2013 by the Ministry of Business, Innovation and Employment. Part of this proof-of-concept is to conduct feasibility studies of the suitability of different pest types for TFT application, based on mathematical modelling. This report presents the findings of such feasibility studies for pasture weevils and varroa mite, in addition to simulations of laboratory fruit-fly populations to guide population control trials (another component of the TFT proof-of-concept).

Objectives

- Provide guidance to laboratory trials aiming to demonstrate cross-generational control of fruit fly populations by the Trojan Female Technique.
- Assess the feasibility of the Trojan Female Technique to control pasture weevils and varroa mites impacting New Zealand agricultural production.

Methods

- Simulation models of laboratory fruit fly populations and field pasture weevil populations were constructed by adapting the most applicable existing mathematical models available in the peer-reviewed literature.
- Models were adapted by including the capacity to simulate both TFT and wildtype mtDNA haplotypes, the capacity to introduce individuals containing the TFT haplotype with varying effects on male fertility, stochastic genetic drift of haplotypes over time, and other stochastic effects where such were not already included and sufficient data was available in the literature to allow appropriate parameterisation.
- Model construction was not carried out for varroa mite, as a fundamental characteristic of its lifecycle renders the Trojan Female Technique unsuitable for persistent effective population control.
- Models simulated a range of scenarios applicable to either demonstrating fruit fly population control by the TFT in the laboratory, or pasture weevil population control by the TFT in the field.

Results

- For laboratory fruit fly populations, clear demonstrable effects of TFT haplotypes were predicted. Model exploration demonstrated that the size of population reduction achieved and maintained by the TFT (i.e. population control) in laboratory trials is predicted to increase with both greater sterilising effects, and greater starting frequencies, of the TFT haplotype. Based on an expected 60% male fertility reduction effect of the TFT mtDNA haplotype to be used in our fruit fly laboratory population

control trials, statistical power analyses indicate that 20 subpopulations per treatment will give greater than 80% power to detect significant differences in size between populations of 100% wildtype flies, 60% wildtype and 40% TFT flies, and 20% wildtype and 80% TFT flies. These sample sizes would still be able to demonstrate a significant effect of the TFT even if the actual reduction in male fertility was only 20%.

- For field pasture weevil populations: (1) while single large releases of TFT individuals were predicted to cause persistent reductions in population size, the observed control effect was much greater when the same number of individuals were trickle released over time (frequently causing eradication); (2) releases into overwintered adult weevil populations were predicted to be more effective than releases into first or second generation summer adult populations; and (3) while the impact of multiple mating on the predicted population control effect was low, increasing male fertility of the TFT haplotype (from full sterility) increased the release effort required for control.
- For varroa mite, females carrying the TFT mtDNA haplotype would incur very high inclusive fitness costs due to the brother-sister mating that occurs between their offspring, within honeybee brood cells, impacting their daughters' reproductive success. This would result in the rapid elimination of TFT haplotypes from populations into which they were introduced.

Conclusions

- Satisfactory statistical power for the demonstration of Trojan Female Technique effects is expected to be achieved in laboratory fruit fly population trials within the number of subpopulation replicates that we have the capacity to run (approximately 100).
- If TFT haplotypes can be developed that cause large reductions in male weevil fertility, the Trojan Female Technique is predicted to be an effective approach to pasture weevil population control. Since the TFT is more effective at lower pest population densities, due to the release of TFT individuals establishing TFT haplotypes at higher frequencies, the potential to combine the TFT with existing parasitoid biocontrol effects has particular promise. Such a combination may enable pasture weevil eradication, which is not possible with parasitoid biocontrol alone.
- The Trojan Female Technique is not a suitable control approach for varroa mite.

Recommendations

- Laboratory trials to demonstrate Trojan Female Technqiue control of fruit fly populations should proceed.
- Pasture weevils are to be considered suitable targets for TFT control of real world invertebrate pests impacting New Zealand's agricultural productivity.
- Of critical import to TFT application to pasture weevil population control will be the identification of mtDNA haplotypes conferring large reductions in male fertility.

1 Introduction

A new approach to pest fertility control has been proposed, called the 'Trojan Female Technique' (TFT). The TFT is a novel twist on the successful Sterile Male Technique (SMT) paradigm. Rather than releasing large quantities of sterile males on a yearly basis (as in the SMT, with the sterile males normally created through irradiation), the TFT involves the release of females carrying naturally occurring mitochondrial DNA haplotypes that reduce the fertility of their male offspring. The large potential cost-savings of the TFT being self-perpetuating in nature (as mtDNA haplotypes with little or no impact on females are minimally or not selected against, due to mtDNA being maternally inherited), could make it economically viable to apply to a wider range of invertebrate species under a wider range of contexts than the SMT, and even to vertebrates for which the SMT is not cost-effective.

Proof-of-concept development work for the Trojan Female Technique was awarded 'Smart Ideas Phase 1' funding in 2013 by the Ministry of Business, Innovation and Employment. Part of this proof-of-concept is to conduct feasibility studies of the suitability of different pest types for TFT application, based on mathematical modelling. Specifically, feasibility of application is to be modelled for the top six terrestrial vertebrate and invertebrate pests (in terms of economic impact, according to Nimmo-Bell 2009) currently facing New Zealand agriculture. These are clover root weevil, Argentine stem weevil, rabbits, possums, pest birds and varroa mite. This report presents the findings of such feasibility studies for the invertebrate pests (the two pasture weevils and varroa mite), in addition to simulations of laboratory fruit fly populations to guide population control trials (another component of the TFT proof-of-concept).

2 Background

While it is well established that mtDNA haplotypes can have dramatic consequences for individual fitness, causing degenerative diseases and cancer through extreme disruptions of the oxidative phosphorylation system essential for aerobic respiration, more subtle effects of mtDNA haplotypes on male fertility are only now being realized. Most tissues can function well when mitochondrial capacity is reduced by as much as 80 percent. However, the dependence of sperm vigour on mitochondrial capacity, together with the relatively small numbers of mtDNA in a sperm, means that even a modest reduction in mitochondrial capacity can impact male fertility substantially. Due to this asymmetry between egg and sperm dependence of mtDNA function, mtDNA haplotypes can potentially cause large reductions in male fitness while having little or no effect on female fitness; a view supported by empirical data from mice and more recently flies (see references in Gemmell et al. 2013).

With mtDNA being maternally inherited, theory predicts that regardless of the selective pressure in males, mtDNA haplotypes will be maintained over generations in a population at an equilibrium frequency determined solely by the relative fitness of a female bearing the mutation (except under rare situations where there may be high levels of positive-assortative mating and strong inclusive fitness costs to females). The predicted effects of such mtDNA haplotypes on the viability of small populations of endangered species are already recognised. The purposeful introduction of females carrying TFT mtDNA haplotypes into pest populations thus offers a novel approach to their management via fertility control.

A generic modelling study has already been conducted to explore the potentially efficacy of the Trojan Female Technique in controlling pest populations across the full spectrum of the birth and death rates that such species may have (Gemmell et al. 2013). This study showed that the TFT does indeed have the potential to be a novel persistent approach for pest control. The study showed that both single large releases and relatively few small repeat releases of Trojan females can theoretically provide effective control within relatively few generations. Although greatest efficacy was predicted for high-turnover species, the additive nature of multiple releases made the TFT applicable to the full range of pest life histories modelled.

Although the generic finding of greatest TFT applicability to high-turnover species is encouraging for the likelihood of being able to control real-world invertebrate pest populations using the TFT (with such species generally characterised by high birth and death rates), there are a range of biological complexities that could act to reduce any control effects realised. These include stochasticity (random effects, such as the genetic drift of TFT mtDNA haplotypes out of populations), female multiple mating (whereby mating with fertile males could counter any infertile male impacts), social structure (whereby females could suffer reductions in 'inclusive fitness', and TFT alleles thus be selected against), immigration of fertile mtDNA haplotypes from other populations, and populations not being limited by breeding output. This last complexity is of particular concern for TFT application to invertebrate pests, since such populations are frequently determined more by extrinsic factors such as weather and climate that by intrinsic factors such as birth rates.

3 Objectives

Construct and simulate species-specific mathematical models of Trojan Female Technique use to control invertebrate pest populations, including greater biological realism than a previous generic approach, to:

- Provide guidance to laboratory trials aiming to demonstrate cross-generational control of fruit fly populations by the Trojan Female Technique;
- Assess the feasibility of using the Trojan Female Technique to control pasture weevils and varroa mites impacting New Zealand agricultural production.

4 Methods

Literature searches were conducted to identify the most applicable existing peer-reviewed mathematical model for the invertebrate species in question, which could be adapted and simulated to explore Trojan Female Technique effects. Key considerations governing the choice of model to adapt were (1) sufficient biological complexity ensuring that the main determinants of population size and dynamics were included, (2) sufficient biological complexity ensuring that any social or mating system considerations with potentially large effects were included, and (3) stochasticity in life history rates. Models obtained were adapted by including (1) the capacity to model populations containing both TFT and wildtype (i.e. males fully fertile) mtDNA haplotypes, (2) the capacity to introduce individuals containing the TFT haplotype with varying effects on male fertility, (3) stochastic genetic drift of haplotypes over time, and (4) other stochastic effects where such were not included and sufficient data was available in the literature to allow appropriate parameterisation.

4.1 Fruit fly laboratory population model

4.1.1 Underlying model

The underlying model used is the stochastic model developed by Rodriguez (1989), constructed to simulate laboratory subpopulations of *Drosophila melanogaster* maintained in a standard serial-passage format. The model is parameterised from, and simulates, subpopulations maintained as discrete generations in 8-dram (35 ml) vials with 10 ml of food. The model explicitly includes density-dependence effects of pre-adult density on both pre-adult survival and adult fecundity, and adult density on adult fecundity. By using this model, TFT simulations will thus encompass any release from density-dependence that might buffer populations from reduced male fertility effects. The model allows for stochasticity in pre-adult survival, adult fecundity and adult sex ratio, with all parameter values and levels of variation based on empirical data gathered from the subpopulations. The incorporation of such stochasticity is necessary for the realistic simulation of drosophila populations. The Rodriguez (1989) stochastic model can be represented by:

$$n_{t+1} = pn_t . e^{(S - sn_t)} . e^{(F - f_1(n_t e^{(S - sn_t)}) - f_2 n_t)} \quad \text{(i)}$$

This is a 'recursion' equation giving the number of eggs output per generation (n_{t+1}) as a function of number of eggs input (n_t). The component pn_t calculates the number of female eggs (p is the proportion of females). The component $e^{(S - sn_t)}$ calculates the sub-adult survival rate, where S relates to density-independent survival, and s relates to density-dependent reductions in survival. The component $e^{(F - f_1(n_t e^{(S - sn_t)}) - f_2 n_t)}$ calculates the female fecundity rate, where F relates to density-independent fecundity, f_1 relates to the density-dependent effect of adult density on adult fecundity, and f_2 relates to the density-dependent effect of pre-adult density on adult fecundity.

4.1.2 Incorporation of TFT effects

To incorporate the effects of an mtDNA haplotype reducing male fertility (with no other effects), we let x represent the proportion of the subpopulation that carries the wildtype mtDNA, with all others carrying the reduced male fertility haplotype (the 'TFT' haplotype). The probability of an adult female receiving wildtype male sperm is determined by this frequency and the number of males with which each female mates. This species re-mates at a relatively low rate (Markow 2002), observed to result in 21% of females mating twice with an 83% level of sperm displacement (Griffiths et al. 1982). Based on this the 'effective' frequency of the wildtype mtDNA haplotype (x_E), i.e. taking multiple mating into account, can be calculated as:

$$x_E = 0.79x + (0.21 \times 0.17)x + 0.21 \times 0.83(1 - (1 - x)^2)$$

$$x_E = 1.1743x - 0.1743x^2 \quad \text{(ii)}$$

If a represents the fertility of TFT haplotype males (as a proportion of wildtype fertility), this 'effective' frequency can then be used to calculate the fertility rate of eggs input into each generation (i.e. the proportion of the input eggs that are fertile) as:

$$q = x_E + a - x_E a \quad \text{(iii)}$$

This term can then be included into equation (i), such that the recursion of eggs output per generation as a function of eggs input is influenced by the proportion of the input eggs that are fertile:

$$n_{t+1} = pqn_t . e^{(S - sqn_t)} . e^{(F - f_1(qn_t e^{(S - sqn_t)}) - f_2 qn_t)} \quad \text{(iv)}$$

4.1.3 Stochastic parameterisation

S, s, F, f_1 and f_2 are considered as normally distributed random variables with distribution parameters calculated as in Rodriguez (1989) and listed in Table 1. The number of female fertile eggs input (pqn_t) is generated by multiplying p by a random variable with binomial distribution approximated by a normal with a mean of $0.5qn_t$ and a standard deviation of $\sqrt{0.25qn_t}$ (Rodriguez 1989).

In addition to this stochasticity in pre-adult survival, adult fecundity and adult sex ratio, we consider the frequency of the wildtype mtDNA haplotype (x) to be susceptible to genetic drift. Birky et al. (1983) show how for sexually reproducing individuals in which organelles are homoplasmic and inheritance is solely maternal, the variance of the change in frequency of an mtDNA haplotype caused by stochastic sampling of a population from one generation to the next can be approximated by $x(1-x)/N_f$, where N_f is the number of adult females (in our case, the number of adult females that would have laid the input eggs). The wildtype frequency for each successive generation is thus randomly sampled from a normal distribution with a mean equating to the frequency in the previous generation, and this variance (calculated from the previous generation), with lower and upper limits of 0 and 1, respectively (at which point the wildtype haplotype is either lost from the population or fixed in the population, and no further drift occurs).

4.1.4 Model simulation and statistical power analyses

The non-stochastic version of the Rodriguez (1989) model has an equilibrium population size of approximately 140 eggs output per generation. Thus, we initiate all model iterations with n_t = 140 eggs, and a corresponding initial value for N_f of 35 (calculated from Eqn 8 in Rodriguez 1989). Each model iteration is run for 10 generations, with 1000 iterations per scenario, and fly population size in the tenth generation of the 1000 iterations reported on for each scenario. Twenty-five different scenarios are simulated: all combinations of initial x (wildtype mtDNA frequency in the initial input eggs) varied from 1 to 0 in steps of -0.2, and a (the fertility of TFT haplotype males as a proportion of wildtype fertility), likewise varied from 1 to 0 in steps of -0.2. The outcomes of these scenarios are then explored in statistical power analyses (using the web tools available at http://www.stat.ubc.ca) to identify what the experimental design for laboratory trials to demonstrate fruit fly population control using the TFT should be. These calculations are conducted on the basis of empirical data showing that the TFT mtDNA haplotype to be used in our laboratory control trials causes a fertility reduction of 60% (i.e. females mating with TFT males have 60% less fertile eggs than females mating with wildtype males; Dowling et al. unpublished manuscript), and a laboratory capacity to run a maximum of approximately 100 subpopulation replicates.

Table 1 Mean and standard deviation for the normally distributed random variables included in the fruit fly model

Parameter	Mean	Standard deviation
S	-0.510581	0.037306
s	0.001335	0.000060
F	2.28345	0.303361
f_1	0.0112493	0.003278
f_2	0.000855	0.000114
pqn_t	$0.5qn_t$	$\sqrt{0.25qn_t}$
x	x	$\sqrt{x(1-x)/N_f}$

4.2 Pasture weevil population model

4.2.1 Underlying model

The underlying model used is based on the life-history structure and associated stage-specific mortality rates for Argentine stem weevil populations on ryegrass pasture in Canterbury, New Zealand (Figure 1, Table 2, Goldson et al. 2011). The Argentine stem weevil is bivoltine in New Zealand, with first generation egg-laying commencing in late September following the cessation of weevil overwintering diapause. Eggs are laid in the sheaths of grass tillers and the resulting larvae develop through four larval instars. First generation adult eclosion occurs December–February. A second larval generation develops over the summer with second generation adults appearing March–June. This second generation, along with some first generation adults, overwinter in a state of reproductive diapause. Being parameterised from almost a decade's worth of empirical field data, the Goldson et al. model is by far the most comprehensive platform available on which to base a simulation model for a preliminary assessment of the potential of the TFT to control populations of this pest species. We have assumed that the model based on this information will be indicative of New Zealand pasture weevil populations in general, and therefore also applicable for a preliminary assessment of the potential for the TFT to control clover root weevil populations.

Capacity to model the potential of the TFT is included as an additional life-history stage between 'eggs' from overwintered adults and 'pp instar', denoted 'fertile spring eggs', and an additional life-history stage between 'eggs' from first generation adults and 'pp instar', denoted 'fertile summer eggs'. The mortality rates going into the 'eggs' stages from overwintered adults ($k_{f,1}$) and first generation adults ($k_{f,2}$) are maintained and implemented as detailed in Table 2. The number of eggs transitioning into the two 'fertile' eggs stages is determined by multiplication by a 'viability rate' (q; ranging from 0–1) that is dependent on the 'effective' frequency of the wildtype mtDNA haplotype (x_E), i.e. taking any simulated multiple mating into account, and the fecundity of females mated with TFT haplotype males as a proportion (a) of their fecundity when mated with wildtype males: $x_{E=}1-(1-x)^y$, where x is the actual frequency of the wildtype mtDNA haplotype (as opposed to the TFT haplotype) in the population and y is the average number of male weevils that each female mates with for each generation. This incorporation of multiple mating assumes that mating with a single wildtype male is sufficient for all TFT effects to be negated. The 'viability' rate (q) is then calculated as: $q = x_E + a - x_E a$.

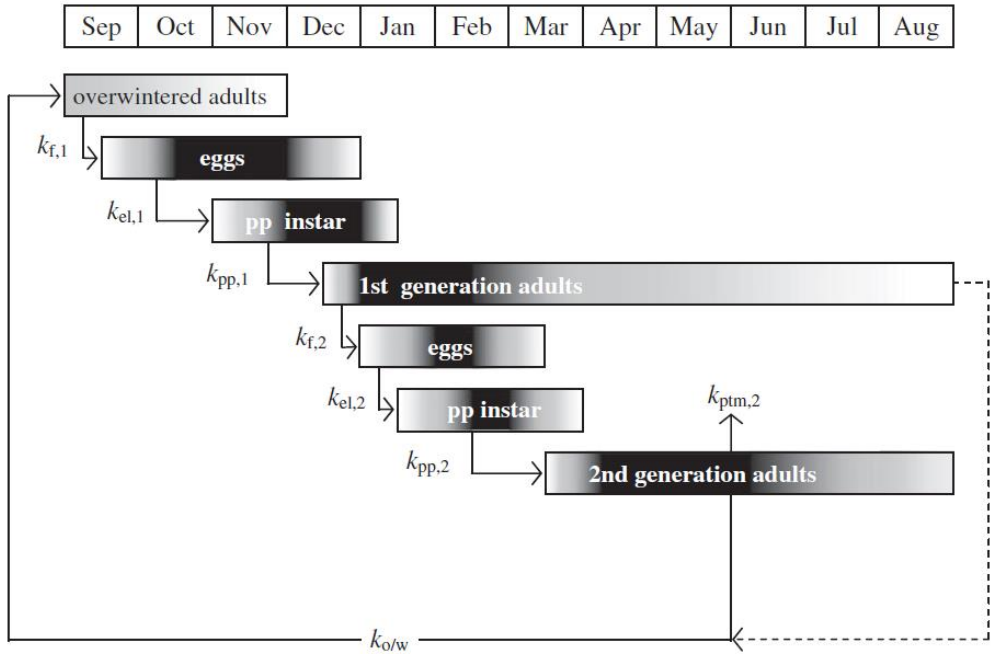


Figure 1 Diagram showing the Argentine stem weevil life history structure simulated here (from Goldson et al. 2011). Shading of the life-stages indicates relative abundance, with the darkest shading indicating peaks in abundance. Mortality rates between life-stages are expressed as k-values, as described in Table 1. The dashed line indicates that some of the late-emerging (February) first generation adults go directly into reproductive diapause and contribute to the overwintering adult generation. Note that while $k_{ptm,2}$ is the rate of second generation adult weevil loss due to parasitism by the parasitoid *Microctonus hyperodae* in the Goldson et al. (2011) model, here we simulated parasitoid-free weevil populations.

Table 2 Argentine stem weevil life-history rates. See text for the determination of q. See Goldson et al. (2011) for *k*-value formulation (determining how they are implemented here)

Symbol	Rate	Implementation in model simulation
$k_{f,1}$	Unrealised fecundity in the founding of the first generation per season	$spring\ eggs = 10^{(\log(160 \times overwintered\ adults) - k_{f,1})}$
q	Viability rate	$fertile\ spring\ eggs = q \times spring\ eggs$
$k_{el,1}$	Mortality of the egg and early instar larval stages in the first generation	$spring\ pp\ instar = 10^{(\log(fertile\ spring\ eggs) - k_{el,1}}$
$k_{pp,1}$	Mortality of the fourth instar / prepupal and pupal stages in the first generation	$first\ generation\ adults = 10^{(\log(spring\ pp\ instar) - k_{pp,1}}$
$k_{f,2}$	Unrealised fecundity in the founding of the second generation per season	$summer\ eggs = 10^{(\log(160 \times first\ generation\ adults) - k_{f,2})}$
q	Viability rate	$fertile\ summer\ eggs = q \times summer\ eggs$
$k_{el,2}$	Mortality of the egg and early instar larval stages in the second generation	$summer\ pp\ instar = 10^{(\log(fertile\ summer\ eggs) - k_{el,2}}$
$k_{pp,2}$	Mortality of the fourth instar / prepupal and pupal stages in the second generation	$second\ generation\ adults = 10^{(\log(summer\ pp\ instar) - k_{pp,1}}$
$k_{o/w}$	Overwintering mortality	$overwintered\ adults = 10^{(\log(second\ generation\ adults) - k_{o/w})}$

4.2.1 Stochastic parameterisation

The life-history analysis of Goldson et al. (2011) identified density-dependence in mortality rates $k_{pp,1}$ and $k_{f,2}$. The values of these two rates each modelled year are thus considered as normally distributed variables with means calculated as per the relevant equations in Goldson et al. (2011), and standard deviations calculated from the equation fits to field data illustrated in Figure 3 of the same paper (Table 3). All other *k*-values each modelled year are considered as normally distributed random variables with means and standard deviations calculated from the ranges presented in Table 1 of Goldson et al. (2011). Adult sex ratio is assumed to be maintained at 50/50 each generation; previous exploration of stochastic drosophila model populations has indicated that variation around this ratio has little influence on model outcomes.

In addition to the stochasticity in *k*-values, we consider the frequency of the wildtype mtDNA haplotype (x) to be susceptible to genetic drift. Birky et al. (1983) show that for sexually reproducing individuals in which organelles are homoplasmic and inheritance is solely maternal, the variance of the change in frequency of a mitochondrial mtDNA haplotype caused by stochastic sampling of a population from one generation to the next can be approximated by $x(1-x)/N_f$ where N_f is the effective adult female population size. Here we assume that the population of weevils in 1 ha, i.e. 10,000 m^2, represents the effective population with regards to genetic drift. The wildtype frequency for each successive generation is thus randomly sampled from a normal distribution with a mean equating to the frequency in the previous generation, and this variance (assuming a 50/50 adult sex ratio), with lower and upper limits of 0 and 1, respectively (at which point either the wildtype haplotype is lost from the population or fixed in the population, and no further drift occurs).

Table 3 Means and standard deviations for normally distributed pasture weevil model rates

Rate	mean	sd
$k_{f,1}$	0.9018	0.2016
$k_{el,1}$	0.4240	0.3385
$k_{pp,1}$	-1.42 + 0.803log(*spring pp instar*)	0.2821
$k_{f,2}$	-0.319 + 0.764log(*second generation adults*)	0.1962
$k_{el,2}$	0.2971	0.4124
$k_{pp,2}$	1.3699	0.5277
$k_{o/w}$	0.3120	0.1029

4.3 Varroa mite

The process of considering existing mathematical models of varroa mite *(Varroa destructor)* infesting honeybee *(Apis mellifera)* colonies highlighted that a fundamental characteristic of the varroa mite lifecycle renders the TFT unsuitable for their persistent effective population control. A critical requirement of the TFT is that the females carrying the TFT mtDNA haplotype do not themselves incur a relative fitness cost that would cause the TFT haplotype to be selected against and lost from the pest population over time (Gemmell et al. 2013). Such a fitness cost could occur either directly, through females with the TFT haplotype having lower reproductive success than their wildtype counterparts, or indirectly, for example through 'inclusive fitness' costs.

Inclusive fitness is defined as the ability of an individual organism to pass on its genes to the next generation, taking into account the shared genes passed on by the organism's close relatives. In the context of the TFT, inclusive fitness costs would thus occur if the relatives of an individual carrying the TFT haplotype were disadvantaged relative to the wider population. Such effects are unavoidable in the varroa mite lifecycle (Figure 2).

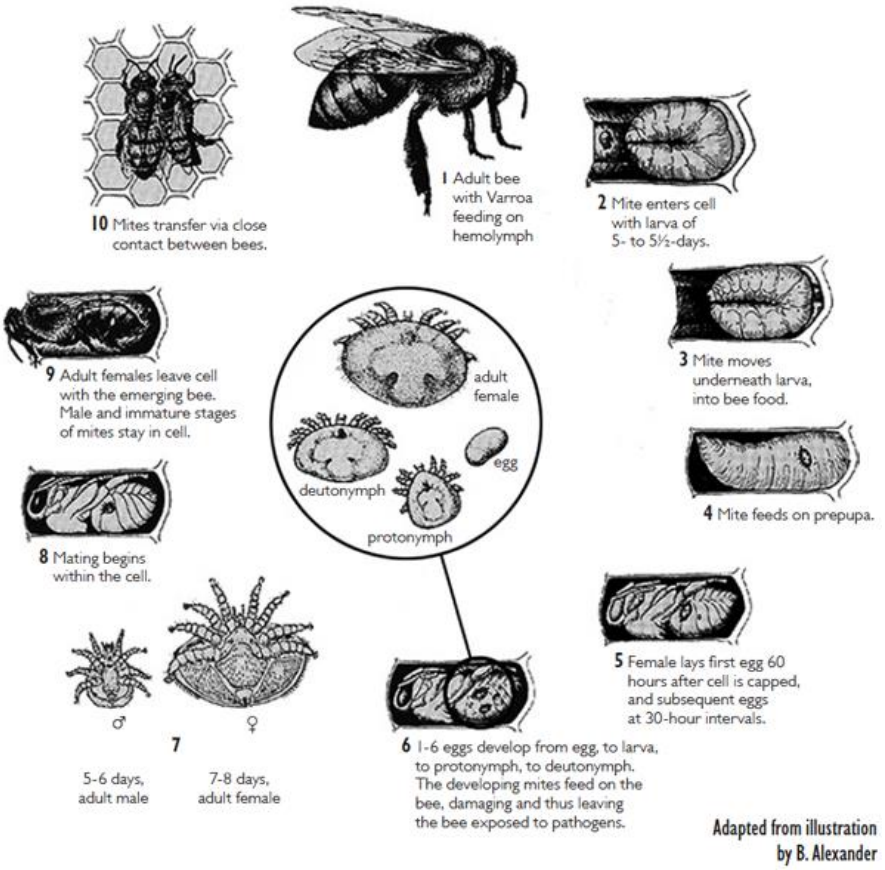


Figure 2 *Varroa destructor* life cycle, from Henderson and Morse (1985) where it is adapted from the illustration by B. Alexander

Varroa mites reproduce within honeybee brood cells. Mature female mites (from the phoretic population living on adult bees) invade open cells and, after 60 hours post-capping (with the cell containing a honeybee larva), lay eggs at 30-hour intervals. Of critical import to the TFT is that the first egg laid is typically a haploid male with the rest being females (Sammataro et al. 2000). In cells invaded by single mites, which is the norm in light infestations, brother-sister mating occurs between the offspring within the cell, with the mother mite and matured female offspring leaving the cell with the emerging bee. Since the male offspring of a TFT haplotype female entering a cell will have impaired fertility, the reproductive success of the mothers' daughters will be reduced (relative to the daughters of a wildtype female, who will have mated with their fertile brother in the cell). With such inbreeding associating male fertility traits with mitochondrial matrilines (Wade and Brandvain 2009), this very high inclusive fitness cost of the TFT haplotype would result in its rapid elimination from the population (Unckless and Herren 2009). While inbreeding is reduced in heavy infestations, in which multiple females often breed in the same cell allowing more diverse mating, TFT females will always be disadvantaged through the unavoidable association between their daughters and their fertility compromised sons. These considerations pre-empt the need for more complex mathematical modelling of TFT application to varroa mite control.

5 Results

5.1 Fruit fly laboratory population model

5.1.1 Baselines dynamics

As in Rodriguez et al. (1989), the model produced highly variable fruit fly population dynamics, very similar to those observed in laboratory populations. Wildtype fruit fly populations (i.e. with no TFT individuals) fluctuated haphazardly with adult densities generally occurring between 50–100 per vial (Figure 3a).

5.1.1 Trojan Female Technique dynamics

Despite the variation observed in the baseline dynamics, clear demonstrable effects of TFT haplotypes on the laboratory fruit fly populations modelled were predicted. Under the assumption that the TFT haplotype caused complete male sterility, simulated fruit fly populations initiated at a 50/50 wildtype/TFT mtDNA haplotype mix were on average approximately half the size of the fully wildtype populations (Figure 3b). If the assumption of complete sterility was relaxed, and it was assumed that the TFT haplotype resulted in only a 50% reduction in male fertility (in terms of fertile eggs produced by females mated with, compared to wildtype), simulated populations again initiated at a 50/50 wildtype/TFT mtDNA haplotype mix were still on average reduced by over 20% (Figure 3c). These effects were predicted to be maintained over the multiple fruit fly generations simulated, but with the variance in population size across iterations predicted to increase over time for the mixed wildtype/TFT scenarios, due to genetic drift effects becoming apparent in the small population sizes considered here. Note that the variance across iterations was predicted to be greater for the complete male sterility scenario (Figure 3a) due to greater sampling effects on haplotype frequencies (genetic drift) between generations at lower population sizes.

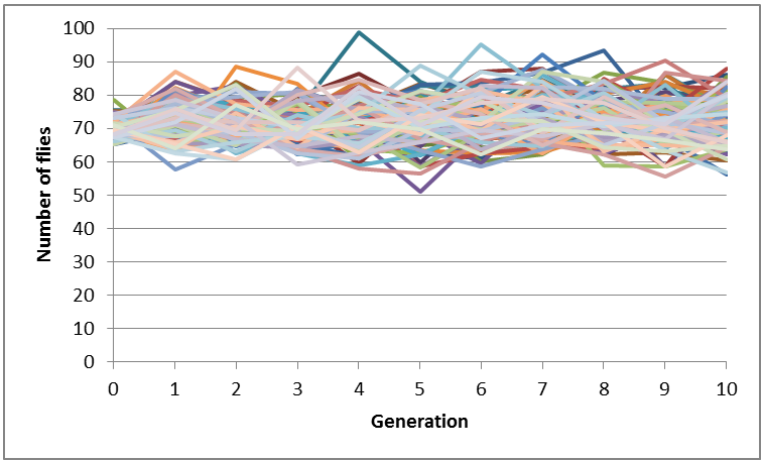
5.1.2 Model exploration

The model exploration predicted that the size of population reduction achieved and maintained by the TFT (i.e. population control) in laboratory trials would increase with both greater sterilising effects, and greater starting frequencies, of the TFT mtDNA haplotype (Table 4).

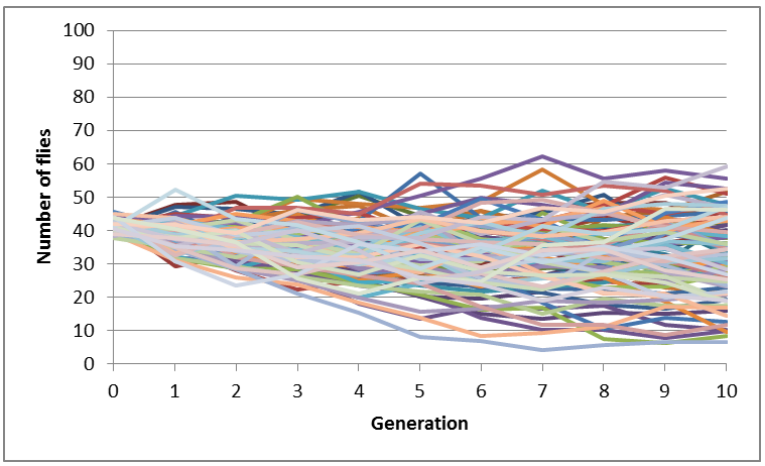
Table 4 Adult fly numbers (mean ± standard deviation) at the tenth generation, for the 1000 model iterations of each scenario simulated

		TFT mtDNA male fertility as a proportion of wildtype fertility (a)					
		0	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
Wildtype mtDNA haplotype frequency (x)	**1**	73.2 ± 6.1	73.2 ± 6.1	73.2 ± 6.1	73.2 ± 6.1	73.2 ± 6.1	73.2 ± 6.1
	0.8	60.7 ± 7.5	63.4 ± 6.8	66.0 ± 6.4	68.5 ± 6.2	70.8 ± 6.2	73.2 ± 6.1
	0.6	43.8 ± 10.5	51.0 ± 8.2	57.4 ± 6.8	63.2 ± 6.2	68.3 ± 6.1	73.2 ± 6.1
	0.4	21.9 ± 12.6	40.2 ± 10.2	46.6 ± 7.3	56.9 ± 6.1	65.6 ± 6.0	73.2 ± 6.1
	0.2	9.5 ± 10.1	16.1 ± 8.9	33.3 ± 7.0	49.5 ± 5.6	62.5 ± 5.7	73.2 ± 6.1
	0	0	0	14.8 ± 2.4	40.4 ± 4.1	59.1 ± 5.2	73.2 ± 6.1

(a)



(b)



(c)

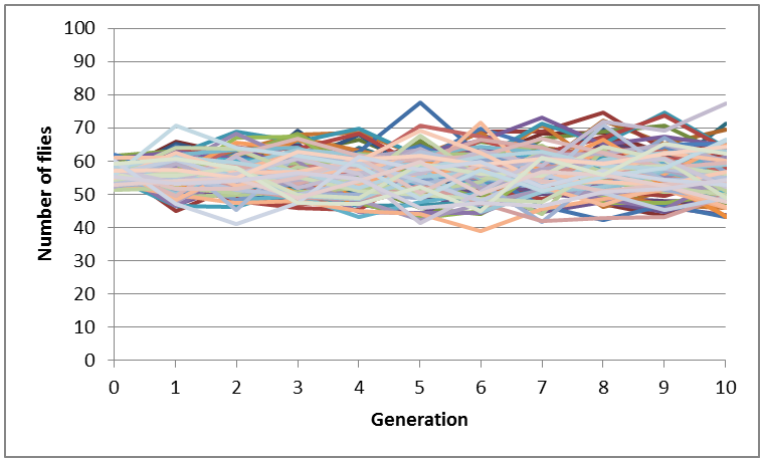


Figure 3 Example output of 100 fruit fly model iterations with (a) wildtype frequency = 1 (i.e. no TFT haplotype in the subpopulations); (b) wildtype frequency = 0.5 and TFT male fertility = 0.0 (i.e. complete male sterility); and (c) wildtype frequency = 0.5 and TFT male fertility = 0.5 (i.e. a 50% reduction in fertility).

5.1.3 Statistical power analyses

Statistical power analyses indicate that 20 subpopulations per treatment will give greater than 80% power to detect significant differences in size at the $p < 0.05$ threshold between populations of (1) 100% wildtype flies, (2) 60% wildtype and 40% TFT haplotype flies, and (3) 20% wildtype and 80% TFT haplotype flies. This is based on the predicted means and standard deviations of trial outcomes reported in Table 4, and empirical data showing that the TFT mtDNA haplotype to be used in our laboratory control trials causes a fertility reduction of 60% (i.e. females mating with TFT males have 60% less fertile eggs than females mating with wildtype males; Dowling et al. unpublished manuscript). These sample sizes would still be able to demonstrate a significant effect of the TFT even if the actual reduction in fertility was only 20%.

5.2 Pasture weevil population model

5.2.1 Baseline dynamics of wildtype populations

As expected with the stochastic processes incorporated, the model produced highly variable pasture weevil population dynamics, very similar to those observed in the field (Goldson et al. 1998; McNeil et al. 2003; Goldson et al. 2011). Wildtype weevil populations (i.e. with no TFT individuals) fluctuated haphazardly with first generation adult densities generally 0–200 m^{-2}, but occasionally reaching peaks three times higher (Figure 4).

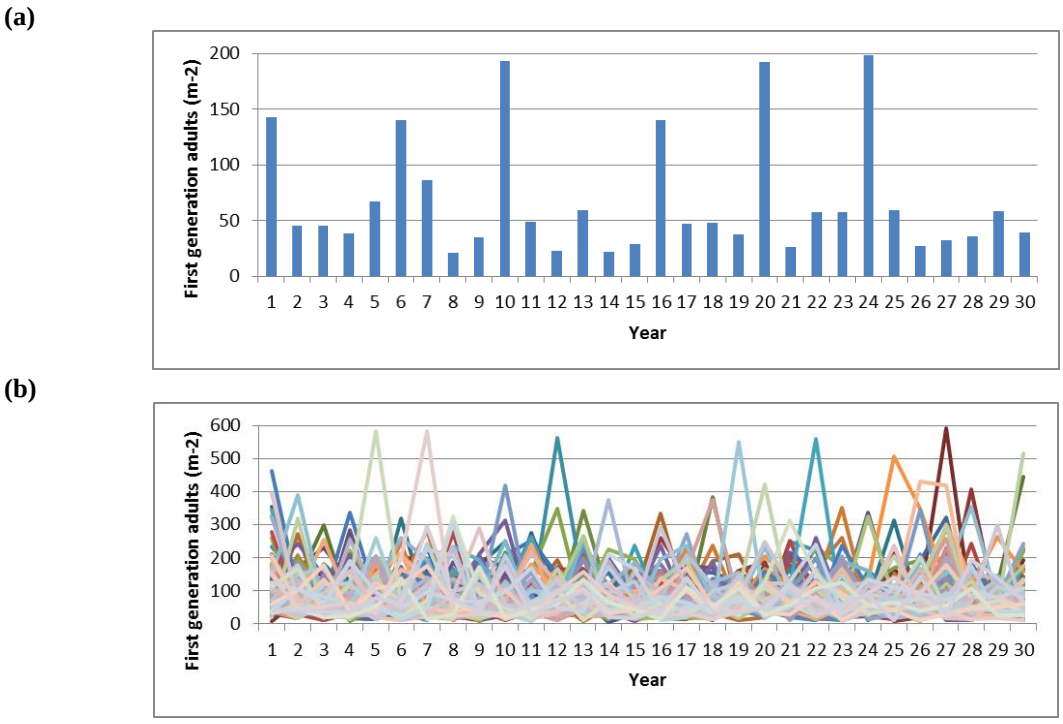


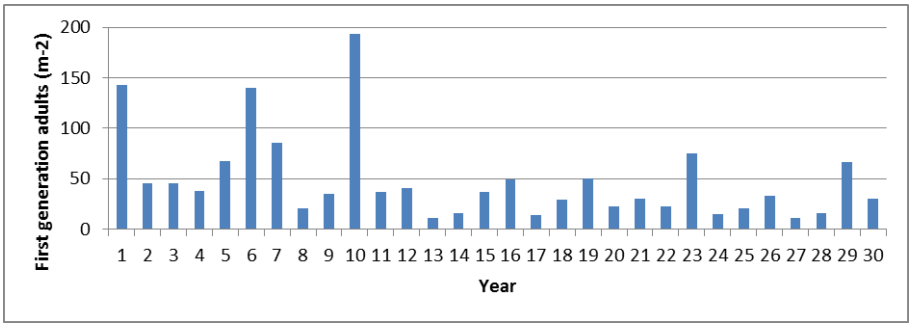
Figure 4 Pasture weevil model simulating purely wildtype population dynamics. (a) Detail of a single model iteration. (b) Variation produced by 100 model iterations.

5.2.2 Single versus 'trickle' releases of TFT individuals

The performance of single versus trickle TFT release strategies was initially compared under the following model variables: adult female weevils assumed to mate only once each generation (i.e. $y = 1$), the TFT haplotype assumed to cause complete male sterility (i.e. $a = 0$), and the release of TFT individuals (at a 50/50 sex ratio) into the overwintered adult population (Figure 1).

During initial model exploration under this variable set, a single introduction of 100 TFT individuals per square metre into the overwintered adult population reduced subsequent weevil population density the year following introduction and maintained this effect over time (Figure 5). The delay is due to the overwintered adult population having mated the previous autumn. Interestingly, when the same numbers of TFT individuals were introduced into the model system over a ten year period (i.e. ten TFT individuals introduced per square metre into the overwintered adult population for each of ten consecutive years), the observed control effect was much greater. The single release of 100 TFT individuals resulted in first generation adult densities generally occurring between 0 to less than 100 individuals per square metre (Figure 6), while the trickle release resulted in weevil populations being eradicated in all iterations.

(a)



(b)

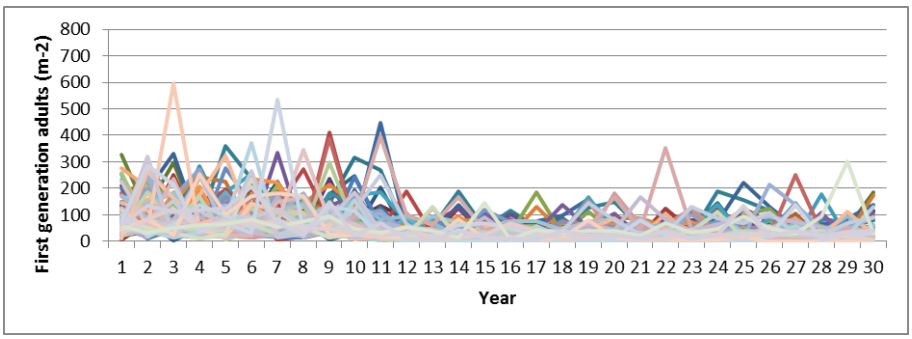
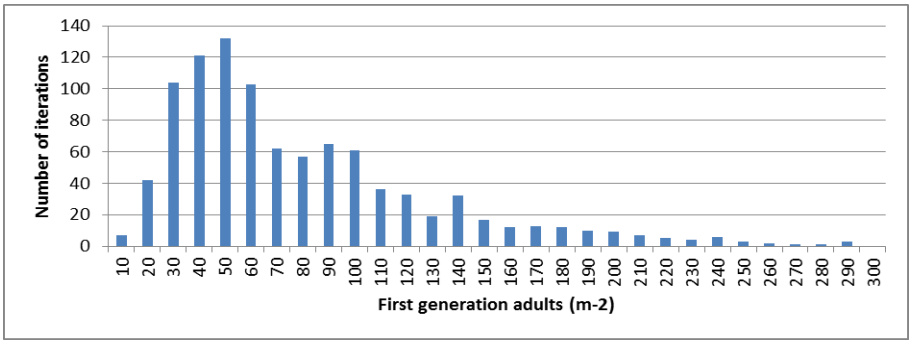


Figure 5 Single introduction of 100 TFT individuals per square metre, with the TFT haplotype causing complete male sterility and the assumption of no multiple-mating. (a) Detail of a single model iteration. (b) Variation produced by 100 model iterations.

(a)



(b)

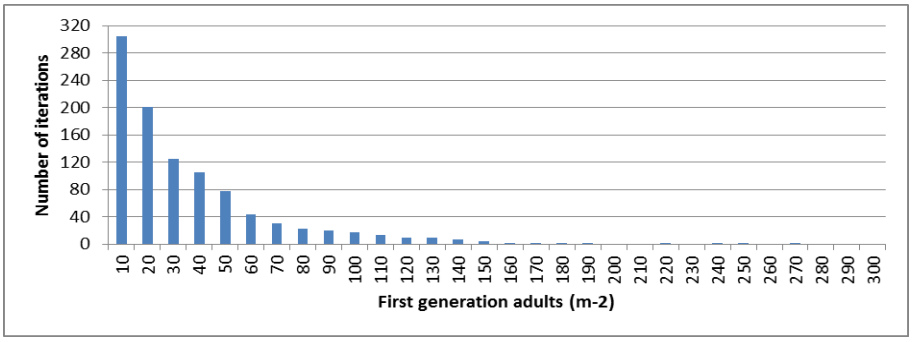


Figure 6 Distribution of first generation adult densities at the 30 year simulation time-point, produced by 1000 model iterations for each of (a) wildtype populations (as per Figure 4), and (b) single introductions of 100 TFT individuals per square metre into the overwintered adult population (as per Figure 5). All TFT simulations are under the assumption that the TFT haplotype causes complete male sterility and there is no multiple-mating. Note that for the wildtype histogram there are an additional 23 occasions on which density exceeded 300 m^{-2}.

5.2.3 TFT releases into overwintered, first generation and second generation adult populations

Given the high level of impact seen with the trickle release of 10 TFT individuals per square meter for each of 10 consecutive years, whereby all simulated weevil populations were eradicated, here we explore timing effects with a trickle release of 1 TFT individual per square metre for each of 10 consecutive years. The trickle release of TFT individuals into overwintered, first generation and second generation adult populations was simulated to explore whether timing of introduction influences the resulting population control effect. All other model variables are kept as before (i.e. the TFT haplotype causing complete male sterility and the assumption of no multiple-mating).

Trickle releasing TFT individuals gave greater population control when they were introduced to the overwintered adult population, as opposed to the first or second generation summer adult populations (Figure 7). Control was also greater for introductions into the second generation summer adult population than the first. These timing effects are explained by the size of adult population into which the TFT individuals are being introduced: the larger the population, the more the introduced TFT haplotype is swamped by the wildtype, reducing the TFT haplotype frequency subsequently established. This is also why trickle-releasing is more effective in general than a single large release of TFT individuals as, with the large annual variation in weevil densities observed, it gives a greater chance of TFT individuals being introduced to a low density population and thus establishing a larger TFT haplotype frequency.

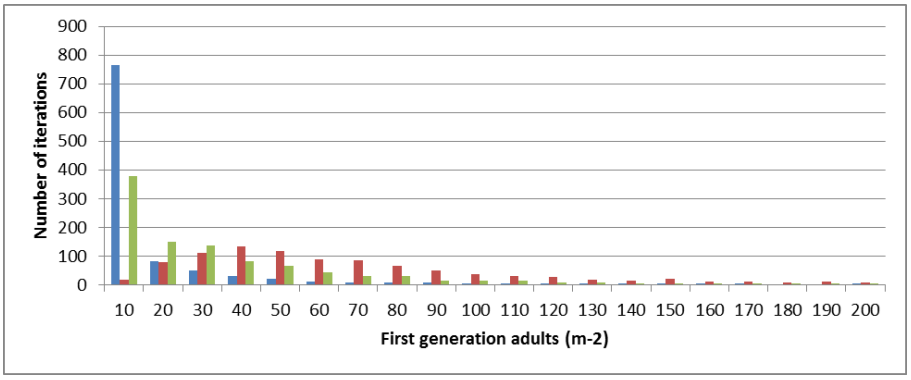


Figure 7 Distribution of first generation adult densities at the 30 year simulation time-point, produced by 1000 model iterations for the trickle release of one TFT individual per square meter for each of 10 consecutive years into the overwintered adult population (blue bars), the first generation summer adult population (red bars), and the second generation summer adult population (green bars). The TFT haplotype simulated causes complete male sterility, and no multiple-mating is assumed. Note that there are an additional 52 and 11 occasions on which density exceeded 200 m^{-2} for the first and second generation summer adult introductions, respectively.

5.2.4 Influence of TFT efficacy on weevil population control

We explored the influence of decreasing TFT efficacy (i.e. incrementally increasing male fertility of the TFT haplotype from infertile, as simulated thus far, to close to wildtype fertility) on weevil population control, by observing how the 30-year first generation adult outcome altered with the fecundity of females mated with TFT males (as a proportion of wildtype) increasing up to 0.9 in steps of 0.1. All other model variables were kept as above, with the assumption of no multiple mating and ten years trickle releasing of one TFT individual per square metre each year into overwintered adult populations.

Increasing male fertility of the TFT haplotype reduced the population control potential of trickle releasing one TFT individual per square metre (Figure 8). At $a = 0.2$ (i.e. the fecundity of females mated with TFT males was one fifth that of females mated with wildtype males) the mean first generation adult population size was predicted to be over 50% of wildtype populations. Conducting the same simulations for trickle releases of 10 and 100 TFT individuals per year demonstrated how this loss of efficacy could be offset to some degree through increasing control effort (Figure 8). Similar offset effects were also achieved by increasing the length of release period to longer than 10 years (data not shown).

5.2.5 Influence of multiple mating on weevil population control

Given the opportunity, female weevils of other species are known to mate with multiple males, resulting in multiple paternity offspring. Given that such mating could counteract the efficacy of introducing TFT individuals for population control, here we simulated increases in the mean number of different male mates per female from one (the default used thus far) to three, assuming that a single wildtype male mate is sufficient to result in full egg fertilisation. However, the frequency of multiple mating is expected to be low in the field, particularly at the periods of low density when most TFT control effects are predicted to be realised.

As expected, multiple mating reduced the predicted population control effect. However, its impact was relatively low compared to the effect of varying TFT male fertility. In contrast to a mean first generation adult population size of only two weevils with no multiple mating (when trickle releasing one TFT individuals per square metre for each of 10 consecutive years, and TFT males are infertile), mean population sizes of 23 and 32 per square metre were observed when the mean number of mates was increased to two and three respectively. However, increasing the number of TFT individuals released per year to ten resulted in eradication in almost all iterations for both two and three mates.

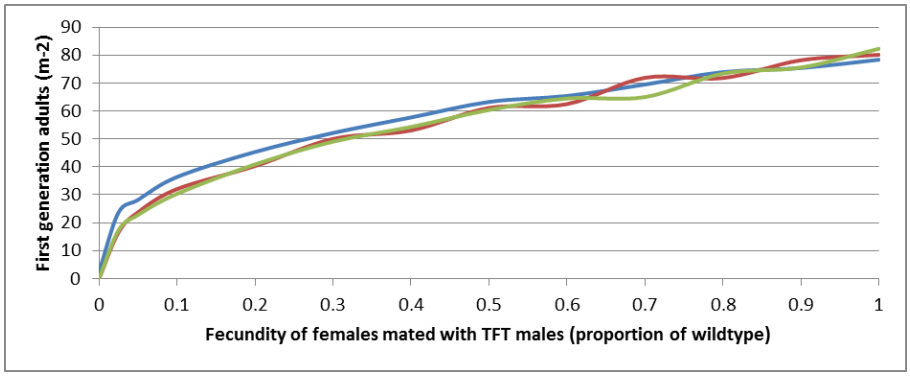


Figure 8 Relationship between mean first generation adult densities at the 30-year simulation time-point and the fecundity of females mated with TFT males, generated by 1000 model iterations for the trickle release of 1 (blue line), 10 (red line) and 100 (green line) TFT individuals per square meter for each of 10 consecutive years in the overwintered adult population.

6 Conclusions

6.1 Fruit fly population model

- Control effects of the Trojan Female Technique on laboratory fruit fly populations (populations held at lower numbers than they would otherwise be across multiple generations) are predicted to be clearly demonstrable in laboratory trials, even with model simulations that include realistic representations of stochasticity, multiple mating, and compensatory releases from density dependence.
- Satisfactory statistical power for the demonstration of TFT effects is expected to be achieved in laboratory fruit fly population trials within the number of subpopulation replicates that we have the capacity to run (approximately 100).
- Note that the simulations conducted here were for the purpose of guiding the design of laboratory trials to provide the first proof-of-concept demonstration of the TFT and do not inform on the utility of the approach to control natural fruit fly populations. For such a purpose, multiple releases, as are conducted for the Sterile Male Technique, are likely to be more effective than the one-off releases modelled here, although theoretically requiring much less effort than the SMT approach.

6.2 Pasture weevil population model

- If TFT haplotypes can be developed that cause complete male weevil sterility, the Trojan Female Technique is predicted to be an effective approach to pasture weevil control.
- Model exploration indicated that a trickle-release approach will be more effective than single large releases, due to multiple releases giving a greater chance of TFT individuals being introduced to a low density population and thus establishing a higher TFT haplotype frequency.
- Model exploration also indicated that introductions to the overwintered adult weevil population will be more effective than introductions to either the first or second generation summer adult populations, again due to higher TFT haplotype frequency being established when introductions are made to lower density populations.
- Two key factors were identified that could reduce the efficacy of the TFT for controlling pasture weevil populations. First, the occurrence of multiple mating in weevil populations was predicted to reduce the population control effect of introducing TFT individuals by a relatively small degree. Second, if TFT males had residual fertility (rather than being sterile) the predicted population control effect was reduced, increasing the release effort required for controlling to any particular level.
- Optimal strategies for TFT application to pasture weevil populations can now be developed (requiring a spatial modelling framework). Since the TFT is more effective at lower pest densities, due to the release of TFT individuals establishing TFT haplotypes at higher frequencies, the potential to combine the TFT with existing parasitoid biocontrol has particular promise. Such a combination may enable the step to pasture weevil eradication, a desirable outcome that is not possible with parasitoid biocontrol alone.

6.3 Varroa mites

- The Trojan Female Technique is not a suitable control approach for varroa mite.

7 Recommendations

- Laboratory trials to demonstrate Trojan Female Technique control of fruit fly populations should proceed.
- Pasture weevils are to be considered suitable targets for TFT control of real world invertebrate pests impacting New Zealand's agricultural productivity.
- Of critical import to TFT application to pasture weevil population control will be the identification of mtDNA haplotypes conferring large reductions in male fertility.

8 Acknowledgements

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