Project title	The use of Sterile Insect Technique to increase the success of IPM in horticultural crops
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Project leader:	Dr Jonathan Knight, Imperial College London
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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Grower Summary

Headline

Male and female sterility can be induced by the irradiation of tomato leafminer pupae, releasing irradiated females increases the success of SIT.

Background and expected deliverables

Sterile Insect Technique (SIT) has been applied successfully to a range of pest species in some parts of the world, most notably the Mediterranean fruit fly. SIT has considerable potential to increase the use of Integrated Pest Management (IPM) in UK horticultural crops. The use of SIT slows down the initial population growth of pests, allowing natural enemies and/or released biological control agents more time to become established before the pest reaches critical economic damage thresholds. This would have the potential to greatly reduce the need for prophylactic applications of pesticides in situations where IPM has not yet been adopted and could also reduce the number of applications of second line of defence chemicals in existing IPM programmes. Although it is anticipated that this concept could ultimately contribute to control programmes against a wide range of important horticultural pests (e.g. cabbage root fly, carrot fly, onion fly), a pest of glasshouse crops was chosen as the experimental system because glasshouse containment allows more control over both the pest and its natural enemy populations.

Biological control of the tomato leafminer (*Liriomyza bryoniae*) using the parasitic wasp *Diglyphus isaea* has been practised in protected edible crops in the UK for over 15 years. However, many growers continue to experience difficulties in maintaining the pest below economic damage thresholds. This has led to the application of non-specific insecticides that have disrupted biological control of other pests leading to the breakdown of the whole IPM programme in conventionally grown crops. Biological pollination with bumble bees has also been seriously impaired in tomato crops. The situation is worse in organic crops because there are no such products available so tomatoes suffer unacceptable levels of damage. A possible solution would be to slow down the growth rate of the pest population which would allow the parasitoids more time to become established. This can be achieved by releasing sterile male *Liriomyza bryoniae* into the glasshouse when the first flush of pest activity occurs. This would prevent the need for pesticide use and it allows the continuation of other biocontrol activities to go on in the move toward pesticide-free production systems.

Summary of the project and main conclusions

The success of a control program based on sterile insect technique (SIT) is dependent on the production of high quality, sterile male flies that can compete effectively with males of the pest population and mate successfully with the wild females. In the past SIT programs have failed because of poor quality sterile males that were unable to mate with the females of the pest population. The failure has been resolved by developing a quality control system that monitors the production of sterile flies.

End users of the SIT product need to be confident that they are using a high quality, efficient, consistent product to ensure success of its use in an IPM programme. As such, the production of high quality mass-reared males is of great importance. In order to achieve success the males released in this project needed to be both sterile and of comparable quality to the pest population or wild type males. Exposure to too great a dose of radiation to induce sterility affects the fitness of the adult males so it was important to determine the optimum dose of radiation required.

The aim of this work is to produce both male and female adults that after irradiation are unable to produce viable offspring. Ideally releases of sterile flies should be male only, but it is also important to produce sterile females. Females that have been irradiated are unable to produce larvae. They still cause puncture damage to the leaves to lay non-viable eggs but no mines develop. Similarly, wild females that mate with sterile males will puncture leaves but mines do not develop from the puncture marks. Thus irradiated females do not have a negative effect on the performance of SIT pest control. However, although releasing extra non-sterile female leafminers has a significant impact on the success of the control program, it requires high release rates of sterile males to counteract this, which makes it expensive.

At present there is limited information on the population sizes and the economic effects of *Liriomyza bryoniae* infestations. Some tomato cultivars, such as Piccolo, are much more susceptible to leafminer damage than others. In order to develop a comprehensive cost/benefit analysis on the feasibility of SIT to control leafminer infestations data needs to be collected. The success of a SIT program depends on whether or not it is economically feasible to use. It is also important to know the size, particularly the density and distribution of leafminer infestations in order to calculate the numbers of sterile males that would have to be released in order to achieve pest suppression. A questionnaire survey will be used to collect data from growers on the perceived loss and the cost of current control methods. The population size and distribution will be estimated by greenhouse surveys and through crop walking.

Conclusions

- Releasing irradiated females increases the success of SIT as it has been shown, in this project, to reduce the number of mines on leaves.
- Sterility can be induced by the irradiation of pupae with 160 Gy of gamma radiation.
- Further studies to examine the fitness of the irradiated males are needed to produce males with a relatively high level of sterility (less than 0.7 mines per female) but that have a comparable fitness to wild males in order to produce high quality sterile males.

Financial benefits

Questionnaire surveys will be used to collect information on the economic costs of leafminer infestations and the cost of control. Glasshouse surveys and crop walking will be used to collect data on the density and distribution of leafminer infestations within glasshouses. Financial benefits may be quantified after collection of this data.

Action points for growers

None to date

Science Section

Introduction

The overarching objective of this project is to enable the production of a high quality crop of tomatoes with pest damage controlled to below an economic threshold. This is currently not being met due to, amongst other things, infestations of the tomato leafminer, *Liriomyza bryoniae*, damaging the leaves of the tomato plants, reducing the photosynthetic area and therefore reducing yield of the crop (Spencer, 1973). The presence of damage to the leaves also makes the crop less saleable to buyers. This objective is defined by the growers who need to produce the tomatoes to a required standard to satisfy the buyers who set the quality targets. Currently, release of the parasitoid wasp *Diglyphus isaea* is the main method of leafminer control in an organic glasshouse sometimes coupled with the application of Eradicoat a physically acting pesticide which can be applied in greenhouses to the upper leaves of tomato plants to prevent female leafminer oviposition (Jacobson, 2008). However, the *D.isaea* biocontrol is not an effective control measure on its own. Principally because releases of *D.isaea* are started after *L.bryoniae* are found at one mine per plant (Jacobson, pers. comm), however, this means the population of *D.isaea* lags behind the *L.bryoniae* population. Even when the *D.isaea* population is fully established the level of control achieved is insufficient to reduce the damage to a level below that causing financial loss and it is still highly visible to buyers.

Sterile Insect Technique

Sterile Insect Technique, or SIT, is the release of large number of sterile males into an area to overflood the wild males in the population and compete to mate with the females (Knipling, 1955). The females that mate with the sterile males would produce non-viable eggs due to dominant lethal mutations within the eggs (Robinson, 2005). In doing so this reduces the reproductive potential of the females. Successive releases of sterile males leads to a reduction in the size of wild population and the pest population can be suppressed, or even eradicated (Knipling, 1955).

This was first developed and successfully implemented to eradicate the New World Screwworm, *Cochliomya hominivorax* (Coquerel), from the Southern United States to Panama as an area-wide control program (Baumhover *et al*., 1955; Bushland & Hopkins, 1953; Lindquist, 1955; Knipling, 1988; Vargas-Terán *et al*., 2005). It has now been developed and used in both area-wide suppression and eradication programs for tsetse flies (*Glossina spp*.), onion root flies (*Delia antiqua*) and many species of fruit flies, such as the

Mediterranean fruit fly (*Ceratitis capitata*) and the melon fly (*Bactrocera curcubitae*), to name just a few (Klassen & Curtis, 2005).

Sterile Insect Technique and Leafminers

Studies have already been done on the development of SIT for the chrysanthemum leafminer, *Liriomyza trifolii* (Burgess), (Kaspi & Parrella, 2002; 2003; 2006) and the interaction between sterile male *L.trifolii* and *D.isaea* (Kaspi & Parrella, 2008), which is a parasitoid used for the biocontrol of both *L.trifolii* and *L.bryoniae.* A dose of 155 Gy gamma radiation can produce sterile male *L.trifolii* that also exhibit comparable competitiveness with wild males (Kaspi & Parrella, 2002; 2003; 2006). It has also been shown that there is synergistic interaction between sterile males and *D.isaea*, which when released simultaneously increases the overall control to a greater level than expected (Kaspi & Parrella, 2008).

Dose Optimisation and quality controls

The success of an area-wide integrated pest management (A-W IPM) program based on sterile insect technique (SIT) is dependent on the production of high quality, sterile male flies that can compete effectively with males of the pest population and mate successfully with the wild females (Calkins & Parker 2005). In the past when A-W SIT programs failed it was thought to be due to a lack of sterile males. These failures were countered by an increase in the release rate of sterile males. Only after complete failures in the control of the New World screwworm, *Cochliomya hominivorax* (Coquerel) however, was the quality of the sterile male flies examined (Klassen & Curtis 2005; Vargas-Terán *et al*. 2005). The quality control of mass-reared insects has emerged as an important discipline, especially in SIT, as a result of the investigations into these program failures (Calkins & Parker 2005). In the case of the New World screwworm the failures were resolved by developing a quality control system to monitor the production of sterile flies. Regular strain renewal was also introduced to increase the genetic variation in the mass-reared population (Calkins & Parker 2005). Most of the work on quality control in mass-rearing has been developed since 1976 with the first ideas being published on mass-rearing sterile fruit flies (Boller & Chambers 1977; Boller 2002). To date the most developed protocols for quality control of mass-reared insects is for fruit flies (FAO/IAEA/USDA 2003), although there are also quality control protocols for the mass-rearing of tsetse fly under development (FAO/IAEA 2006).

In order to achieve success in an AW-IPM SIT program, the males released need to be both sterile and of comparable quality to the pest population males (Cáceres *et al*. 2007). Exposure to radiation to induce sterility, however, also affects the fitness of the adult males

(Bakri *et al*. 2005a). There is a trade-off between sterility and male fitness (Robinson 2005). The relationship between radiation dose and the percentage of sterile males is asymptotic. The dose of radiation needed to achieve sterility in 100% of the mass-reared flies is such that the radiation has a deleterious effect on the fitness of the males. This effect is so great that the release of sterile males is ineffectual, as the mass-reared males will be unable to compete with wild males for mates, and so the expected population decrease is not achieved. In the West Indian Fruit fly, *Anastrepha obliqua* (Macquart), a dose of 25 Gy was able to achieve greater then 98.2% sterility. At doses above 25 Gy, whilst the percentage sterility increased to almost 100%, there were, however, significant reductions in the flight ability of the males and increased male mortality rates (Toledo *et al*. 2004). Sterile males that do not have comparable fitness to wild males will be outcompeted by the wild males for copulations with females (Toledo *et al*. 2004).

The trade-off between sterility and male fitness required is dependent on what the AW-IPM program is trying to achieve: complete eradication or population suppression. The trade-off and whether the aim of the program is suppression or eradication have important economic consequences as total eradication is more costly than suppression (Mumford 2005). The trade-off between sterility and male fitness therefore has an important role in balancing the efficiency and cost of an SIT program (Parker & Mehta, 2007). Males that have a high level of sterility but do not have comparable fitness to the pest population males require higher release rates to achieve population suppression than high quality competitive sterile males and are therefore more expensive to produce. In the development of SIT for the serpentine leafminer *Liriomyza trifolii* (Burgess) sterility was defined as the development of less than 0.7 mines per female (Kaspi & Parrella 2003). This is the definition of sterility used in this research. Dose optimisation is therefore important to ensure sterility is induced but with a dose that minimises damage to the somatic cells of the developing pupa (Bakri *et al*. 2005a).

The production of high quality sterile males is the key to a successful SIT program as the performance of the sterile males released is the end product (IAEA 1992). The end users of the SIT product want to be sure that they have a high quality, efficient, consistent product so that there are no failures in their AW-IPM program. As such, the production of high quality mass-reared males is of great importance. To achieve this in mass-reared fruit fly production quality control protocols have been developed (FAO/IAEA/USDA 2003). Quality control management systems provide feedback on the mass-rearing process and allow the identification of problems and the instigation of improvements (Barnes *et al*. 2007). These protocols can be divided into production quality control, process quality control and product quality control (Calkins & Parker 2005). Process quality control looks at how steps in the production process are carried out (Calkins & Parker 2005). How plants are infested, pupae are collected and how they are irradiated are important processes for which quality control protocols are developed (Parker 2005).

To establish an optimum sterilising radiation dose the ability of irradiated male and female adult *L.bryoniae* to mate and produce viable offspring needs to be examined. The aim of this is to produce both male and female adults that after irradiation are unable to produce viable offspring. Whilst ideally the releases of sterile flies should be male only, it is important to produce sterile females too as the females are capable of the damaging effects of the leafminers: puncturing and laying eggs that develop into the leaf-mining larvae. Releasing extra non-sterile female leafminers would have a significant impact on the success of the control program and would require high release rates of sterile males to counteract this, which in turn would be expensive.

Studying the percentage emergence of adults from irradiated pupae will see if the irradiation process has any adverse effects on pupal development and adult eclosion. Similarly the sex ratio of the emerging adults will also be analysed to see if irradiation has greater detrimental effects one sex more than the other. The effect of the irradiation process on the fitness of the males is also to be compared to wild, non-irradiated male *L.bryoniae.* To assess this, the longevity of males under environmental stress (lack of food and water) and the flight ability will be compared for irradiated and non-irradiated individuals. Sterile males should be able to live a comparable length of time as wild males so that the area can remain over-flooded with sterile males. This is particularly important in *L.*bryoniae as the females have multiple matings. The flight ability is also a good indicator of male competiveness as irradiated males need to be able to fly well in order to seek out wild females to mate with. The methods used in this project have been adapted from the quality control methods developed for Tephritid fruit fly production, tsetse fly production and from work on developing SIT for the chrysanthemum leafminer *L.trifolii* (FAO/IAEA/USDA 2003; FAO/IAEA 2006; Kaspi & Parrella 2003, 2006, 2008).

The aim of the work so far has been to establish the optimum dose of gamma radiation in order to produce high quality sterile males. The first study will examine the effect of different radiation doses on the sterility of both male and female leafminers. This is to determine a dose that can induce a sufficient level of sterility, defined as less than 0.7 mines per female (Kaspi & Parrella 2003).

Materials and methods

Population Establishment

Liriomyza bryoniae pupae were collected from commercial glasshouses with naturally occurring infestations and reared on tomato plants, *Lycopersicum esculentum* cv. Moneymaker, grown from seed under glasshouse conditions. Four to six week old tomato plants were exposed to adult *L.bryoniae* inside a cage (63x51x63) at 20±5ºC, 70±5% relative humidity (RH) and 16L:8D photoperiod. The cage also had a Petri dish streaked with diluted honey (30% honey solution) as a sugar source for the adults. Female adult *L.bryoniae* puncture the leaves for feeding and oviposition. Adult males feed from the pits punctured by the females. After 48 hours infested plants were removed from the cage and returned to the glasshouse. The leafminers develop from egg, through larval stages, to the pupa under greenhouse conditions (approximately 20±5ºC), taking approximately 10-12 days. When the leafminers were in the third larval instar and just about to pupate, the tomato plants were cut close to the soil level and hung upside-down over a funnel constructed out of grease-proof paper. The pupae dropped off leaves into a funnel and were collected in a pot. The pupae were incubated in Petri dishes (9 cm) on damp filter paper under controlled conditions (20±5ºC, 70±5% RH and 16L:8D). After 10 days of pupation, the Petri dishes were placed in the cage so that the adult flies emerged from pupation directly into the cage.

Dose Optimisation

Liriomyza bryoniae were reared as described above. The pupae collected were irradiated after 8 to 10 days of pupation using a gamma (γ) radiation emitting Caesium-137 source – a Gammacell 3000 Elan (MDS Nordion, Ottawa, Canada) with a dose of 150 Gy or 160 Gy. Half of the pupae were not irradiated as a control group. The control and irradiated Petri dishes (9 cm) were allocated at random. Emerging adults were removed individually and sexed within a few hours of eclosion to ensure virginity of female adults. 10 virgin males and 10 virgin females released into a plexiglass and insect netting cage (20x20x20 cm) containing a four-week old tomato plant. Each cage had one of the following combinations:

10 irradiated males (IM) x 10 irradiated females (IF)
10 irradiated males (IM) x 10 control females (CF)
10 control males (CM) x 10 irradiated females (IF)
10 control males (CM) x 10 control females (CF)

This will be replicated 14 times in total. Seven replicates of 160 Gy and seven replicates of 150 Gy. So far there have been 4 replicates of 160 Gy and 2 of 150 Gy. After 20 days the number of developing mines and the number of pupae produced per cage were counted. All analyses were computed using R (www.r-project.org). The proportion of the plant's leaves that are undamaged (D), punctured (P) and punctured with mines (M) was recorded. Leaf damage was catergorised into leaves that were Damaged (P+M) and Undamaged (D). The results were also grouped as Low economic damage (D+P) and High economic damage (M). Both of these result groupings were analysed using a Tukey's Honest Significant Difference test. [Once the full data set has been collected then this will be analysed as a Generalised Linear Mixed Effects Model (glimm) as an lmer with binomial errors. Currently there are too few data points to make a valid model.]

The data on the number of mines and pupae per female were analysed using a mixed effects model (lmer) with poisson errors, as an analysis of deviance (Appendix 1). Maximal models were fitted and non-significant terms with the least influence on model likelihood were deleted sequentially to achieve an optimal model (Crawley 2007).

Results of Dose Optimisation

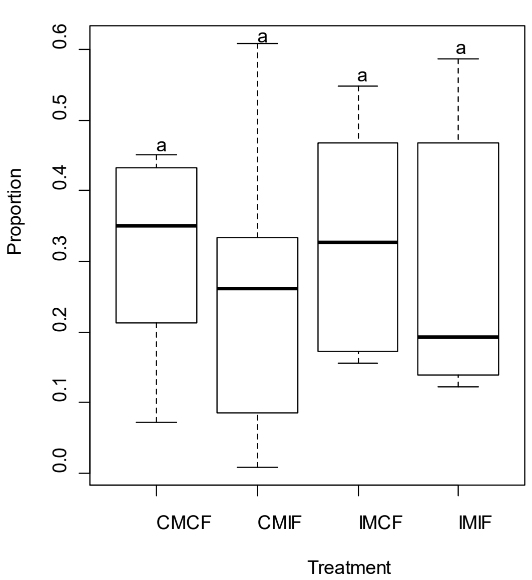

Figure 1: Proportion of leaves with damaged levels (P+M). Boxes labelled a are not significantly different (P>0.05) to each other as analysed by Tukey's Honest Significant Difference.

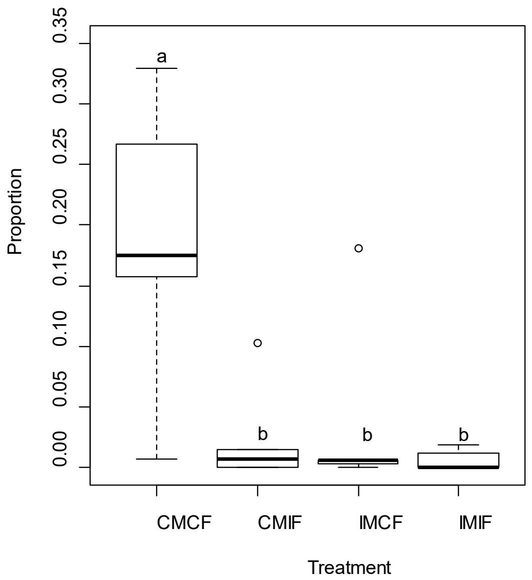

Figure 2: Proportion of leaves with economic damage levels (M). Boxes labeled a and b are significantly different (P<0.05) to each other as analysed by Tukey's Honest Significant Difference.

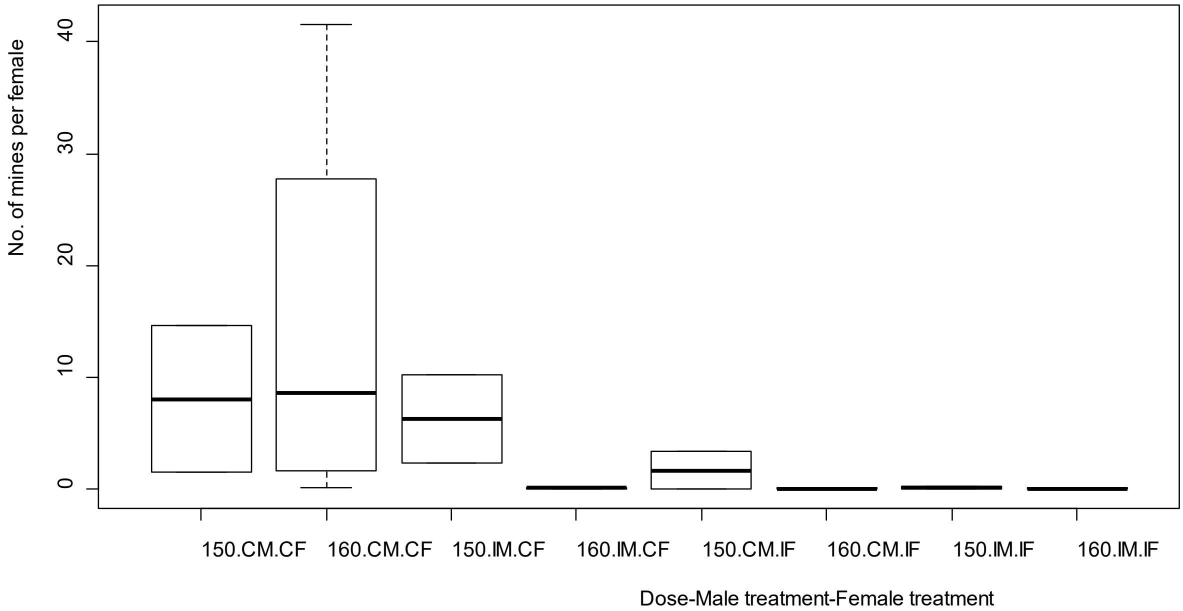

Figure 3: Boxplot of the number of mines that develop per female for different treatments CM: Control males; IM: Irradiated males; CF: Control females; IF: Irradiated females

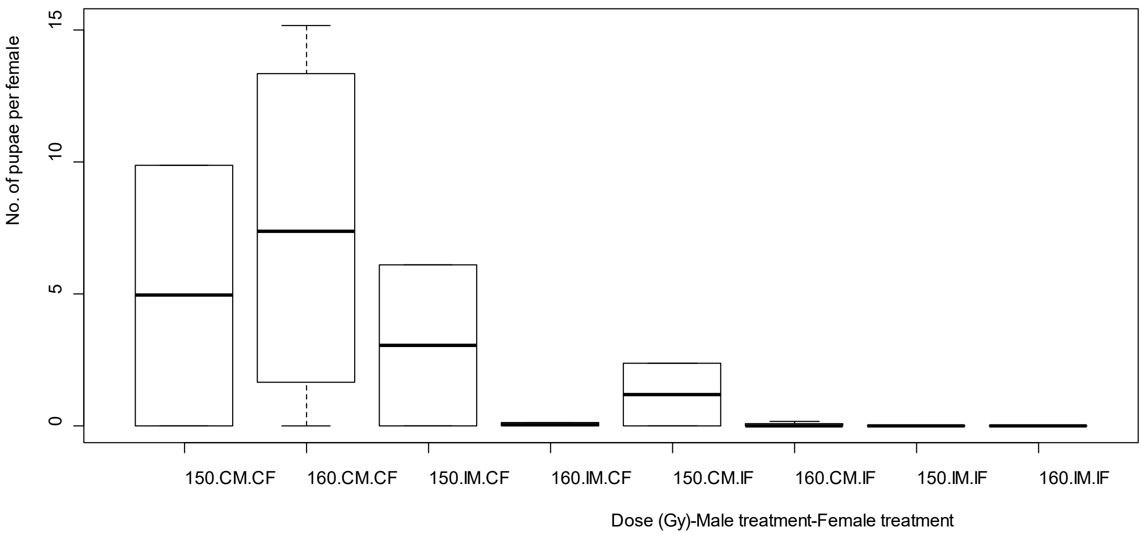

Figure 4: Boxplot of the number of pupae produced per female for different treatments CM: Control males; IM: Irradiated males; CF: Control females; IF: Irradiated females

The proportion of leaves that had leafminer damage i.e. leaf puncturing and mines compared with leaves with no damage (Figure 1) was non-significant between the different treatments at (P>0.05). When the proportion of the leaves with high damage levels were compared

(Figure 2) there was a significant difference between the control cross (CMCF) and the crosses with irradiated individuals ($P<0.05$) (CMIF, IMCF, IMCF).

When the number of mines per female for the different radiation doses and the different treatment crosses (Figure 3) were examined, the irradiated females had a significant effect on the minimal adequate model ($P<0.001$). The irradiated males and the dose did not have an effect on the model individually but were found to have a significant interaction effect ($P<0.005$) (Appendix 1). The number of pupae produced per female (Figure 4) was also affected by the females ($P<0.001$) and by the interaction between the dose and the males ($P<0.05$) (Appendix 1).

Discussion

There was no significant difference ($P>0.05$) between the different treatment groups when the proportion of leaves with leafminer damage was compared (Figure 1). This shows that there is comparable leaf puncturing by control females that have mated with irradiated males. This is a good indication that the control females are unable to select against mating with irradiated males and are still puncturing the leaves and laying eggs, as in the control crosses. This is also the same for irradiated females that have been crossed with the control males. With the same number of leaves being punctured for each of the four treatments this is in line with my previous findings that irradiated females and females mated with irradiated males puncture leaves a similar amount.

When the leaves were examined in terms of economic damage there is a different result (Figure 2). The leaves that have both punctures and mines on them are classed as high damage leaves and those with only punctures and with no damage are classed together as low damage. The proportion of leaves with high, economic damage is significantly greater ($P<0.05$) in the control cross (CMCF) than for the crosses where at least one of the sexes was irradiated.

The irradiated females and the interaction between the dose and the irradiated males both have a significant effect on the number of mines produced per female. Fewer mines were produced in crosses with at least one irradiated sex compared to a higher number of mines in the control groups (Figure 3). The number of pupae produced per female (Figure 4) exhibit the same results as for the mines per female; although there are fewer pupae than mines. This is logical as the pupae emerge from the mines and where there are no mines on a plant there will be no pupae.

There are no mines produced at 160 Gy in crosses between irradiated males and control females (IMCF), there is a significant interaction between the dose and the males ($P<0.005$). This shows that sterility can be induced by irradiation of the pupae with a dose of 160 Gy. Fig. 3 also indicates that there is a higher level of sterility achieved with a dose of 160 Gy than for 150 Gy. This is true for both irradiated male and irradiated females (IMCF and CMIF).

Irradiated females also have induced sterility from irradiation (Figure 3) and the presence of irradiated females has a significant effect on the number of mines and pupae produced ($P<0.001$). With the exception of the cross with control males (CMIF) at 150 Gy, there are no mines produced by irradiated females. This means that if irradiated females are released, whether knowingly or by accident, they will not be producing offspring for the next generation of the pest population. SIT programs that use male-only releases are more efficient in terms of the rate of pest suppression and in terms of the numbers of males needed per release. As a result of this male-only release programs are more cost effective (Mumford 2005). In the case of *L.bryoniae*, however, if it is not possible to produce male-only releases, as it is at present, then any females released will be sterile also and will have a less deleterious effect on the efficacy of the SIT program.

Conclusions

Dose optimisation results so far show that sterility can be induced by the irradiation of pupae. Both male and female adults are unable to produce viable offspring after irradiation. Similar numbers of leaves with puncture damage indicate, however, that females (both control and irradiated) puncture the leaves a similar amount suggesting that they still oviposit the same amount even if the eggs are unable to develop past the embryonic stage. The initial results also suggest that a dose of 160 Gy has a greater sterilising effect than a 150 Gy; but the dose is part of an interaction with the irradiated male flies. Further studies to examine the fitness of the irradiated males are needed to produce males with a relatively high level of sterility (less than 0.7 mines per female) but that have a comparable fitness to wild males.

Technology transfer

None to date

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Appendices

Appendix 1: Summary of optimal mixed effects model output for mines and pupae

Variable	Optimal model terms	*n*	z	P	Terms deleted from full model
Mines per female	Intercept	24	3.14	<0.005	Males x Females
	Dose		-0.49	0.62	Dose x Males x Females
	Males		-1.15	0.25	
	Females		-3.71	<0.001	
	Dose x Males		-3.11	<0.005	
	Dose x Females		-1.79	0.07	
Pupae per female	Intercept	24	0.84	0.40	Dose x Females
	Dose		0.31	0.75	Males x Females
	Males		-1.41	0.16	Dose x Males x Females
	Females		-4.51	<0.001	
	Dose x Males		-1.96	<0.05	