



**The application of sterile
insect technique against
the tomato leafminer
*Liriomyza bryoniae***

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Abstract

The tomato leafminer *Liriomyza bryoniae* (Kaltenbach) (Diptera: Agromyzidae) is an economically important pest of greenhouse tomato crops in the UK, which at high infestations can reduce the value of the crop by up to 20% . Sterile insect technique, SIT, is the release of sterile insects to overflood and mate with the pest population. Wild females that mate with sterile males lay eggs that contain dominant lethal mutations and are unable to develop into larvae. SIT has been proposed as a novel, alternative method of *L.bryoniae* pest suppression that could be used in conjunction with the current biological controls, such as *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae). This thesis presents information on the development of SIT against *L.bryoniae* and examines the feasibility and compatibility with existing pest management methods. Work was carried out that determined high quality, sterile adult *L.bryoniae* were produced when late stage pupae were irradiated with a dose of 160 Gy gamma radiation. Both male and female *L.bryoniae* were sterile after irradiation with 160 Gy, which is important given the current inability to separate pupae by sex. A multi-treatment cage experiment was used to compare bi-sex and male-only releases and showed that the release of sterile females did not have a detrimental effect and did not add to the pest problem. The multi-treatment cage experiment also compared the use of *D.isaea* with sterile male releases both separately and concurrently. Whilst the study conditions favoured the optimal environment for *D.isaea* oviposition and development, the concurrent release of sterile male *L.bryoniae* and *D.isaea* were compatible. SIT is a suitable method for *L.bryoniae* suppression; but further work to develop a more time and cost-efficient mass-rearing technique and greater knowledge of the market are required in order for it to become a financially viable pest management option. Overall, the irradiation of *L.bryoniae* pupae with 160 Gy produced sterile adults that have comparable fitness to wild-type adults, do not produce viable offspring and have the potential to suppress a *L.bryoniae* infestation.

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Declaration

I herewith certify that all material in this dissertation which is not my own work has been properly acknowledged:

Chapter 5: The questionnaire was distributed by and returned to Becky Turner, HDC Research Manager, and Louise Arculus, HDC Communications Administrator.

Signed

Catherine Sophie Walker

Date

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1. Introduction

1.1 Problem statement

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, releases of the parasitoid wasps *Diglyphus isaea* and *Dacnusa sibirica* are the main methods of leafminer control in an organic greenhouse. This can sometimes be 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). In conventional tomato crops, non-specific insecticides such as spinosad are often only applied as a last resort to avoid disruption to biological control agents maintaining low numbers of other pest species. Releases of *D.isaea* and *D.sibirica* are started after *L.bryoniae* are found at one mine per plant (Jacobson pers. comm); however, this means the parasitoid populations lag behind the leafminer population. Even when the parasitoid 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.

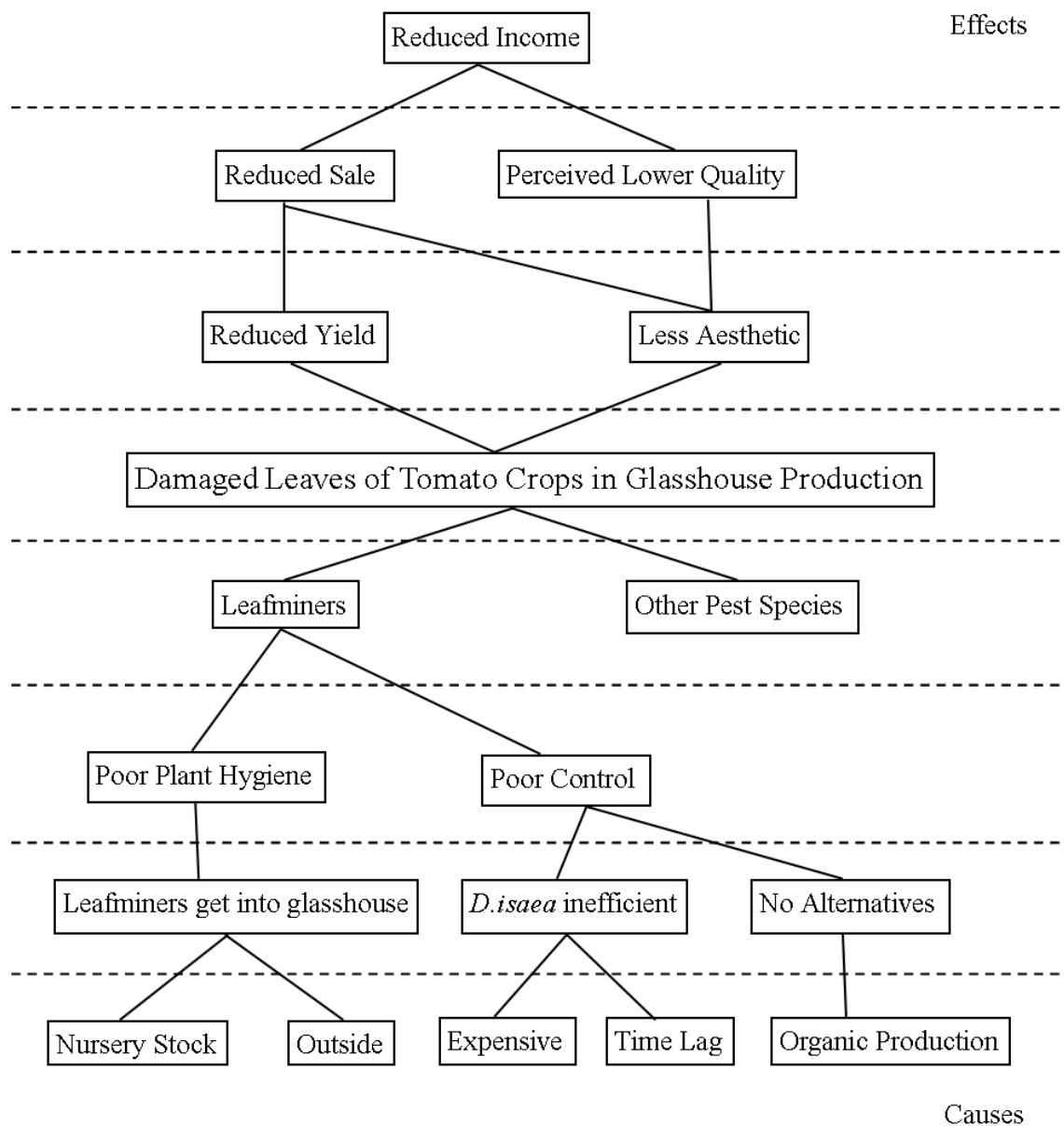


Figure 1.1: The causes and effects of damage to the leaves of tomato crops by *Liriomyza bryoniae*.

1.2 Biology of the tomato leafminer

The tomato leafminer, *Liriomyza bryoniae* (Kaltenbach) (Diptera: Agromyzidae), is a highly polyphagous species of leafminer (Spencer 1973). In the UK *L. bryoniae* is an important economic pest of greenhouse tomato and herb production.

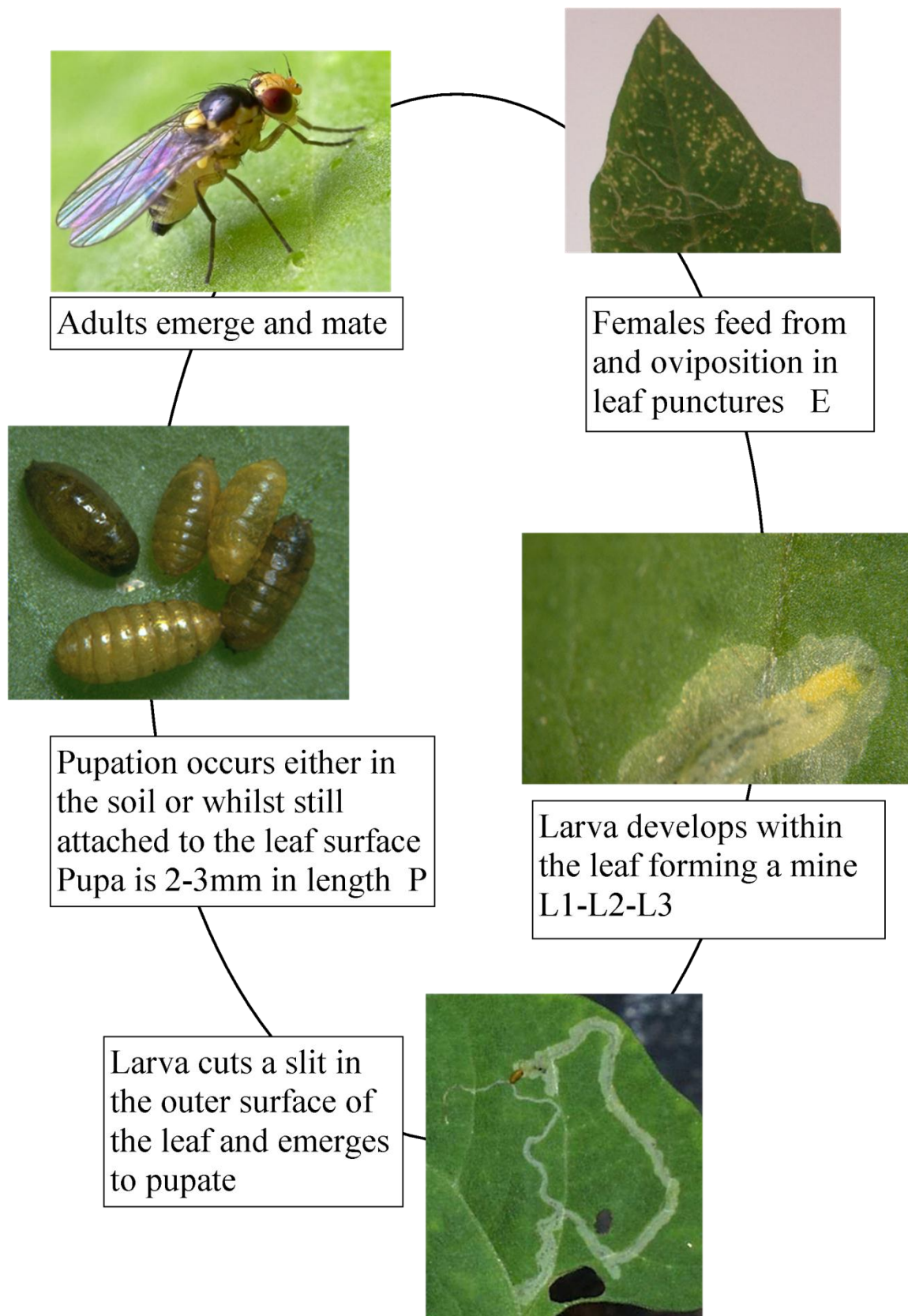


Figure 1.2: Lifecycle of the tomato leafminer *Liriomyza bryoniae*.

On average, adult females oviposit 163 eggs per female on tomatoes at 20°C during a mean lifespan of 6.6 to 9.0 days (Tokumaru & Abe 2003). Females puncture leaves with their ovipositor and then reverse back over the puncture marks to feed from the plant exudates (figure 1.2). In a closely related species *Liriomyza trifolii* (Burgess), the serpentine leafminer, females make two different types of leaf punctures: large, fan-shaped punctures and tubular punctures (Benthke & Parrella 1985). The females feed from both type of leaf puncture; but they only oviposit in the tubular punctures. It is also thought that *L. trifolii* does not deposit epideictic pheromones to mark the position of oviposition. The females often puncture the leaf in clusters using the ovipositor to test for host plant suitability and quality (Benthke & Parrella 1985). *Liriomyza trifolii* has been observed to puncture the leaves of non-host species; but they do not oviposit in these punctures (Benthke & Parrella 1985). A detailed study into the feeding and ovipositional behaviour is lacking for *L. bryoniae*. However, it can be assumed that female *L. bryoniae* exhibit similar puncturing, feeding and oviposition strategies to *L. trifolii*, at least to the extent that the females feed from all of the puncture marks and oviposit in a proportion of these punctures.

A few days after oviposition, the beginning of the larval mines can be observed. The larva that emerges from the egg feeds rapidly on the mesophyll cells of the leaf. As they eat they form a distinctive irregular mine between the upper and lower leaf surfaces where the larva has eaten the photosynthetic leaf cells (Spencer 1973). The leaf mine widens as the growing larva develops through three larval stages (L1, L2, L3,) (figure 1.2). Before pupation the larva cuts a slit in the leaf surface so that the pupa forms on the outside of the leaf surface. Quite often the pupa is found on the underside of leaves; but not exclusively as pupae can also be found on the upper leaf surfaces. The mechanism for this is currently unknown. During pupation some pupae become detached from the leaf surface and fall into the soil beneath the host plant. Some pupae remain attached to the leaf surfaces and a few fail to emerge from the slit in the leaf surface before they pupate.

The development time of *L. bryoniae* from oviposition to adult emergence when reared on kidney bean plants, *Phaseolus vulgaris*, at 20°C is 25.5 ± 1.3 days (Tokumaru & Abe 2003). This development time is temperature dependent; decreasing with increased temperature; and increasing with decreased temperature (table 1.1). The lethal temperature, which

inhibits development, is 35 °C in both *L.trifolii* and *L.sativae*, the pea leafminer (Tokumaru & Abe 2003). There are no data on the lethal development temperature of *L.bryoniae*; however, personal observations would suggest that the lethal temperature is somewhere between 38 and 40°C.

Table 1.1: The effect of temperature and photoperiod on the development of *L.bryoniae*

Temperature (°C)	Photoperiod (L:D)	Host Plant	Developmental period (day)	Reference
18	15:9	<i>Phaseolus vulgaris</i>	29.9 ± 1.9	a
20	15:9	<i>Phaseolus vulgaris</i>	25.5 ± 1.3	a
25	15:9	<i>Phaseolus vulgaris</i>	19.3 ± 0.8	a
25		<i>Lycopersicum esculentum</i>	17.1	b
35	15:9	<i>Phaseolus vulgaris</i>	No development	a

a: Tokumaru & Abe (2003); b: Minkenberg & Helderma (1990).

1.3 Economic importance of the tomato leafminer

The tomato leafminer, *L.bryoniae*, is an economically important pest of commercial tomato crops in greenhouses in the UK. At high infestation densities, the area of the photosynthetic material lost from the leaves greatly reduces the plant's ability to photosynthesize (Parrella *et al.* 1985). A reduction in photosynthesis has a knock-on effect and reduces the yield and the quality of the tomatoes produced (Ledieu & Helyer 1985). High *L.bryoniae* infestations can lead to a 17% reduction in yield, a reduction in the quality of the tomatoes produced and consequently a loss of 20% of the monetary value of the crop (Ledieu & Helyer 1985). The presence of leafminer damage, both mines and puncture marks, is aesthetically damaging. Tomato buyers that notice this leaf damage, even when the quality of the tomato is unaffected, may be less willing to pay as high a price as for an undamaged crop (Jacobson pers. comm). The tomato production industry in the UK is currently valued at £175 million per annum with an estimated 75,000 tonnes of tomatoes produced by UK growers in 2011 (Tomato Growers' Association, no date).

In the greenhouse herb industry basil, thyme and mint are economically affected by the presence of *L.bryoniae* (Horticultural Development Company, no date). Parsley and coriander on the other hand are not susceptible to leafminer damage. The punctures and mines on the leaves cause the most damage as the leaves are the harvestable part in herb production. Herb plants with damage to the leaves cannot be sold (Horticultural Development Company, no date).

1.4 Conventional and organic production

In conventional tomato production, plants are grown in rockwool and fertilisers are added via the irrigation system. Organic tomatoes are grown in the soil and gain all of their nutrients directly from the soil as defined by the Soil Association (2011). Organic farmers are limited in the resources available to control pest and weed infestations as they cannot apply chemical fertilisers and pesticides (Soil Association 2011). Organic tomato production in the UK in 2011 accounted for 5.2 % of the production area, which is approximately 9.6 ha (Source: Tomato Growers' Association Statistics). The increased amount of organic tomato production has been driven by higher prices for organic produce. The majority of the tomatoes produced in the UK, however, are still grown conventionally with a production area of approximately 178 ha. There has, however, been a shift away from the use of chemical pesticides to control pest outbreaks even in conventionally grown tomato crops. Many growers use biological controls, or biocontrols, as part of an integrated crop management scheme to achieve pest free crops (British Tomato Growers' Association, no date). The inundative release of predatory mites and parasitoid wasps are used to control insect pests and managed hives of bumblebees are used to pollinate crops.

Driving forces for change in integrated pest management (IPM) are the EC Directive 91/414/EEC (European Parliament 1991) and the EC Regulation No. 396/2005 (European Parliament 2005). These regulations have reduced the number of pesticides available to growers by removing those that were considered too great a risk or too hazardous to humans, animals and the environment. Maximum Residue Levels (MRLs) were introduced under EC Regulation No. 396/2005, which was updated from EC Directive 91/414 (European Parliament 1991, 2005). MRLs are the highest level of pesticide that is acceptable to be found in food when pesticides are applied correctly. This is to reduce risks for consumers.

Some supermarkets, as major buyers of tomato crops, have also set even more stringent MRLs for the products that they sell. These factors have encouraged growers to become less reliant on chemical pesticides and to use more biocontrols for pest management (Jacobson 2008). In herb production, MRLs vary between herb species and this is an important factor in the introduction of biocontrol based IPM (Horticultural Development Council, no date).

Pesticide resistance occurs in some pest species of both tomato and herb pests. Switching to a biocontrol based IPM program, with the occasional use of chemical pesticides to treat problem infestations, minimizes the risk of developing pesticide resistance (Horticultural Development Company, no date).

1.5 Development of biological controls in protected crops

Encarsia formosa Gahan was the first parasitoid wasp commercially available for the pest management of the glasshouse whitefly *Trialeurodes vaporariorum* (Westwood) (Helyer *et al.* 2003, Copping 2009). It was used in commercial greenhouses in the late 1920s and 1930s. The invention of broad spectrum organochlorine pesticides, such as dichlorodiphenyltrichloroethane (DDT), after World War II and synthetic pyrethroids during the 1970s slowed the development and uptake of biocontrols in greenhouse pest management (Helyer *et al.* 2003). The emergence of pesticide resistance in pest species and the subsequent importance of pests that were previously considered minor led to the re-examination of predators and parasitoids for pest management. The development of biocontrols is less costly and less difficult than the development of selective pesticides (Helyer *et al.* 2003). Biocontrol agents do not have the same environmental concerns that are associated with organochlorine pesticides, nor can resistance be developed against biocontrols. As such biocontrols have been developed for a number of species and are now widely used in protected crops to control pest infestations as part of an integrated crop management (ICM) strategy.

1.6 Current IPM compatible leafminer control methods

1.6.1 *Diglyphus isaea*

Diglyphus isaea (Walker), is a parasitoid wasp (Hymenoptera: Eulophidae) which is a biological control that is inundatively released to control *L.bryoniae* infestations in protected

crops. *D.isaea* is a polyphagous parasitoid; but it is mainly a parasitoid of agromyzid leafminers. Commercially it is released to control *L.bryoniae*, *L.trifolii* and *L.huidobrensis* (Copping 2009). The parasitoid stings and paralyses the late instar (L3) leafminer larva and lays an egg on to the outside of the leafminer larva. The parasitoid develops attached to and feeding off of the paralysed leafminer larva. Releases in tomato greenhouses in Japan found that *D.isaea* can provide up to 95.1% parasitism in *L.trifolii* under experimental conditions (Ozawa *et al.* 1999). Releases to control *L.bryoniae* in commercial greenhouse were initially less successful as populations of *D.isaea* were slow to establish (Sampson & Walker 1998). Action thresholds of one new mine per week were set for the release of *D.isaea*, which meant that populations established faster and that it was a more reliable control method (Sampson & Walker 1998). The use of *D.isaea*, however, is still unable to completely control *L.bryoniae* infestations. The population of established *D.isaea* lags behind the leafminer population and is unable to suppress the pest population to a level that is satisfactory for growers (Jacobson pers. comm). During the early tomato growing season low temperatures, of around 15°C, and low pest densities are unfavourable for *D.isaea* population establishment (Barclay 1987, Minkenberg 1989, Bazzocchi *et al.* 2003). Parasitoids, such as *D.isaea*, are more effective at pest population reduction at higher densities (Barclay 1987). *D.isaea* also is more effective at higher temperatures (Minkenberg 1989). The development time at 25 °C is 9.8 days, more than twice the rate than at 15 °C when the development time is 25.5 days (Minkenberg 1989). By the time in the growing season when the average temperature favours optimal *D.isaea* development (25 – 30 °C) the *L.bryoniae* population is already well established and damage is above economic thresholds. The cost of control using *D.isaea* is expensive for growers. Control requires a high release density at a rate of 1200 to 2400 parasitoids per hectare to achieve population suppression (Copping 2009), which can cost up to £3000 per hectare annually.

1.6.2 *Dacnusa sibirica*

Dacnusa sibirica (Telenga) (Hymenoptera: Brachonidae) is a solitary koinobiont parasitoid wasp currently used as a biocontrol against *L.bryoniae* and *L.trifolii* (Abe *et al.* 2005, Copping 2009). Females lay an individual egg into the leafminer larva. Females can determine whether a leafminer larva is already parasitised and choose non-parasitised larvae to oviposit in (Copping 2009). Female *D.sibirica* can manipulate the sex ratio of their offspring

depending on the age of the host larva, as a measure of host quality (Croft & Copland 1995). As the age of the host increases the sex ratio becomes female biased as the development time of females is less than for males (Croft & Copland 1995). The *D.sibirica* larva develops inside the larva until the leafminer pupates at which point the host is killed. The adult *D.sibirica* emerges from the leafminer puparium instead of an adult leafminer (Copping 2009). *D.sibirica* is used as a pest management method in greenhouse tomatoes from early on in the growing season until July. This is when the temperature is between 15 and 20°C the optimum for *D.sibirica* development (Minkenberg 1990, Copping 2009). Above 20°C the rate of oviposition, fecundity and longevity decreases in *D.sibirica* (Minkenberg 1990). *D.sibirica* are released at a rate of 1200 to 5000 parasitoids per hectare, or one parasitoid to ten leafminers, for inundative biocontrol pest management in greenhouses (Copping 2009).

1.6.3 Spinosad

Spinosad is a non-specific insecticide used to control leafminers, caterpillars, thrips and foliage feeding beetles (Copping 2009). Spinosad is made up of two compounds: Spinosyn A and Spinosyn D, which are derived from the secondary metabolites of *Saccharopolyspora spinosa* (Mertz & Yoa) an Actinomycete found in the soil (Copping 2009). Spinosad affects the nicotinic acetylcholine and GABA receptors in the target insects. It is non-toxic to predatory insects; but it is toxic to honeybees and other pollinators directly after application when it is still wet (Copping 2009).

1.6.4 Eradicoat

Eradicoat and Eradicoat T are physically acting insecticides that block the spiracles of the adult leafminers (Root *et al.* 2008). When applied to the upper section of tomato crops it prevents adult oviposition in these newest leaves. Based on the plant starch Maltodextrin both products have been cleared for use on organic crops. They have a zero day harvest interval, which means that crops can be harvested on the same day as application. Eradicoat can be used alongside biocontrol agents as a leafminer control method in an IPM program as it does not have any residual toxicity to affect the biocontrols (Root *et al.* 2008). The physical nature of the insecticide, however, means that any biocontrol agents that come into direct contact with the spray will also suffocate and die as it is non-specific. This will have a negative effect on the dynamics of the IPM programs used to control other pests of

the tomato crop, such as the predatory mite *Phytoseiulus persimilis* (Athias-Henriot) used to control the greenhouse spidermite *Tetranychus urticae* (Koch). It is therefore only possible to use the Eradicoat on the top part of a crop and so it can only be used to prevent leafminer oviposition in the youngest tomato leaves.

1.7 Sterile insect technique

Sterile insect technique, or SIT, is the release of a large number of sterile males into a wild population of the target species to over-flood the area with sterile males. Females of the wild population that mate with the sterile males fail to produce viable offspring and in the next generation the population size is reduced. Successive releases of sterile males over a sustained period will lead to a reduction in the pest population size with eradication possible (Knipling 1955). SIT is also inversely-density dependent. As the pest population reduces in size, successive releases of the same number of sterile males have a greater effect on the pest because the ratio of sterile to fertile males increases. This makes SIT an effective control method, even at low population densities (Knipling 1955).

The concept of SIT was independently conceived by three individuals. Serebrovskii's work, based in the USSR, inducing sterility through the development of homozygotes with chromosomal translocations (Klassen & Curtis 2005) was, however, unable to be completed due to World War II. Vanderplank and his colleagues in East Africa were able to induce sterility through the crossing of tsetse fly species, *Glossina* spp., and the development of sterile hybrids (Vanderplank 1944). Vanderplank suggested that these sterile hybrids could be used to control tsetse fly populations.

In the 1950s Knipling came upon the work of Muller (1927) who had shown that exposure of *Drosophila melanogaster* to ionizing radiation induced dominant lethal mutations in their eggs. Knipling (1955) and his colleagues developed this further and used ionizing radiation to produce sterile males of the New World screwworm, *Cochliomyia hominivorax* (Coquerel). The mass-production and release of sterile New World screwworms is the first example of a successful area-wide integrated pest management (AW-IPM) program using SIT and has eradicated this economically important pest of livestock from the Southern United States, down through Central America to the Panama Canal (Klassen & Curtis 2005).

The use of X-rays and chemical mutagens were also evaluated to sterilize insects. The use of chemosterilants, however, was considered too high risk because of the issues with toxicity and residues (Breland *et al.* 1974); and so radiation has been chosen in most of the successful AW-IPM SIT programs to date. Recently the use of X-ray irradiators has been re-investigated for use in SIT programs (Mastrangelo *et al.* 2010, Mehta & Parker 2011). The fear of terrorism and the production of a 'dirty bomb' using radioactive materials have led to increased restrictions on the international transportation of radioactive isotopes (IAEA 2007, Mastrangelo *et al.* 2010, Mehta & Parker 2011). The new, self-shielded X-ray irradiators that have been developed are powered by electricity and only produce X-rays when switched on and as such they require less shielding than for gamma irradiators (IAEA 2007). As they do not contain any radioactive isotopes there are no transboundary shipment restrictions and cost much less, only US \$5000, to transport rather than the US \$50,000 required to transport a gamma irradiator (IAEA 2007). Studies on two species of Tephritid fruit flies *Ceratitis capitata* (Wiedemann) and *Anastrepha fraterculus* (Wiedemann) have been used to determine the dose of X-rays required to produce 99% sterility in comparison to gamma radiation doses (Mastrangelo *et al.* 2010).

1.8 History of sterile insect technique

1.8.1 New World screwworm

Knipling (1955) identified the myiasis-causing New World screwworm as a candidate species for SIT for a few key reasons. Firstly it was an economically important pest that was very damaging to livestock. The adult females oviposit in wounds on livestock and the larvae that emerge burrow into the flesh of the animal making the wound larger and preventing it from healing up. This encourages more females to lay eggs in the enlarged wound. The screwworm causes a large amount of suffering to infested animals and is hard to prevent, thus it is a significant pest for livestock producers in areas where it is present.

Secondly, whilst males mate multiple times, females only mate once. This means that the females need to mate with only one sterile male for the control method to have an effect; whereas the sterile males can mate with more than one female so fewer males are needed to over-flood the area.

Lastly, the population of New World screwworm was naturally controlled each winter. The colder temperatures limited the range of the populations to the North and reduced the population size. This meant that releases after the winter would require fewer sterile males to eradicate the already decreased population. Knipling realised that SIT was a numbers game and developed mathematical models to assess the effect of sterile male releases on the screwworm populations (Knipling 1955, Abbot 1925).

Irradiation of screwworm pupae with 25 Gy of gamma radiation two to three days prior to emergence produced a high proportion of sterile males (Bushland & Hopkins 1953). Field trials of releases of sterile screwworms on the islands of Sanibel, off the West coast of Florida, and Curacao, off the coast of Venezuela, proved successful and the method was thought to be promising for the eradication of New World screwworm from the Southern states of the United States (Baumhover *et al.* 1955, Lindquist 1955). Full scale releases of sterile males started in Florida and the New World screwworm was eradicated in sequential stages across the Southern United States. A barrier of sterile male releases was maintained at each stage to prevent immigration from screwworm infested areas (Klassen & Curtis 2005).

The sterile males for this SIT AW-IPM program were mass-reared on an artificial diet composed of ground meat (Melvin & Bushland 1936). This was the first time that an obligate parasite, like the screwworm, had been reared on an artificial medium. Now the artificial medium is a more complex composition of bovine blood, inedible egg, milk substitute, water and either a polyacrylate gelling agent, such as Aquatain, or a cellulose fibre (Taylor & Mangan 1987, Chaudhury & Skoda 2007).

For the eradication program in Florida the sterile males were mass-reared at research facilities in Florida with 60 million flies released each week (Klassen & Curtis 2005). When the eradication program spread southwards into Mexico a large screwworm release zone acting as a barrier was maintained to prevent immigration and a new factory was built to assist the already existing mass-rearing facility in Texas. The mass-rearing facility in Mexico was able to produce 500 million flies per week (Klassen & Curtis 2005).

Since the eradication of the New World screwworm from the southern United States, the program has been extended down through Mexico in 1974, Belize, Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica and finally Panama, which was declared screwworm free in 2001. There is now a permanent sterile screwworm barrier maintained in Panama at the border with Colombia (Klassen & Curtis 2005, Vargas-Terán *et al.* 2005).

1.8.2 Onion root fly

The onion root fly *Delia antiqua* (Meigen) is a severe pest of onion crops across The Netherlands (Loosjes 2000). The larvae of the onion root fly feed and damage the bulbs of the onion plants. Sterile insect technique has been developed and sterile males are available commercially from a private firm de Groene Vlieg. Onion root flies are mass-reared by de Groene Vlieg and are sent to Belgium for irradiation before being released. This SIT program is not part of an AW-IPM and growers buy SIT on an individual farm basis. The Dutch Government provided financial backing for the set-up and first two years but now the program is solely run by de Groene Vlieg.

Flies are released by hand from small cages containing up to 40,000 sterile males. The majority of released flies do not migrate out of the field they are released in. An advantage of this technology allows farmers to opt in on a farm-to-farm basis. However, this is also a disadvantage as it is not an area-wide control program. Fields where SIT is used are subject to immigration of fertile males from neighbouring farms so eradication is impossible. Equally, growers that opt out of using SIT but are surrounded by SIT-using fields can selfishly gain benefits from the sterile male releases without having to pay for them. Another disadvantage of the onion root fly SIT program is that growers have distrust for the SIT program. At high pest densities pesticides are more effective than SIT. Growers also put more trust in pesticides as they apply them themselves and can see an immediate effect.

Although the SIT of onion root fly works on a localized scale, the success of the program depends on the acceptance and wide-spread uptake of the program by growers.

Unfortunately this appears not to be the case due to mistrust in the ability to control high density populations, widespread reliance on insecticides and the lack of government support (Loosjes 2000).

1.8.3 Serpentine leafminer

The serpentine leafminer *Liriomyza trifolii* (Burgess) is an economic pest of greenhouse ornamental and vegetable crops and in particular of greenhouse chrysanthemums (Kaspi & Parrella 2003). As with *L.bryoniae*, the serpentine leafminer larva develops within the leaf, eating the mesophyll and forming an enlarged serpentine shaped mine as it develops (Spencer 1973). The development of sterile insect technique (SIT) for *L.trifolii* was initiated after it was realised that it could provide growers with an additional form of control against the leafminer. This could either be used in conjunction with, or instead of, the biological control agents using parasitoids that are currently available for leafminer control, such as *D.isaea* and *D.sibirica* (Kaspi & Parrella 2003).

It was found that $170 \pm 5\%$ Gy of gamma radiation was sufficient to sterilize both the male and female leafminers (Kaspi & Parrella 2003, 2006). Sterility was defined as less than 0.7 mines produced per female in males; and less than 0.1 mine per female for adult females. The optimum dose was determined by examining the emergence of the adults, the quality of the males and the ability of the irradiated males to mate successfully with the wild-type females (Kaspi & Parrella 2003). After irradiation of the pupae at one to two days before adult eclosion with 170 Gy it was found that there was not a significant difference in the percentage of adult emergence, male longevity whilst under stress, or in the flight ability of the sterile males in comparison to un-irradiated males (Kaspi & Parrella 2003). There were also no significant differences in the ability of the sterile males to copulate with and transfer sperm to un-irradiated females (Kaspi & Parrella 2003).

In small cage trials releases of sterile male *L.trifolii* at ratios of 3:1, 5:1 and 10:1 sterile to fertile the sterile males were able to significantly reduce the size of the next generation of the pest population, shown as a reduction in mine damage (Kaspi & Parrella 2006). This was also shown to be true in large cage trials. The release of sterile males at a ratio of 10:1 (sterile: fertile) combined with the release of the parasitoid *Diglyphus isaea* completely eradicated the pest population from the large cage within the experimental time period. The sterile males and parasitoids were released once a week for seven weeks, after two weeks of pest population establishment, and the pest population was eradicated after 11 weeks. The level of population reduction when sterile males were released with *D.isaea* was

found to be greater than the sum of the reduction of both controls on their own (Kaspi & Parrella 2006, Kaspi & Parrella 2008a). This suggests that there is a synergistic relationship between the *D.isaea* and the sterile males (Kaspi & Parrella 2008a). This may be due to the fact that the two control methods target different life stages of the leafminer with *D.isaea* controlling the larvae and SIT reducing the reproductive potential of the adult females (Kaspi & Parrella 2008a). It may also be because, as the pest population declines, the sterile males exert a greater pressure on the population because the sterile to fertile male ratio increases (Knipling 1955, Kaspi & Parrella 2008a). Another reason for the synergistic interaction could be that the high leafminer presence elicits a response in the *D.isaea* to increase its rate of oviposition (Kaspi & Parrella 2008a).

1.9 Biological basis of sterile insect technique

Understanding the biology and ecology of a target pest species is critical to determine whether it is a candidate species for use in a SIT program. The biology of the species not only influences the decisions involved in the mass-rearing and distribution of sterile insects; but also determines whether the SIT program can be successful (Lance & McInnis 2005).

It is important to understand the abundance, distribution and population dynamics of a pest species as an estimate of the absolute pest density are required to determine the size and frequency of sterile insect releases (Itô & Yamamura 2005). The number of sterile males required depends upon the population size, the area which it covers, whether the releases are male-only and lastly the goal of the control program: suppression, or eradication (Lance & McInnis 2005). Knipling (1955) hypothesized that for populations with stable dynamics a ratio of 2:1 sterile to fertile males would achieve eradication after four or five generations. The assumption of a stable population dynamic is, however, unrealistic for almost all actual insect populations (Lance & McInnis 2005) and as such more realistic models have been developed to take fluctuating population dynamics into account (Barclay 2005, Itô & Yamamura 2005).

There are several biological aspects that make a candidate species amenable for use in SIT. Firstly, and most importantly, the species has to be exclusively sexual reproductive (Knipling 1955, Lance & McInnis 2005). This is because SIT targets the reproductive potential of adult

females through mating with sterile males. Species that have parthenogenesis or facultative sexual reproduction are unsuitable for SIT programs.

The more complex the mating system the more difficult it is to develop a successful SIT program for that species. Lepidopteran species, for example, often have complex mating systems involving the production of species-specific sex pheromones, courtship behaviours and leks (aggregations of males competing for mates) with female mate choice being important (Lance & McInnis 2005). Dipteran species, on the other hand, often have more simple mating systems where females passively accept the first mate they encounter and frequently do not produce sex pheromones.

The adult stage released must not be the stage which causes the damage or presents a problem, such as a vector (Lance & McInnis 2005). The sterile insects released must not add to the pest problem. In the New World Screwworm the larvae are the damaging stage causing myiasis in livestock, whereas the sterile males do not have a negative effect.

Another key question to answer is whether it is possible to mass-produce and sterilize enough males to achieve a sterile to fertile ratio for population suppression, or even eradication. Species that have a low intrinsic rate of increase require lower release rates to control; but consequently require higher costs to mass produce sterile males (Lance & McInnis 2005). Species where mass-rearing is possible at a relatively low unit cost are therefore more suitable for use in a SIT program. It is also necessary for the species to be handled and sterilized with relative ease. Species that have a quiescent stage, such as holometabolous species that pupate, or adults that can enter diapause, can be easily sterilized using radiation (Lance & McInnis 2005). Dipteran pupae, for example, are easily manipulated and packaged for sterilization, transport and release. Adult sterile onion root flies are stored in diapause to allow sterile males to be stockpiled for release (Loosjes 2000). The mass-rearing and sterilization must also not have a detrimental effect on the behaviour and quality of the sterile males. Quality control management systems and optimization of the radiation dose can be implemented to ensure the production of high quality sterile males that can compete effectively for mates (Calkins & Parker 2005).

It was originally thought that SIT would only be effective in species where the females only mate once, however this has been shown to be untrue (Knipling 1955, Barclay 2005). The serpentine leafminer *L.trifolii* is a polyandrous species and females mate multiple times, however, it is possible to suppress and at higher release ratios (10 sterile:1 fertile) even eradicate the pest population (Kaspi & Parrella 2006, Kaspi & Parrella 2008b). However, for the reproductive potential of the females to be reduced by mating with sterile males, the sperm produced by the sterilized males must be able to compete with the sperm of normal males. In other words, sperm competition must occur (Knipling 1955). Sterile males must also be able to produce post-copulatory factors, such as accessory gland proteins, to signal to the female that she has mated successfully. In some species the accessory gland proteins and sperm are important as they decrease the receptiveness of females to further mating (Ringo 1996, Lance & McInnis 2005).

The ability of an insect species to disperse is an important factor in assessing suitability for the SIT. Whilst it is important to ensure that sterile males can disperse from release sites, species that can disperse long distances are unsuitable for SIT programs as the control program would be under threat from immigrating fertile males and eradication would be impossible to sustain (Lance & McInnis 2005). SIT is therefore a good control option for small isolated pest populations, such as islands or small isolated habitat patches, which are not under a constant threat from immigration. Insect populations often have an over-dispersed, or clumped, distribution due to the distribution of suitable habitat patches in an environment. Sterile insects must therefore be released at a scale for optimal distribution throughout the control area (Itô & Yamamura 2005).

1.10 Genetic basis of sterile insect technique

In order for a SIT program to be a successful method of pest suppression, sterile males need to be able to compete effectively with wild males to mate with wild females. Male flies that are unable to mate or to produce sperm to transfer to females are technically sterile; but they will not have an effect on the wild population (Robinson 2005). To have an effect on the reproductive potential of a wild female, sterile males must be able to compete for matings, mate successfully with wild females and transfer sperm that is able to fertilise the eggs of the female (Robinson 2005). The chromosomes contained within the sperm,

however, must contain mutations so that after fusion of the sperm and egg, dominant lethal mutations will occur inside the developing zygote, which will lead to the death of the zygote (Robinson 2005).

Exposure to radiation causes mutations in cells (Muller 1927). Reproductive cells are more susceptible to damage than somatic cells. Ideally in a SIT program an optimum dose of radiation induces mutations into the genetic material of the gametes, whilst the somatic cells remain undamaged (Robinson 2005). Irradiation causes breaks to form in the chromosomes of the gametes. Irradiated gametes are able to fuse together as normal to form a zygote. The dominant lethal mutations, however, become apparent during nuclear division. Broken chromosomal fragments can fuse together to form a dicentric chromosome, a chromosome with two centromeres, and an acentric fragment, with no centromere. This is known as a breakage-fusion-bridge cycle. As mitosis continues an imbalance of chromosomal abnormalities builds up in the zygote leading to cell death and, eventually, the death of the zygote (Smith & von Borstel 1972, Robinson 2005).

The structure of the centromere of a chromosome is thought to be one of many factors that have an effect on whether an insect species can be sterilized using radiation. Insects that are monokinetik, with a localized centromere, such as the Diptera, Coleoptera and Hymenoptera, are susceptible to radiation. Holokinetik species with diffuse centromeres, such as the Lepidoptera and Homoptera, are more resistant to radiation exposure (Robinson 2005). There are always exceptions to this rule however (Robinson 2005). In Lepidopteran species, such as flour moth *Ephesia kuehniella* (Zeller), the chromosomes do not form breakage-fusion-bridge cycles and so genetic imbalances do not accumulate and this is thought to be due to presence of holokinetik centromeres (Tothová & Marec 2001). This differs to monocentric species where the accumulation of dicentric and acentric fragments leads to zygote lethality (Tothová & Marec 2001).

Dose-response curves of the sterility, or percentage of dominant lethal mutations, to radiation dose can be plotted using percentage egg hatch as a measure of sterility (LaChance & Graham 1984, Robinson 2005). In leafminers egg hatch is assessed by counting mines, with sterility in *L.trifolii* defined as less than 0.7 mines per female (Kaspi & Parrella

2003). At low radiation doses the dose-response curve exhibits a linear relationship as both increase (Robinson 2005). With increasing doses, as the sterility approaches 100%, the relationship becomes asymptotic. A disproportionately large increase in dose is required to achieve a relatively small increase in percent sterility (Robinson 2005). The shape of the dose-response curve is species-specific (LaChance & Graham 1984) and depends on the radiation sensitivity of the species. The asymptotic dose-response relationship is important when choosing a radiation dose for a SIT programs. At levels close to 100% sterility in the population the dose required to achieve is much greater than to achieve 80%, for example, and the radiation is more damaging to the somatic cells. The mutations in the somatic cells have a detrimental effect on the fitness of the adult males. The success of a SIT program depends on the production of males that are both sterile and exhibit a comparable level of fitness to the wild males so that they can compete for mates. It is therefore important to find an optimum radiation dose where the percentage of males released are sterile so that they have an effect on the reproductive potential of the wild females; whilst at the same time the dose should not have a negative impact on the fitness of the males (Toledo *et al.* 2004, Robinson 2005, Bakri *et al.* 2005b).

1.11 Sterile insect quality

To achieve success in a SIT AW-IPM program the male flies produced must be both sterile and exhibit a comparable level of fitness to that of the wild males (Bakri *et al.* 2005b, Calkins & Parker 2005). An optimum radiation dose must be used to sterilize the males to find a balance in the trade-off between sterility and competitiveness (Cáceres *et al.* 2007a). The production of high quality males is important for the development of a cost efficient SIT control program as fewer sterile males are required for population suppression when they are able to compete effectively for mates (Parker & Mehta 2007).

The production of high quality males is dependent on the mass-rearing process and on the dose of radiation used for sterilization. Quality protocols to evaluate and improve the mass-rearing of sterile insects were first introduced for Tephritid fruit flies (Boller & Chambers 1977, Boller 2002). The quality control protocols for the mass-rearing of Tephritid fruit flies are now well developed and universally used to assess the quality of the sterile males being produced (FAO/IAEA/USDA 2003). Comprehensive quality controls have also been

developed for the mass-production of other species, such as tsetse flies, *Glossina* spp. (FAO/IAEA 2006). Quality control protocols focus on three main areas: production, process and product quality controls (Calkins & Parker 2005, Parker 2005). These focus on the inputs into the mass-rearing facility; the methods used for the mass-rearing and sterilization; and the quality of the end product (sterile males) and how they are transported and released (Calkins & Parker 2005, Parker 2005, Cáceres *et al.* 2007b).

The assessment of the quality of sterile males is through comparison of longevity and flight ability, for example, in sterile and wild males (FAO/IAEA/USDA 2003, Toledo *et al.* 2004). Pupal size and adult emergence rates can be used to assess the mass-rearing methods, such as food quality, and the effect that the sterilization process has on the quality of the males produced (FAO/IAEA/USDA 2003, FAO/IAEA 2006). The results of quality control systems feedback into the mass-rearing system by identifying problems and allow for improvements to be initiated (Barnes *et al.* 2007). Studies on the effects of the irradiation environmental conditions have lead to improvements to produce high quality sterile males (Bakri *et al.* 2005b). Hypoxic and nitrogen-rich atmospheres in fruit fly irradiation reduce the damage to the somal cells (Bakri *et al.* 2005b, Nestel *et al.* 2007).

1.12 Suitability of the tomato leafminer for sterile insect technique

The tomato leafminer *Liriomyza bryoniae* is a suitable species for the development of SIT because it has a simple mating system that has exclusively sexual reproduction (Spencer 1973). There is currently no evidence to suggest that sex pheromones are used for mate selection and it is thought that females passively choose males to mate with. On the other hand females are polyandrous and mate multiple times, as in the serpentine leafminer *L. trifolii* (Kaspi & Parrella 2008b); but this will only affect the ratio of sterile to fertile males needed to achieve population suppression. *Liriomyza bryoniae* is a dipteran species so it has holokinetic chromosomes, so breakages in the chromosomes on exposure to radiation lead to the development of dominant lethal mutations. Females that mate with sterile *L. bryoniae* males will lay non-viable eggs. *L. bryoniae* has a quiescent pupal stage, which will allow for easy irradiation, packaging and transportation. A disadvantage of the pupal stage is that it is not possible to sort the pupae by sex. Releases of sterile insects that are not male-only

require higher release rates and therefore are more costly. *L.trifolii* pupae cannot be sexed by fine gradient size separation or by the timing of adult emergence (Parrella pers. comm).

SIT is an effective control method for use in greenhouses as each greenhouse acts as an isolated habitat, as with an island. There is little migration between greenhouses due to phytosanitary measures. This means that releases of sterile leafminers only have to act on the population within the greenhouse. *L.bryoniae* is a highly polyphagous species (Spencer 1973). There is currently no exact data on the dispersal ability of *L.bryoniae* or on the density or distribution of leafminer infestations within greenhouses; although some herb species and tomato cultivars are more susceptible to infestation than others (Jacobson & Morley 2007). Cherry and cocktail cultivars are more susceptible to leafminer damage than the classic and beef cultivars (Jacobson & Morley 2007).

Previous work examining the irradiation of *L.bryoniae* pupae using gamma radiation found that some sterility was observed with an absorbed dose of 150 and 160 Gy (Walker 2007). Irradiated males crossed with un-irradiated females showed reduced mine damage at 150 and 160 Gy in comparison to crosses with un-irradiated males (Walker 2007). Pupae that were irradiated with 180 Gy showed both a reduction in adult emergence after pupation and reduced adult longevity (Walker 2007). This suggests that irradiation with 180 Gy damages somatic cells as well as the reproductive cells in the *L.bryoniae* pupae.

L.bryoniae is an economic pest of both the greenhouse tomato and herb industry. Current control methods are expensive and fail to prevent infestations from reaching damaging economic levels. The development of a control method using SIT could provide a valuable additional option for *L.bryoniae* suppression for greenhouse tomato growers. SIT could be used as a stand-alone control method, or the more likely option, as part of an IPM control strategy if it is compatible with biocontrols. This depends, not only on the logistical feasibility of mass-rearing and sterilization of the *L.bryoniae*; but also on the socio-economic factors that would encourage growers to use this technology (Lance & McInnis 2005; Mumford 2005).

1.13 Aims and objectives

- Examine the dose-sterility relationship in male *Liriomyza bryoniae* through irradiation of late stage pupae with gamma radiation.
- Examine the effect of radiation on female *L.bryoniae* – can females also be sterilised using the same dose as males?
- Determine the absorbed dose at which an acceptable level of sterility is induced in male *L.bryoniae*.
- Investigate the effect of irradiation on pupae by measuring the sex ratio and proportion of adult emergence after pupation.
- Examine the quality of irradiated males in order that sterile male *L.bryoniae* have comparable fitness to wild males to compete effectively for mates.
- Examine the efficacy of sterile adults to suppress an established *L.bryoniae* infestation.
- Compare male-only and bi-sex releases to determine feasibility of releasing sterile females alongside sterile males.
- Compare release of sterile male *L.bryoniae* with the inundative release of the parasitoid *Diglyphus isaea*. Compare the separate and concurrent release of these two methods to determine the compatibility of SIT with current biocontrols.

1.14 Thesis outline

Chapter 2 examines the dose-sterility relationship in male *L.bryoniae* using a range of doses from 70 to 160 Gy. Factorial-designed crosses between irradiated un-irradiated flies examine the sterility of males and females using doses of 150 and 160 Gy.

Chapter 3 examines the effect of irradiation on late stage pupae using a dose of 160 Gy. The sex ratio and the rate and timing of adult emergence after pupation are used to assess the effect of irradiation on *L.bryoniae*, as a comparison with un-irradiated pupae. The longevity of male *L.bryoniae* deprived of food and water is used as an indicator of male fitness to compare the quality of irradiated and un-irradiated males.

Chapter 4 is a multi-treatment cage experiment to investigate the efficacy of sterile *L.bryoniae* against an established population. Male-only and bi-sex releases are compared to assess the feasibility of using sterile females as well as sterile males as part of a SIT program. The use of *D.isaea* as a biocontrol is compared to the use of sterile male *L.bryoniae* both separately and concurrently, to assess the compatibility of SIT with current biocontrol methods.

Chapter 5 presents the results of a questionnaire survey of tomato growers. The survey aims to establish knowledge about *L.bryoniae* infestations in greenhouse tomato crops in order to make informed decisions about SIT against *L.bryoniae*.

Chapter 6 is the concluding chapter summarising the overall findings. The feasibility of a SIT program against *L.bryoniae* and the further work required to achieve an economically viable program is discussed. The potential role of SIT in protected crops as part of a future integrated pest management strategy is also discussed.

2. Quantification of the optimum gamma radiation dosage to produce sterile male and female tomato leafminers *Liriomyza bryoniae* (Diptera: Agromyzidae) for use in a sterile insect technique program

2.1 Abstract

The tomato leafminer *Liriomyza bryoniae* (Kaltenbach) is an economically important pest in commercial tomato greenhouses in the UK. Adult females puncture the leaves and oviposit eggs in the punctures and the larvae develop within the leaf tissue forming characteristic mines. The development of sterile insect technique (SIT) against *L. bryoniae* could be used in conjunction with current biological controls. The relationship between sterility and radiation dose in male flies was examined using pupae irradiated with 70 to 160 Gy gamma radiation. The sterility of males and females was examined using factorial-designed crosses between flies that were irradiated as late stage pupae with 150 Gy or 160 Gy and un-irradiated flies. The relationship between sterility and dosage displays a rapid decrease in male fertility from 70 to 110 Gy. Above the critical dose of 120 Gy no viable offspring were produced and sterility was induced. Female *L. bryoniae* are unable to detect sterile males and whether the sperm they receive from mating contain radiation induced mutations. Both male and female *L. bryoniae* can be sterilised after exposure to 160 Gy gamma radiation and this dose should be used in future work to produce behaviourally competitive sterile males as part of a cost effective SIT program.

2.2 Introduction

The tomato leafminer *Liriomyza bryoniae* (Kaltenbach) (Diptera: Agromyzidae) is a highly polyphagous species of leafminer originating from Southern Europe (Spencer 1973). In the UK it is an economically important pest of commercial greenhouse tomato production. Leafminer populations are able to establish because greenhouse conditions closely match the Southern European climate and natural enemies are absent. In 2011 the British tomato market produced an estimated 70,540 tonnes worth around £175 million per annum with the majority of this being the production of speciality varieties, such as cherry varieties, which are the most susceptible to leafminer damage (Jacobson & Morley 2007).

Adult female *L. bryoniae* puncture the upper surface of leaves for feeding and oviposition, the shape of the puncture differing for the purpose: fan-shaped for feeding and tubular for both feeding and oviposition (Benthke & Parrella 1985). Females oviposit an average of 163 eggs during a mean lifespan of 6.6 to 9.0 days at 20°C (Tokumaru & Abe 2003). Three larval instars of *L. bryoniae* develop inside the leaf tissue, eating photosynthetic material forming a mine (Spencer 1973). At high densities these mines can cause a significant loss in the photosynthetic area of the plant, which reduces the tomato crop yield. The presence of mines damage is also visible to buyers and can lead to a crop being sold at a lower price (Jacobson pers. comm).

The tomato growing industry is currently moving towards insecticide-free integrated pest management (IPM) with growers reliant on biological control agents to control the majority of tomato insect pest species (Tomato Growers' Association, no date). Non-specific insecticides, such as spinosad, are only now used as a last result to avoid disruption to these biological control methods. Organic production now accounts for 5.2% of the UK's tomato production area (Source: Tomato Growers' Association Statistics). The only options available to organic growers are the augmentative releases of biological control agents. Augmentative releases of the parasitoid wasps *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) and *Dacnusa sibirica* (Telenga) (Hymenoptera: Brachonidae) are used, either individually or in conjunction, to control *L. bryoniae* infestations. In conventional tomato crops Eradicoat, a physically acting pesticide, is also applied to deter females from puncturing the young leaves

at the top of the crop (Root *et al.* 2008). The high cost of *D.isaea* and *D.sibirica*, however, has made the adoption of biological controls lower than expected (Jacobson pers. comm).

The use of biological controls against *L.bryoniae* may be more effective when used in combination with additional control methods, such as sterile insect technique (SIT) as part of an area-wide integrated pest management strategy (A-W IPM) (Kaspi & Parrella 2008a). SIT is the release of a large number of sterile males of the target pest species to overflood the pest population. Wild females that mate with the sterile males fail to produce viable offspring and the next generation population size is reduced. Successive releases of sterile males over a sustained period will lead to a reduction in the pest population size; and to possible eradication (Knipling 1955). The success of an A-W IPM program based on SIT is dependent on the production of sterile male flies with comparable fitness to males of the pest population to compete effectively with them for successful matings with the wild females (IAEA 1992, Calkins & Parker 2005, Cáceres *et al.* 2007a).

Exposure to gamma radiation induces mutations in cells and is therefore often used to induce sterility in SIT (Muller 1927, Bushland & Hopkins 1953, Knipling 1955). Reproductive cells are more sensitive to radiation than somatic cells (Muller 1927). After fertilisation, mutations in the chromosomes of gametes accumulate and develop into dominant lethal mutations (DLM) causing zygote death (LaChance & Graham 1984, Robinson 2005).

Exposure to radiation to induce sterility, however, also affects the fitness of the adult males (Bakri *et al.* 2005a, 2005b). 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 (LaChance & Graham 1984). 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, such as reduced longevity and poor flight ability. Males that have a high level of sterility but are not behaviourally competitive with wild males require higher release rates to achieve pest population suppression than high quality competitive sterile males (Toledo *et al.* 2004) and are, therefore, more expensive to produce. Determination of the optimum dose of gamma radiation is important to ensure an appropriate sterility rate is induced but that damage to the somatic cells of the developing pupa is minimised (Bakri *et*

al. 2005a, 2005b). Dose optimisation is also necessary in the development of a cost effective SIT program.

To establish an optimum radiation dose the ability of irradiated male and female adult *L.bryoniae* to mate and produce viable offspring needs to be examined. Ideally in a SIT program the releases of sterile flies should be male only (Knipling 1955). However, with *L.bryoniae* it is also necessary to produce sterile females since there is currently no method to separate flies by sex at the pupal stage (Parrella pers. comm). As females are responsible for the damaging effects of the leafminers, puncturing and laying eggs that develop into the leaf-mining larvae, the release of extra non-sterile female leafminers would prevent the success of a SIT program (Knipling 1955, Lance & McInnis 2005).

Previous work on *L.bryoniae* has shown that sterility can be induced with doses of 150 and 160 Gy. However, at a dose of 180 Gy adult longevity was greatly reduced (Walker 2007). The determination of an optimum dose for SIT in the serpentine leafminer *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) was investigated with an initial range of 50 to 210 Gy gamma radiation, with sufficient sterility, defined as 0.7 mines per female, induced between 155 and 170 Gy (Kaspi & Parrella 2003). The optimum dose to induce sterility in *L.trifolii* and used in subsequent work was 170 Gy (Kaspi & Parrella 2003, 2006).

The objective of this study was to establish the optimum dose of gamma radiation required to produce high quality sterile male and female *L.bryoniae*. The aim of the initial experiment was to determine the relationship between radiation dose and male sterility over a range of doses between 70 and 160 Gy. The objective of the second study was to quantify the effect of irradiation on the sterility of both males and females. This was achieved using a fully factorial design using plant damage, as a measure of reproductive potential, after crosses with both irradiated and un-irradiated *L.bryoniae* at 150 and 160 Gy. The doses chosen in the second study were based upon the results from the dose relationship study and from previous work on *L.bryoniae* irradiation (Walker 2007) and SIT development in *L.trifolii* (Kaspi & Parrella 2003).

2.3 Methods

All experiments were conducted in greenhouses at Silwood Park Campus, Ascot, UK (51.507N, 0.640W). The experiments were conducted during the months of April to October yearly from 2009 to 2011. Experimental time was limited by *L.bryoniae* availability.

Liriomyza bryoniae cultures were established yearly from a commercial tomato greenhouse with naturally occurring infestations (Wight Salads Ltd, Isle of Wight) in mid April. The cultures were maintained until early October when the population size decreased and *L.bryoniae* overwintered as pupae (van der Linden 1993).

2.3.1 Leafminer rearing

Liriomyza bryoniae were reared on pest-free tomato plants, *Lycopersicum esculentum* (cv. Moneymaker) under greenhouse conditions. Six week old tomato plants were exposed to adult *L.bryoniae* to allow oviposition for 48 hours at $20 \pm 5^{\circ}\text{C}$, $70 \pm 5\%$ relative humidity (RH) and 16:8 LD photoperiod. The *L.bryoniae* developed from egg to pupa under greenhouse conditions ($20 \pm 10^{\circ}\text{C}$). Pupae were irradiated with a Gammacell 3000 Elan Caesium-137 source (MDS Nordion, Ottawa, Canada). Pupae were irradiated 24 - 48h prior to eclosion in a hypoxic environment to minimise the deleterious effects of irradiation (Kaspi & Parrella 2003, Nestel *et al.* 2007).

2.3.2 Male sterility–dose relationship

Pupae were placed in individual wells (7 mm diameter) in 12-well plates on damp filter paper (5 mm diameter) to ensure the virginity of emerging adults. Trays were then sealed with paraffin film prior to irradiation, creating a hypoxic environment (Nestel *et al.* 2007b). Trays were selected for irradiation using a randomisation process. Pupae were irradiated after 8 to 10 days of pupation (24 - 48h prior to eclosion) with doses from 70 to 160 Gy, in increments of 10 Gy. Five irradiated males and five control (un-irradiated) females were released into a plexiglass and insect netting cage (80 x 20 x 20 cm) containing a randomly assigned eight-week old tomato plant. After 20 days the number of leaves that were undamaged (D), punctured (P) and had mines (M) were counted. The number of mines and pupae per leaf were also counted. The duration of the experiment was longer than the average lifespan of adult flies to determine whether sterility was permanent or temporary and to allow time for eggs to develop into larvae.

2.3.3 Male and female sterility

Pupae collected were irradiated with a dose of 150 Gy or 160 Gy or were not irradiated as a control group at random. 150 and 160 Gy doses were chosen based on previous work (Walker 2007) and from taking the upper range of doses where sterility was induced in the previous sterility-dose relationship study. The higher doses of 150 and 160 Gy were used to ensure sterility would be induced in the males. Ten virgin males and ten virgin females released into a plexiglass and insect netting cage (80 x 20 x 20 cm) containing an eight-week old tomato plant in factorial combinations: irradiated males x irradiated females; irradiated males x control females; control males x irradiated females; control males x control females. There were 4 replicates at 160 Gy and 2 at 150 Gy. After 20 days the proportion of the plant's leaves that were undamaged (D), punctured (P) and punctured with mines (M) was recorded. Leaf damage was categorised into leaves that were damaged (P+M) and those with economic damage (M). The number of developing mines and pupae produced per female were counted.

2.3.4 Statistics

All statistical analyses were performed using R (version 2.10.1, www.r-project.org). The sterility-dose relationship data were analysed using generalised linear models (GLM) with poisson errors. Graphical observation of the variables ensured a normal distribution prior to statistical testing. Leaf damage data were analysed using Tukey's Honest Significant Difference test. The data on the number of mines and pupae were analysed using a mixed effects model (lmer) with poisson errors, as an analysis of deviance. 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).

2.4 Results

2.4.1 Male sterility–dose relationship

The mean total number of leaves per eight-week old plant was 319 (min = 255, max = 485). The number of mines per plant decreased as the dose increased ($z = -9.68$, $p < 0.001$) and at doses > 110 Gy no mines were observed (figure 2.1). The number of mines declined linearly as the dose increased from 70 Gy (mean = 30 mines) to 110 Gy (mean = 6 mines). Using the model fitted to the data it is possible to predict that the dose above which mines do not

develop is 120.7 Gy. However, the dose did not affect the number of leaves punctured per plant ($z = -0.87$, $p > 0.05$) (figure 2.2).

The number of pupae per plant (figure 2.3) also decreased as the dose increased ($z = -7.09$, $p < 0.001$). There is a linear relationship between the number of mines and the number of pupae produced per plant after 20 days ($p < 0.001$, $f = 976.9$, $df = 13$, $r^2 = 0.98$) (figure 2.4).

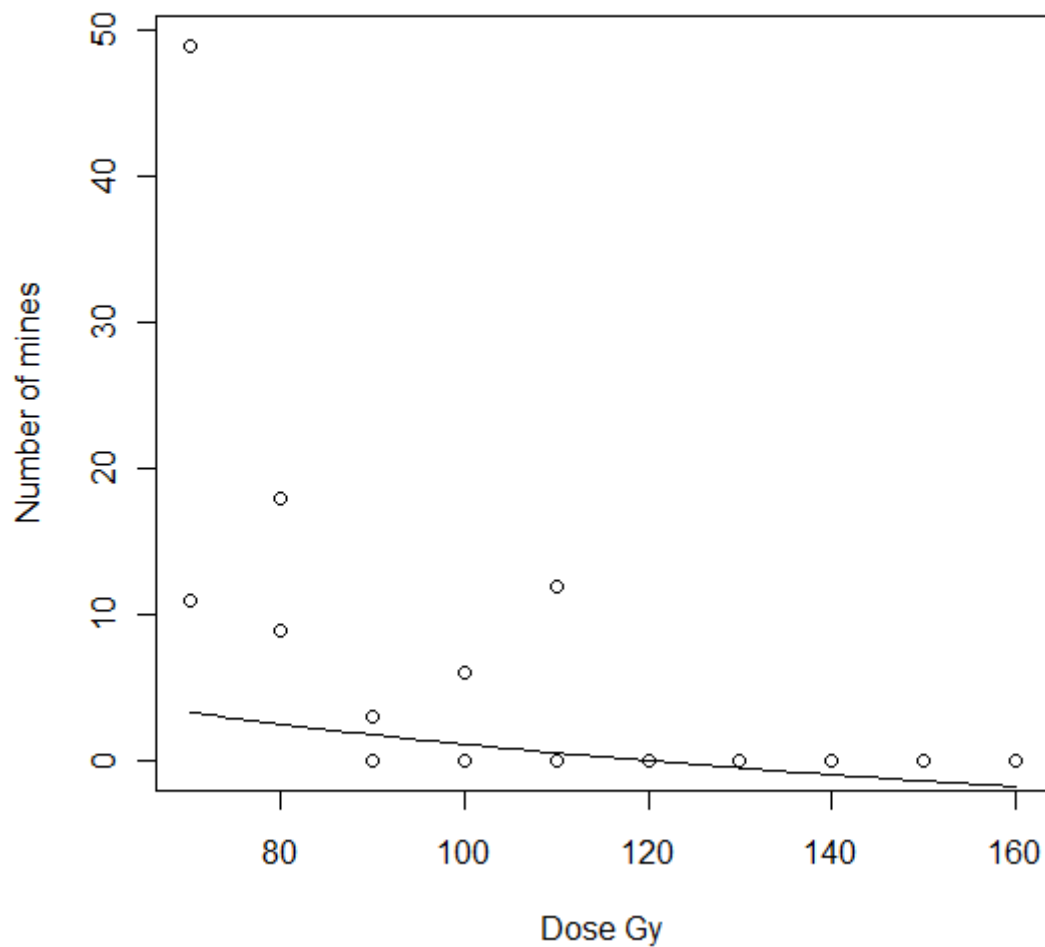


Figure 2.1: Relationship between the number of mines per tomato plant after 5 females are crossed with 5 irradiated males, at different gamma radiation doses (Gy), after 20 days. The number of mines per plant decreases as the dose increases ($z = -9.68$, $p < 0.001$).

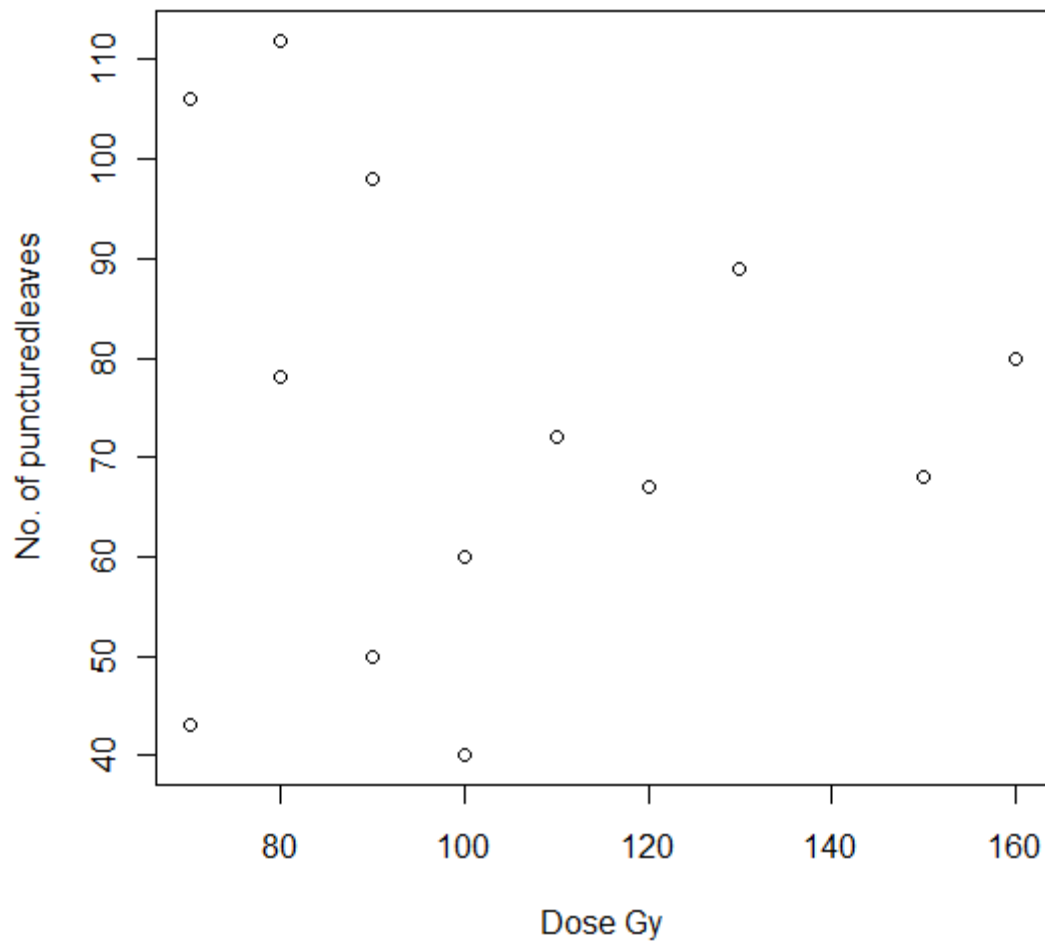


Figure 2.2: Relationship between the number of leaves with puncture marks per tomato plant after 5 females are crossed with 5 irradiated males, at different gamma radiation doses (Gy), after 20 days. Dose does not affect the number of leaves punctured per plant ($z = -0.87, p > 0.05$).

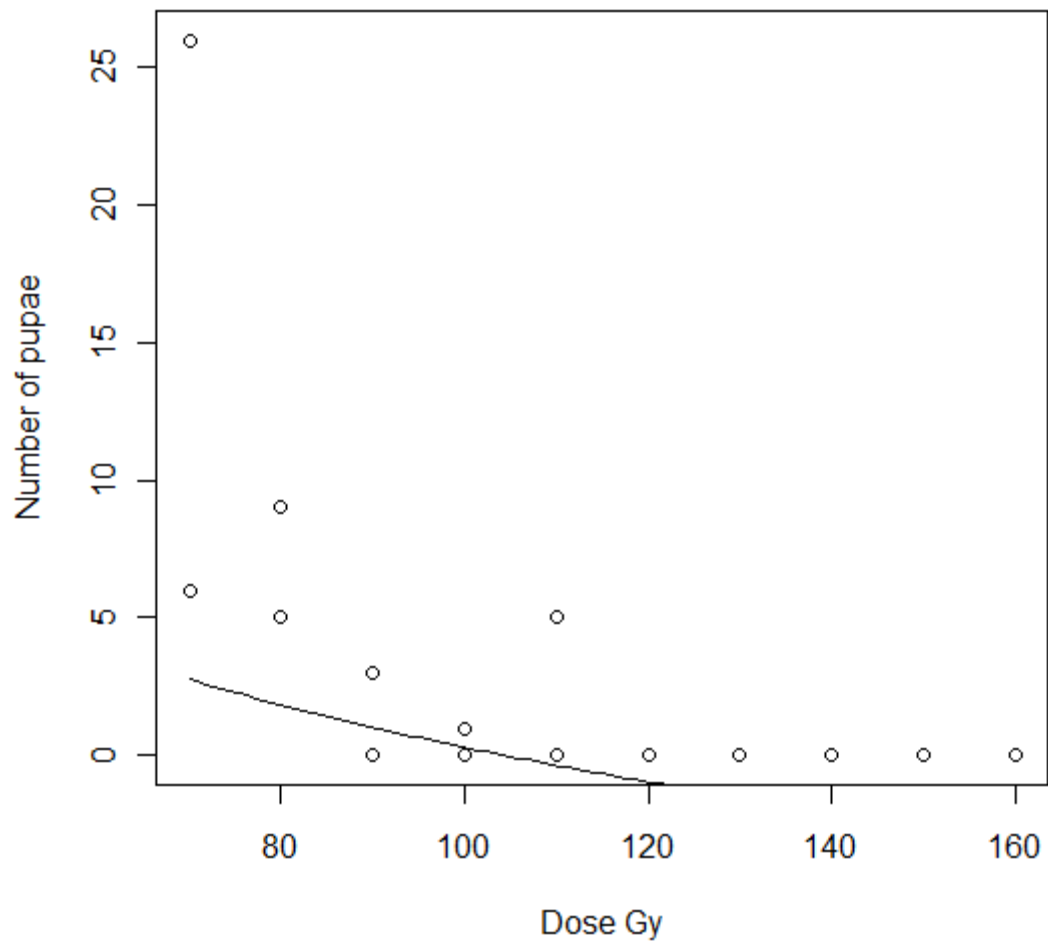


Figure 2.3: Relationship between the number of pupae per tomato plant after 5 females are crossed with 5 irradiated males, at different gamma radiation doses (Gy), after 20 days. The number of pupae per plant decreases as the dose increases ($z = -7.09$, $p < 0.001$).

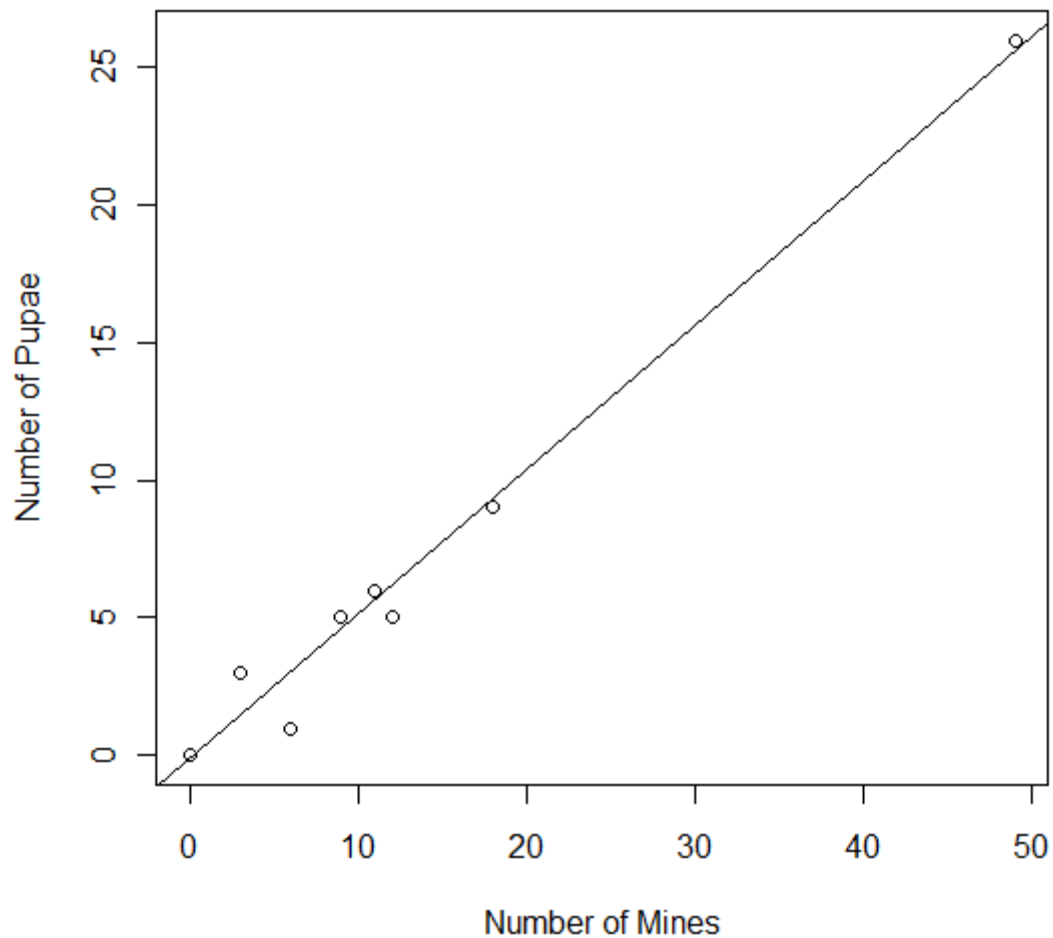


Figure 2.4: Linear Relationship between number of mines and number of pupae per tomato plant from crosses with 5 male and 5 females after 20 days. ($p < 0.001$, $f = 976.9$, $df = 13$, $r^2 = 0.98$, $y = -0.11 + 0.52 x$).

2.4.2 Male and female sterility

The proportion of leaves that had damage (P+M) (figure 2.5) were not significantly different between any of the treatments ($p > 0.05$). The proportion of leaves with economic damage (M) (figure 2.6) was significantly greater ($p < 0.05$) in the Control treatment (mean = 0.18) than the other treatments (Irradiated M, Irradiated F, Irradiated M+F: mean = 0.02); and there was no significant difference between the treatments with irradiated adults ($p > 0.05$).

Increasing the number of females in each treatment increases the number of mines (figure 2.7) and the number of pupae (figure 2.8) ($p < 0.001$). No mines or pupae were produced in treatments where one or both of the sexes had been irradiated at 160 Gy (figures 2.7 & 2.8). At 150 Gy no mines or pupae developed when both the males and females had been irradiated, however, mines (mean = 4) and pupae (mean = 2.1) were observed when only one sex in the treatment was irradiated with 150 Gy. Increasing the number of males did not affect the number of mines or pupae ($p > 0.05$), however, there was a reduction in the number of mines ($p < 0.005$) and pupae ($p < 0.05$) when there were both more males and a higher dosage (table 2.1).

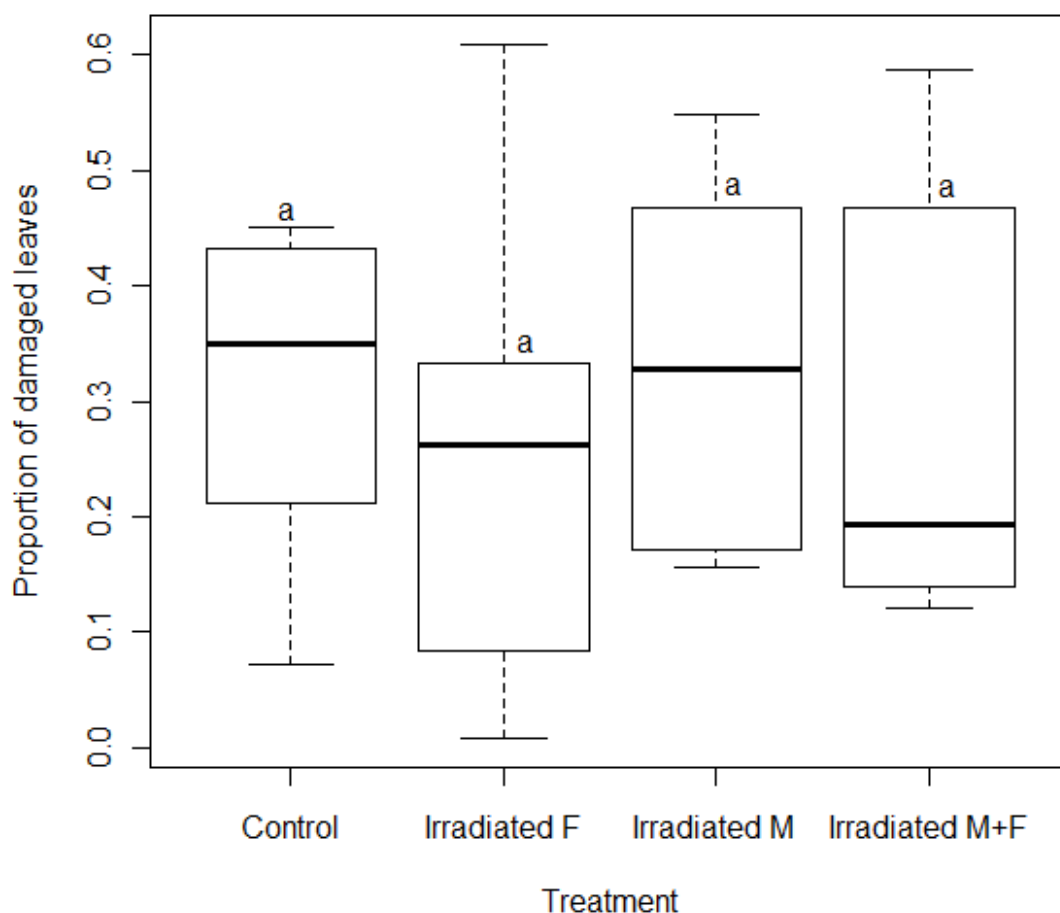


Figure 2.5: Proportion of leaves with damaged leaves (Punctures + Mines). Boxes labelled a are not significantly different ($p > 0.05$) to each other as analysed by Tukey's Honest Significant Difference.

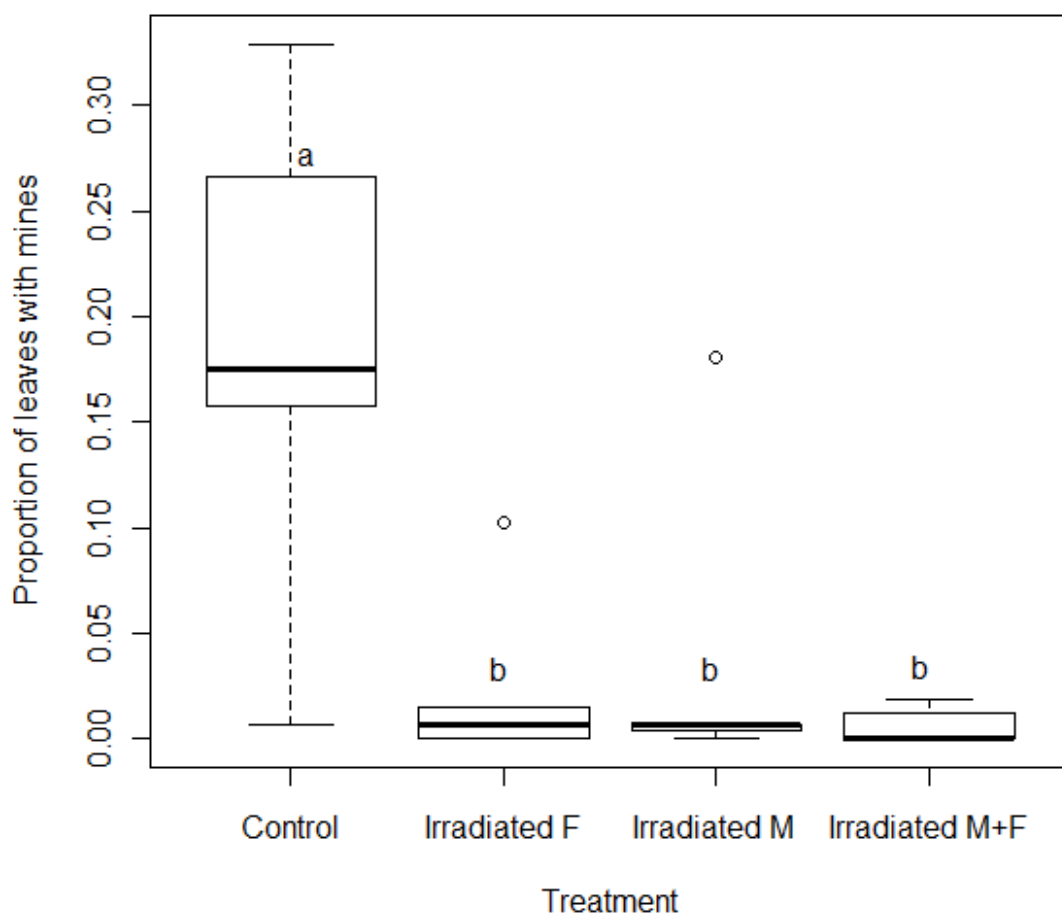


Figure 2.6: Proportion of leaves with economic damage (Mines). Boxes labelled a and b are significantly different ($p < 0.05$) to each other as analysed by Tukey's Honest Significant Difference.

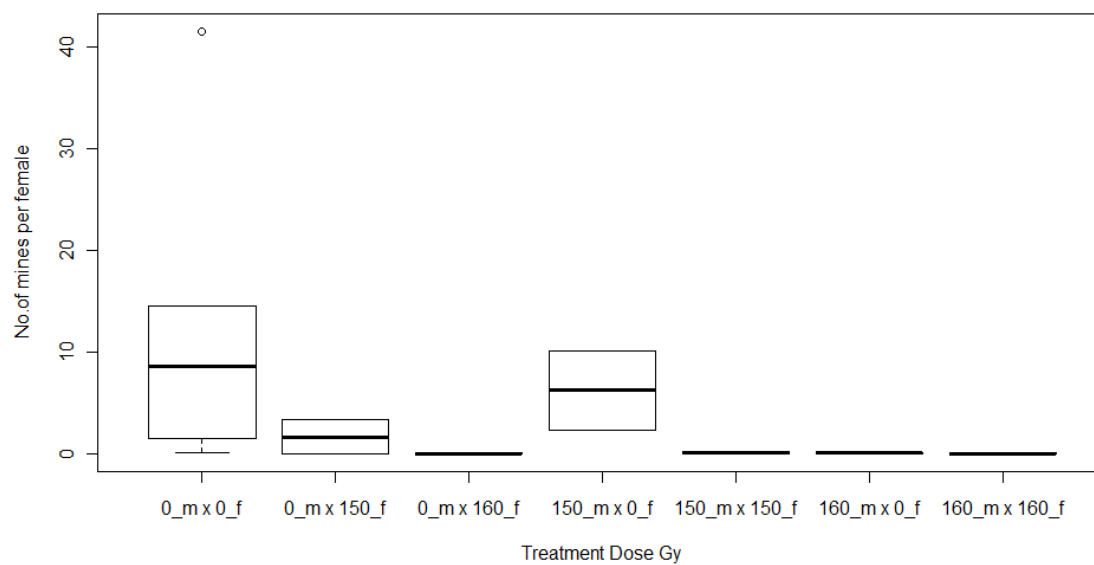


Figure 2.7: Boxplot of the number of mines that develop per female for different treatments

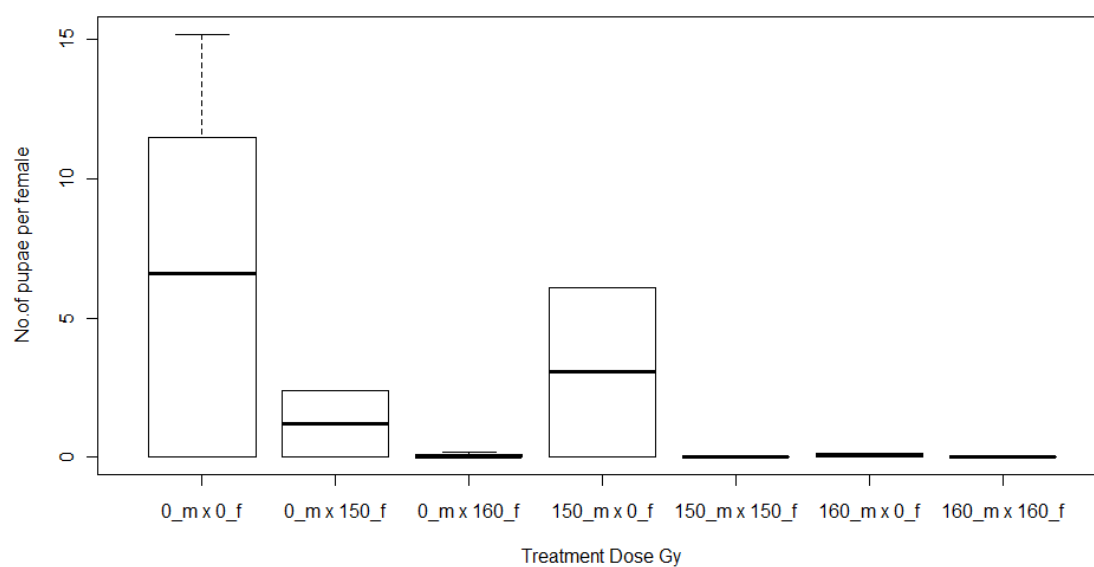


Figure 2.8: Boxplot of the number of pupae that develop per female for different treatments

Table 2.1: Summary of optimal mixed effects model output for mines per female and pupae per female

Variable	Optimal model terms	<i>n</i>	<i>z</i>	<i>P</i>	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	

2.5 Discussion

2.5.1 Male sterility–dose relationship

The data presented in this study are consistent with the findings of La Chance and Graham (1984); that insects display a greater decline in fertility in response to increased radiation at low doses and above a certain critical dose sterility is induced. Males exposed to doses of 120 Gy and above were sterile; there were no larvae developing and mines were not observed. The critical dose was lower than observed for *L.trifolii* in which sterility is induced with doses between 155 and 170 Gy (Kaspi & Parrella 2003). The critical dose, however, was higher than required for other species such as the onion root fly, *Delia antiqua* (Meigan), which only requires 30 to 40 Gy (IDIDAS 2012) (table 2.2).

Liriomyza bryoniae appears to display a “1-hit” response where, as with other Dipteran species such as *Musca domestica* L. (Muscidae) and *Anastrepha fraterculus* (Wiedemann) (Tephritidae), only one dominant lethal mutation (DLM) is required per sperm to ensure zygote lethality (LaChance & Graham 1984, Allinghi *et al.* 2007). Diptera are susceptible to radiation exposure because they have monokinetic chromosomes with a localised centromere (Robinson 2005). As the dose increases the chromosomal DNA acquires more than one DLM per gamete, causing the zygote to accumulate chromosomal abnormalities

and leads to zygote death at a faster rate (Robinson 2005). At higher doses, however, the radiation not only induces DLM into the

Table 2.2: Radiation dose exposed to late stage pupae to produce sterile insects in current and developing SIT programs. Sterility induced in hypoxic conditions, excluding *Cydia pomonella*. (Kaspi & Parrella 2003, Bakri *et al.* 2005b). More extensive list available from IDIDAS (2012).

Insect species	Dose (Gy)
<i>Anastrepha ludens</i> (Loew)	70 - 80
<i>Anastrepha obliqua</i> (Macquart)	80
<i>Bactrocera dorsalis</i> (Hendel)	90
<i>Bactrocera philippinensis</i> (Drew and Hancock)	64 - 104
<i>Ceratitis capitata</i> (Wiedemann)	90 - 145
<i>Cochliomyia hominivorax</i> (Coquerel)	80
<i>Cydia pomonella</i> L.	150
<i>Delia antiqua</i> (Meigan)	30 - 40
<i>Liriomyza trifolii</i> (Burgess)	155 - 170

reproductive cells, but also damages the somatic cells of the developing pupa. This can have an effect on the ability of the pupae to emerge as adults, the longevity and the behavioural competitiveness of these adults. Previous work on *L.bryoniae* has shown that after exposure to 180 Gy of gamma radiation adult longevity was significantly reduced with the majority of adults dying within 24 hours of eclosion (Walker 2007).

There was a significant positive relationship between the number of mines and the number of pupae per plant that developed at different doses, implying that if mines are produced then the larvae will develop successfully through to pupation. This supports the idea that radiation induces DLM into the DNA of the sperm. The broken chromosomal fragments fuse together to create dicentric (two centromeres) and acentric (no centromere) chromosomal fragments chromosomes. During mitosis these chromosomal fragments accumulate as part of a breakage-fusion-bridge cycle. The accumulation of chromosomal abnormalities builds up in the zygote causing cell death and ultimately leads to the death of the zygote (Smith & von Borstel 1972, Tothová & Marec 2001, Robinson 2005). The suppressive action of SIT

targets the egg stage of the leafminers to reduce the next generation size, unlike the parasitoids *D.isaea* and *D.sibirica*, which target the larval and pupal stages respectively.

In the serpentine leafminer *L.trifolii* adult females puncture the leaves to feed on the leaf tissue and then oviposit in a proportion of these punctures (Benthke & Parrella 1985). It can be assumed that this is also true for *L.bryoniae* as previous work showed that the number of punctures was unaffected by increasing the radiation dose experienced by the males (Walker 2007). The amount of female leaf puncturing, as measured by the number of leaves with puncture damage, was not significantly affected by the radiation dose received by the male. This suggests that female *L.bryoniae* are unable to detect whether the sperm they receive from mating contain radiation induced mutations. Radiation therefore is not reducing the probability that males will be able to mate but is reducing the population size of the next generation. It is important in a SIT program that females are unable to detect, not only males that have been sterilised, but also sperm carrying DLM (Robinson 2005).

2.5.2 Male and female sterility

Due to the low level of repetition in the initial dose-sterility relationship study, doses at the upper end of the range previously observed (150 and 160 Gy) were used to examine the reproductive potential of irradiated and un-irradiated *L.bryoniae* of both sexes. 150 and 160 Gy were chosen based on the sterility-dose relationship curve and on previous work with *L.bryoniae* and *L.trifolii* (Kaspi & Parrella 2003, 2006, Walker 2007).

Once sterilised, both male and female *L.bryoniae* do not recover their fertility because the studies ran for 20 days, which is longer than the adult lifespan. Females live longer than males with an average lifespan of 6.6 to 9.0 days at 20°C (Tokumaru & Abe 2003). This is consistent with the findings in *L.trifolii* and other species of Diptera (Kapsi & Parrella 2003, Allinghi *et al.* 2007).

The proportion of leaves with *L.bryoniae* damage (P+M) was not significantly different across the four treatments (150-160 Gy). All the plants were punctured in the same proportion independent of whether the females were irradiated or whether they had mated with irradiated males. Not only are females unable to detect whether sperm contains DLM,

they cannot detect whether their eggs contain DLM and puncture and oviposit regardless of this. *Liriomyza bryoniae* is a polyandrous species and females mate multiple times to maximise their fecundity (Spencer 1973, Kaspi & Parrella 2008b). Usually this is a key factor against the suitability of a species for use in a SIT program (Lance & McInnis 2005). Females that receive high quality sperm during mating have a lower propensity to re-mate (Vreysen *et al.* 2006). Female *L.bryoniae* that are unable to differentiate between viable and non-viable gametes are more likely to mate fewer times, negating the effect of the polyandry.

When the plants were analysed in terms of economic damage (M) i.e. leaves on which both punctures and mines were observed, there was a significant reduction in economic damage in the treatments where one or both of the sexes were irradiated. This confirms that irradiated females, as well as irradiated males, are unable to produce viable offspring and can be sterilised using radiation (150 -160 Gy). Ideally in SIT, releases should be of sterile males only. Bi-sex releases reduce the efficiency of SIT as more flies need to be reared and released to achieve the same effect (Knippling 1955, Barclay 2005). This also has a knock-on effect on the cost of the SIT program. However, bi-sex releases are still feasible if the female adults do not have a detrimental effect on the program by causing more damage. Species where the adult female feeds extensively or acts as a vector, such as mosquitoes, it is unacceptable to have bi-sex releases (Lance & McInnis 2005). In *L.bryoniae* there is currently no known mechanism to separate the sexes at the pupal stage. Separation by size or by emergence timing is not possible (Parrella pers. comm). However, as females can also be sterilised with the same dose as male flies there is a possibility that bi-sex releases will not adversely affect an SIT program. The release of sterile females may even increase the effect by mating with wild males. On the other hand, sterile females could divert the attention of sterile males so the ratio of sterile to wild males would have to be greater to have an effect (Knippling 1955, Barclay 2005). However, the effect of bi-sex releases on the release ratio is unknown and warrants further investigation.

When sterility was analysed for the different treatments, a greater level of sterility was found after irradiation with 160 Gy than with 150 Gy. No mines or pupae developed in treatments where only one sex had been irradiated with 160 Gy. The number of viable offspring was reduced when either sex was irradiated with a dose of 150 Gy in comparison

to the control treatment. However, it was only when both sexes had been irradiated that no viable offspring were produced. At 160 Gy a higher proportion of the males were sterile so there was a higher probability of the females mating with a sterile male than at 150 Gy. Irradiated *L.bryoniae* adults require a level of sterility that produces non-viable offspring after mating with wild adults, to destroy the reproductive potential of the wild adults and to reduce the size of the next generation.

The aim of SIT is to produce high quality sterile males to mate with the wild females and destroy their reproductive potential (Knippling 1955, IAEA 1992). It is also necessary to produce sterile females that do not add to the pest problem as part of bi-sex releases. To achieve this level of sterility in both sexes requires irradiation with a dose of 160 Gy. However, it is also important to produce high quality males that exhibit a comparable level of fitness to that of the wild males (Bakri *et al.* 2005b, Calkins & Parker 2005). The development of a successful SIT program to control *L.bryoniae* requires further investigation into the quality and behavioural competitiveness of sterile males. In the serpentine leafminer *L.trifolii* the dose used to conduct quality testing and to determine the release ratios was 170 Gy, which was the upper end of the dose range capable of inducing sterility from 155 to 170 Gy (Kaspi & Parrella 2003, 2006, 2008a).

2.6 Conclusions

The production of high quality sterile males is the key to a successful SIT program and the main objective of this work was to determine the optimum dose of gamma radiation required to achieve this. It was determined that the irradiation of mature pupae with 160 Gy produces permanently sterile males and females. Quality testing is required, however, before a final dose can be decided upon to ensure the production of high quality sterile males that have comparable fitness to wild males. Wild females that mate with sterile males are unable to detect sperm containing DLM and oviposit at the same rate as after mating with wild males. Sterile females still puncture the leaves in to feed and oviposit. However, since they do not produce viable offspring that would cause mine damage, it is possible to release sterile females as part of bi-sex releases without detrimental effects to the success of the SIT program. Future work should focus on the production of high quality sterile males that are behaviourally competitive with wild males as part of a cost effective SIT program.

3. Quality testing of the tomato leafminer *Liriomyza bryoniae* (Diptera: Agromyzidae) after irradiation with 160 Gy for use in a sterile insect technique programme: the trade-off between sterility and quality.

3.1 Abstract

Liriomyza bryoniae (Kaltenbach) (Diptera: Agromyzidae), the tomato leafminer, is an important pest of greenhouse tomato in the UK. Sterile insect technique, SIT, is a possible novel method of pest suppression that could be used in conjunction with current biocontrol pest management methods in tomato greenhouses. In SIT releases sterile males mate with wild females, who produce non-viable offspring, destroying the reproductive potential of the pest population. The success of an SIT program depends on the production of high quality males that are both sterile and exhibit comparable fitness to wild males so that the sterile males can compete effectively for mates. Dose optimisation is required to balance the trade-off between sterility and competitiveness in order to achieve pest suppression. Late stage pupal irradiation with a dose of 160 Gy gamma radiation produced sterile adult *L.bryoniae* in previous work. The objective of this study was to assess the effect of irradiation with 160 Gy on the fitness of *L.bryoniae*. Percentage emergence and the sex ratio of adults were compared for irradiated and un-irradiated pupae to check pupal viability. The longevity of males deprived of food and water was assessed as a measure of male fitness. Irradiation with 160 Gy did not negatively affect the rate of adult emergence or skew the sex ratio in comparison to the un-irradiated *L.bryoniae*. Male longevity under stress was not affected by irradiation at 160 Gy with comparable longevity observed for both irradiated and un-irradiated males. The results suggests that 160 Gy is a suitable dose to use to produce high quality sterile males for SIT; but the performance of these sterile males against an established *L.bryoniae* population requires further investigation.

3.2 Introduction

Sterile insect technique, SIT, is the release of a large number of sterile males into an area to flood a pest population of the same species (Knipling 1955). Wild females that mate with sterile males lay eggs containing dominant lethal mutations, DLM, and fail to develop. The reproductive potential of the pest population is reduced by the non-viable offspring. Successive releases of sterile males suppress, and in some cases eradicate, the pest population (Knipling 1955). The tomato leafminer *Liriomyza bryoniae* (Kaltenbach) is a polyphagous agromyzid leafminer pest of greenhouse tomato crops in the UK (Spencer 1973). *Liriomyza bryoniae* larvae develop inside the leaf, feeding on the photosynthetic plant material and at high densities reduce the ability of the plant to photosynthesize (Spencer 1973, Parrella *et al.* 1985). Many tomato growers in the UK rely on the use of biocontrols for pest management and use chemical pesticides as a last resort for novel or problematic pest infestations (British Tomato Growers' Association, no date, Parrella 2008). Inundative releases of *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) and *Dacnusa sibirica* (Telenga) (Hymenoptera: Brachonidae) are the current commercially available biocontrols for *L. bryoniae* suppression. SIT has been proposed as a novel method for *L. bryoniae* suppression in tomato greenhouse crops that could be used instead of, or in conjunction with the existing biocontrol parasitoids *D. isaea* and *D. sibirica*.

The success of a SIT program depends on the ability of sterile males to survive in the environment, locate, mate with and inseminate females (Calkins & Parker 2005). The dose-sterility relationship is asymptotic (Robinson 2005). As sterility approaches 100% the increase in radiation required to increase sterility is much greater than at lower sterility levels (Robinson 2005). There is, however, a trade off between the sterility of irradiated males and the ability of the males to carry out the function they were produced for (Bakri *et al.* 2005b, Cáceres *et al.* 2007a). Reproductive cells are more susceptible to radiation damage than somatic cells (Muller 1927). At the higher doses used to achieve 100% sterility somatic cells are also damaged by radiation causing a reduction in male fitness and an associated decline in the probability that sterile males find a mate (Robinson 2005). In the past, SIT programs focussed on producing males that were 100% sterile even though the radiation doses required were to the detriment of male fitness (Klassen & Curtis 2005). Failures in SIT programs caused by low quality over-irradiated males were wrongly

attributed to the sterile to wild male ratio and releases of greater numbers of sterile males were used in an attempt to fix the failures (Calkins & Parker 2005, Klassen & Curtis 2005). However, examination of the competitiveness of sterile males resulted in a reduction in radiation dose and the introduction of protocols to assess sterile male quality to produce high quality sterile males (Boller & Chambers 1977, Boller 2002). Whilst the overall proportion of males that are sterile is reduced by lowering the radiation dose, the sterile males have comparable fitness to wild males and are able to compete more effectively for mates (Lux *et al.* 2002, Calkins & Parker 2005). The trade-off between sterility and fitness, however, needs to be balanced. With too low a radiation dose males may have comparable fitness to wild males; but the proportion of sterile males in the release may be too low for population suppression and instead add to the pest problem.

Comprehensive quality protocols have been designed for the mass-rearing of Tephritid fruit flies and tsetse fly, *Glossina* spp., as part of SIT programs (IAEA/FAO/USDA 2003, IAEA/FAO 2006). These are quality protocols and standards for production that can be universally applied to achieve a uniform product. The quality protocols examine mass-rearing methods, pupal irradiation, the packaging and transport of pupae prior to release and evaluation of the success of sterile males in the field (IAEA/FAO/USDA 2003). The protocols are designed to monitor and provide feedback on the quality and competitiveness of the sterile males, which are the end product of a SIT program (IAEA/FAO/USDA 2003). The fitness of Tephritid fruit fly males is particularly important to the success of SIT due to the complex mating behaviour of fruit flies (Lux *et al.* 2002). In Tephritid fruit flies, males form a lek and display to females who have a choice of mates. Females can detect poor quality males and avoid mating with them (Lux *et al.* 2002). It is therefore important that sterile males have comparable fitness to wild males in order to mate with wild females (Lux *et al.* 2002). *L.bryoniae* has a simple mating system, which does not involve leks or the production of pheromones (Spencer 1973, Kaspi & Parrella 2008b). Female *L.bryoniae* mate multiple times (Spencer 1973, Kaspi & Parrella 2008b) and as such a sterile male is required to mate and inseminate in the same manner as a wild male. The female has to be satisfied that it received good quality sperm with which to fertilise its eggs. This prevents the female from rejecting the sperm and re-mating (Vreysen *et al.* 2006). Females should not be induced to mate more frequently as a result of unsatisfactory sterile matings (Vreysen *et al.* 2006).

L.bryoniae females that mate with males irradiated with 160 Gy are unable to tell that the sperm received contains DLM and is un-viable (chapter 2). Female *L.bryoniae* that mated with irradiated males punctured and laid eggs the same amount as females that mated with un-irradiated males (chapter 2).

Determining the optimum dose required to produce males that are sufficiently sterile to reduce a population but that are of comparable fitness to wild males to compete effectively for mates is an important requirement for a successful SIT program (IAEA 1992, Bakri *et al.* 2005b, Calkins & Parker 2005, Cáceres *et al.* 2007a). The number of competitive partially sterile males required to suppress a pest population is fewer than required using completely sterile low fitness males and improves the cost efficiency of a SIT program (Toledo *et al.* 2004, Parker & Mehta 2007). The trade-off between sterility and male fitness required is also dependent on what the SIT program is trying to achieve: complete eradication or population suppression (Mumford 2005). This has important economic consequences as total eradication is more costly than suppression (Mumford 2005). Dose optimisation therefore has an important role in balancing the efficiency and cost of an SIT program (Parker & Mehta 2007).

Previous work on SIT against *L.bryoniae* (chapter 2) demonstrated that some sterility can be observed in *L.bryoniae* males after irradiation with a dose of 120 Gy. Adult emergence after pupation, however, is reduced by irradiation with doses of 180 Gy and above, presumably due to damaged somatic cells (Walker 2007). Males irradiated with 160 Gy were deemed to exhibit a high level of sterility as they produced no viable offspring when crossed with wild-type females (chapter 2). The previous work suggests that there is 100% sterility with a dose of 160 Gy due to low replication (chapter 2). If the replication of these crosses was increased it is possible that the sterility level would be lower than 100% and actually closer to the 96 to 99% usually required for SIT programmes. As such the fitness of males irradiated with 160 Gy requires comparison to wild males (chapter 2). If male *L.bryoniae* do not exhibit comparable fitness and competitiveness to that of wild, un-irradiated males then the dose should be lowered and re-examined until an optimum dose is determined that can produce both sterile and high quality males.

The aim of this study was to assess the effect of irradiation with a dose of 160 Gy on the viability of pupae as measured by percentage emergence and sex ratio of adults. The timing of adult emergence was also observed. The assessment of adult emergence and sex ratio are two routine quality control tests used to check the fitness and quality of Tephritid fruit flies and tsetse flies (FAO/IAEA /USDA 2003, FAO/IAEA 2006). The negative effects of using too high a radiation dose, causing damage to the somatic cells of the developing pupae, would result in low adult emergence. If the process of irradiation affects one sex more than the other a skewed sex ratio would be observed. The second aim of this study was to compare the fitness of males irradiated with 160 Gy to wild, un-irradiated males. The longevity of irradiated and un-irradiated males under stress, without food and water, was used as an indicator of male fitness. The stress of the lack of food and water imposed on the newly emerged males measures the relative nutrient reserves available. This is to determine whether pupal irradiation with 160 Gy has a detrimental effect on the survivability of sterile males and can be used as a measure of male fitness. The assessment of male longevity under stress is also a standard protocol used to assess the quality of sterile males in Tephritid fruit flies and tsetse SIT programs (FAO/IAEA /USDA 2003, FAO/IAEA 2006).

3.3 Methods

3.3.1 Leafminer rearing

All experiments were conducted at Silwood Park, Ascot, UK in a controlled environment room with artificial lighting $20 \pm 5^{\circ}\text{C}$, $70 \pm 5\%$ RH and 16:8 LD from April to July 2011. A *L.bryoniae* colony was established in late April from a commercial tomato greenhouse with naturally occurring infestations (Wight Salads Ltd, Isle of Wight). The colony was replenished with a second pupae collection at the end of June from the same location to increase the genetic diversity of the population. *Liriomyza bryoniae* were reared on pest free tomato plants, *Lycopersicum esculentum* (cv. Moneymaker) under greenhouse conditions. The greenhouse was set to $20 - 30^{\circ}\text{C}$, humidity was not controlled and natural lighting was used. Six week old tomato plants were exposed to adult *L.bryoniae* for 48 hours at $20 \pm 5^{\circ}\text{C}$, $70 \pm 5\%$ RH and 16:8 LD. The *L.bryoniae* developed from egg to pupa under greenhouse conditions ($20 \pm 10^{\circ}\text{C}$).

3.3.2 Quality testing

The quality assessments used were adapted for use with *L.bryoniae* from the required routine quality control tests on adult emergence, sex ratio and male longevity under stress designed for Tephritid fruit flies (FAO/IAEA/USDA 2003) and for tsetse flies (FAO/IAEA 2006). Pupae were randomly assorted into two treatment groups: irradiated (160 Gy) and un-irradiated (0 Gy). The dose at which male and female *L.bryoniae* exhibited a high level of sterility was determined as 160 Gy in previous work (chapter 2). Pupae were placed in individual wells (7 mm diameter) in 12-well plates on damp filter paper (5 mm diameter) to ensure the virginity of emerging adults. Trays were then sealed with paraffin film prior to irradiation. Pupae were irradiated 24-48h prior to eclosion in a hypoxic environment to minimise damage to the somatic cells (Kaspi & Parrella 2003, Nestel *et al.* 2007) using a Gammacell 3000 Elan Caesium-137 source (MDS Nordion, Ottawa, Canada). There were 12 trays of irradiated pupae and 12 trays of un-irradiated pupae.

3.3.2.1 Male longevity under stress

After irradiation, emerged adults were separated by sex and females were removed. There were 49 irradiated males (160 Gy) and 36 un-irradiated males (0 Gy). Males were placed individually into plastic tubes (38 mm diameter, 60 mm height) which had one end covered with insect netting and sealed around the outside with paraffin film. Males were deprived of both food and water to induce environmental stress. The time of emergence was noted. Males were checked every 6 hours at 0630, 1230, 1830 and 0030 over a period of 96 hours when their status was noted as either alive or dead. The times by which each male had died was noted.

3.3.2.2 Adult emergence: Timing, sex ratio and percentage emergence

After irradiation, trays were examined and emerged adult *L.bryoniae* were sexed and counted every 6 hours at the start of each time period, over a total of 102 hours. Period 1 was from 0030 to 0629; period 2 from 0630 to 1229; period 3 from 1230 to 1829 and period 4 from 1830 until 0029. The lights in the controlled temperature room turned on at 0700 and off at 2300. The percentage adult emergence was calculated per tray with the formula: Adult emergence = (Emerged adults / Total number of pupae) x 100 %. The sex ratio was

calculated per tray using the formula: Sex ratio = Number of adult females / Number of adult males. Sex ratio was expressed as a ratio of females to one male.

3.3.3 Statistics

All statistical analyses were performed using R (version 2.10.1, www.r-project.org). The times at which males under stress died were analysed using a Cox proportional hazards model as a non-parametric analysis of covariance survival analysis. Censoring was not required as all the males were dead by the end of the experimental period. In the adult emergence data, variance was not equal between the two groups ($p < 0.05$) as determined using an F-test. To fulfil the assumptions of the statistical tests the data were analysed using a Welch two-sample t-test. The sex ratio data for irradiated and un-irradiated *L.bryoniae* exhibited equal variance as determined using an F-test ($p > 0.05$). The data were therefore analysed using a two-sample t-test to fulfil the assumptions of the statistical tests. The proportion of adult *L.bryoniae* that emerged in a given time period per day were analysed using a beta regression model. No adults emerged from 0030 to 0629 (period 1) or from 1830 to 0029 (period 4) so these were removed from the model. A beta regression assumes that the proportions lie between 0 and 1, so for data where the proportion was 0 or 1 these were replaced with 0.001 and 0.999 respectively.

3.4 Results

3.4.1 Male adult longevity under stress

There was no difference in the longevity of irradiated and un-irradiated males under environmental stress ($p > 0.05$, figure 3.1). The mean longevity of control males was 63 ± 2.3 (mean $\pm$ se) hours and all were dead after 90 hours. The mean longevity of the males irradiated with 160 Gy was 66.4 ± 2.6 (mean $\pm$ se) hours and all were dead after 96 hours.

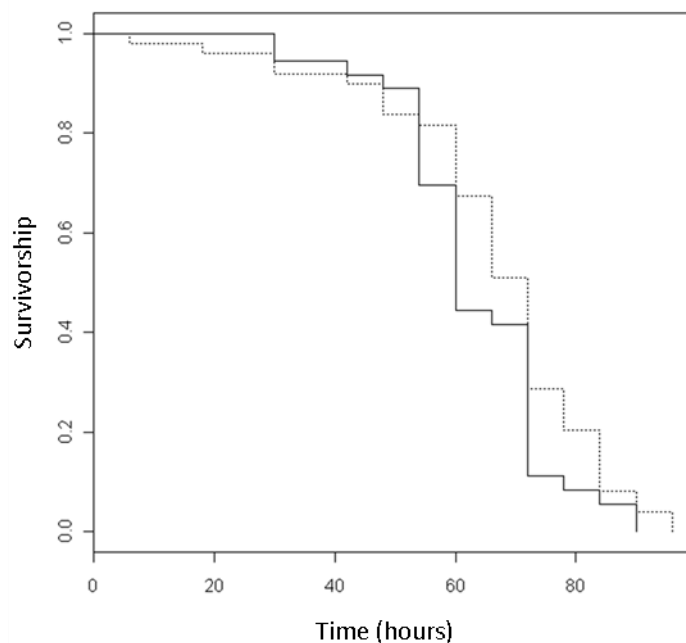


Figure 3.1: Kaplan-Meier survivorship curve of un-irradiated and irradiated (160 Gy) adult male *L. bryoniae* under food and water stress ($p > 0.05$). Solid line is for the un-irradiated males (0 Gy) and dashed line is for irradiated males (160 Gy).

3.4.2 Adult emergence

The percentage of adult emergence was not different between the irradiated and un-irradiated treatments ($p > 0.05$, $t = 0$, $df = 16.5$, figure 3.2a). The percentage of adult emergence in the *L. bryoniae* irradiated with 160 Gy was $75.7\% \pm 4.2$ (mean $\pm$ se) and was $75.7\% \pm 2.2$ (mean $\pm$ se) for the un-irradiated, control group. Even though the mean adult emergence was the same in the two treatments, the range of emergence was greater in the irradiated treatment (50% - 91.7%) than in the control group (58.3% - 83.3%).

There was no difference between the female to male sex ratio in irradiated and un-irradiated *L.bryoniae* ($p > 0.05$, $t = -1.44$, $df = 22$, figure 3.2b). The sex ratio was expressed as a ratio of females. The sex ratio for the *L.bryoniae* irradiated with 160 Gy was 1.2 ± 0.3 (mean number of females:males $\pm$ se) and 0.8 ± 0.2 (mean number of females:males $\pm$ se) in the un-irradiated group. The range of sex ratios in the irradiated group (0.2 - 3.5) was greater than in the un-irradiated treatment (0.3 - 1.7).

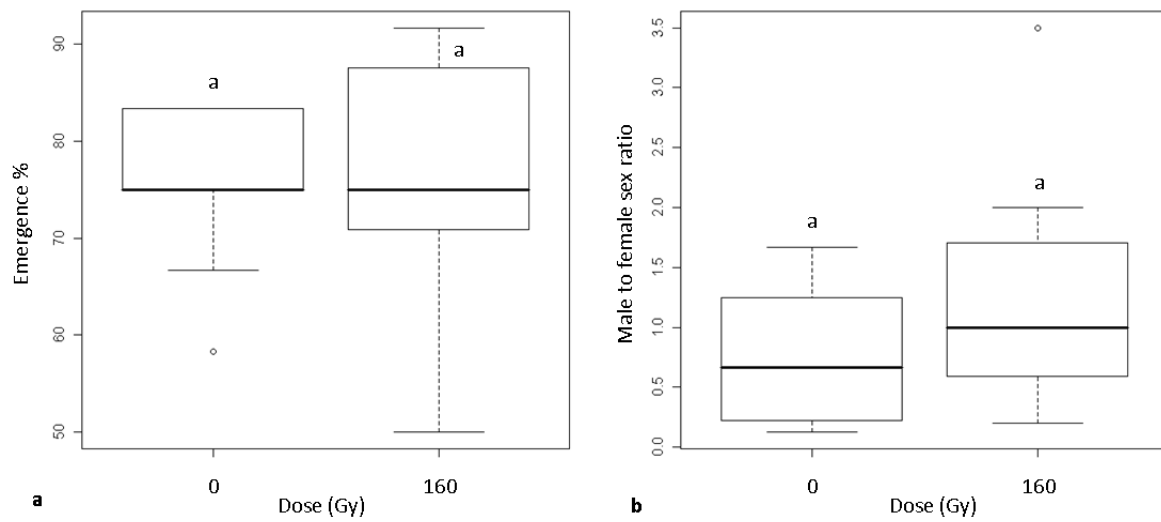


Figure 3.2: Box and whisker plots of a) Percentage of adults emerged from un-irradiated (0 Gy) and irradiated (160 Gy) *L.bryoniae* pupae; and b) Sex ratio (female:male) of un-irradiated (0 Gy) and irradiated (160 Gy) adult *L.bryoniae*. Treatments that were not significantly different ($p > 0.05$) are denoted with the same letter, a.

A greater proportion of adults emerged in the morning, between 0630 to 1229 (period 2) (0.91 ± 0.03 , mean $\pm$ se) than in the afternoon, from 1230 to 1829 (period 3) (0.12 ± 0.02 , mean $\pm$ se) ($z = 5.12$, $df = 9$, $p < 0.001$) (figure 3.3a). No adults emerged from 1830 to 0629 (periods 1 and 4). During period 2, between 0630 and 1229, males and females both emerged in similar proportions, 0.44 ± 0.06 and 0.56 ± 0.06 respectively (mean $\pm$ se) ($p > 0.05$, figure 3.3b).

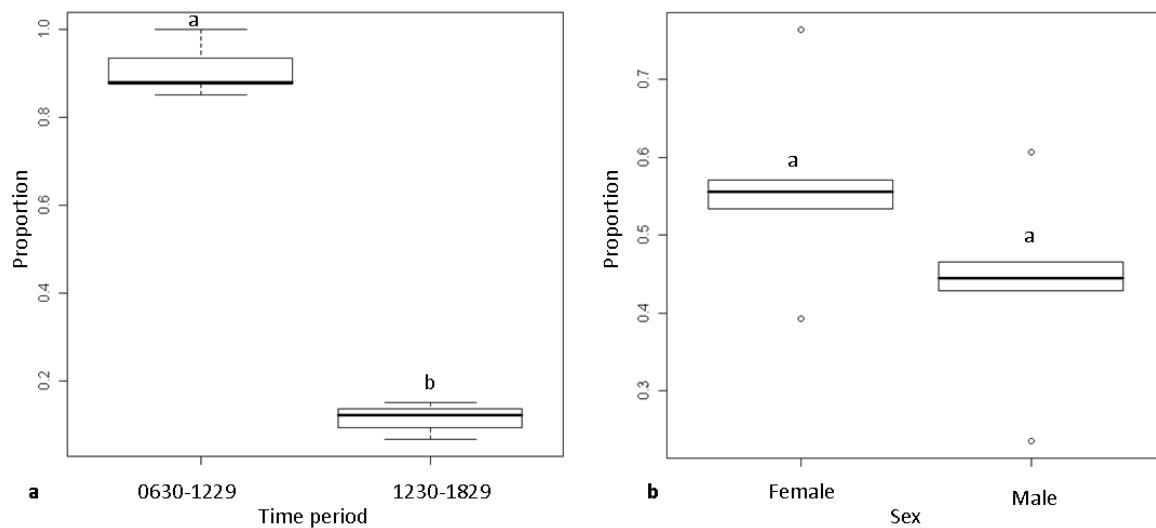


Figure 3.3: Box and whisker plots showing a) the proportion of adult emergence per day in 0630-1229 (period 2) and 1230-1829 (period 3); and b) the proportion of female and male adult emergence during period 2. The letters a and b denote significantly different treatments ($p < 0.05$).

3.5 Discussion

The results in this study are promising for the development of SIT for *L.bryoniae* and are useful in determining the optimum dose to produce sterile high quality males. For an SIT program to be successful, sterile males must be able to compete with wild males for matings, mate and inseminate wild females (Knipling 1955, Robinson 2005). Previous work has shown that *L.bryoniae* females are unable to differentiate between non-viable and viable sperm (chapter 2). In order to compete for matings sterile males must exhibit comparable fitness to that of wild males. Irradiation of pupae 24 to 48 hours prior to eclosion with 160 Gy did not have a detrimental effect on the longevity of sterile males with irradiated males living a comparable time (66.4 hours) to that of un-irradiated males (63 hours). The adult emergence and sex ratio of *L.bryoniae* were also unchanged by irradiation (160 Gy) with irradiated adults emerging at the same rate as un-irradiated (75.7%) with equal sex ratios (1 female:1 male).

This study has demonstrated that the longevity of male *L.bryoniae* deprived of food and water is not affected by irradiation at 160 Gy. When late stage *L.bryoniae* pupae were irradiated under hypoxic conditions the absorbed dose of 160 Gy induced mutations into

the reproductive cells but not into the somatic cells. Reproductive cells are more sensitive to radiation damage than somatic cells (Muller 1927). The relationship between radiation dose and sterility is asymptotic, that is to say, as sterility approaches 100% a disproportionately large increase in dose is required to achieve a relatively small increase in percent sterility (LaChance & Graham 1984, Robinson 2005). *Liriomyza bryoniae* appears to display a “1-hit” dose-sterility relationship, as shown by the dose-sterility relationship in males (chapter 2). Only one mutation is required per gamete, which once fertilised results in the formation of a DLM leading to zygote death (LaChance & Graham 1984, Allinghi *et al.* 2007). At higher radiation doses, not only do the gametes acquire more than one DLM, but genetic mutations also accumulate in the somatic cells. Damage to the DNA of the somatic cells can result in damage to the body of the developing pupa. This affects the ability of the pupa to emerge and also the behaviour of the resulting adult. The results of this study showed that irradiation with an absorbed dose of 160 Gy did not affect the emergence rate. The sex ratio of *L.bryoniae* was unaffected by irradiation at 160 Gy. The longevity of adult males under environmental stress was used to examine the physiological effects of irradiation. There was no difference in male longevity when irradiated as pupae with 160 Gy. Consequently a dose of 160 Gy can be used in further studies to produce *L.bryoniae* adults for SIT that are sterile and exhibit comparable fitness to un-irradiated *L.bryoniae*.

Additional methods were also adopted as part of the study protocol to minimise the deleterious effects of the radiation. Pupae were irradiated 24 to 48 hours prior to eclosion when the majority of pupation had already been completed and the insect had become a pharate (Kaspi & Parrella 2003). During pupation the body structure of the *L.bryoniae* is broken down inside the puparium and then reassembled from the limbless larva into the winged pharate, before emerging from the puparium as an adult. The pupae in this study were also placed in individual wells that were sealed with paraffin film prior to being transported for irradiation, approximately three hours prior to irradiation. During this time, the respiring pupa reduces the amount of oxygen in the well. In a hypoxic environment the rate of respiration slows and the radiation is less likely to cause damage to the somatic cells (Nestel *et al.* 2007). In Tephritid fruit flies, irradiation of pupae in a hypoxic environment minimises the deleterious effects of the radiation and the adult males display a higher

mating competitiveness in comparison to males that were irradiated at ambient oxygen levels (Nestel *et al.* 2007).

On examination of adult emergence it was found that the majority of adult emergence occurred during the period (0630-1229) when the light came on, at 0700. This is true for both sexes, which both emerged during this time. Whilst this study was carried out in a controlled environment room, it would suggest that adult emergence occurs early in the day and, once an adult has fully developed inside the puparium, eclosion is triggered by light. The timing of adult emergence is important to know and can be used in decision making about the release of sterile insects as part of a SIT program. The optimal time to release irradiated pupae in greenhouses would be during the evening, or before sunrise, so that sterile adults can emerge into the greenhouse when eclosion is triggered by sunrise, or by the switching on of artificial lighting. Knowledge of the timing of eclosion is also useful for future studies requiring newly emerged adults so the timing of work can be planned.

Only a few quality parameters were examined in this study: two examined adult emergence and one investigated longevity as a measure of male fitness. For a fully comprehensive measure of male fitness it would be necessary to examine more quality parameters, such as male flight ability, as used for other species (table 3.1). The longevity of males not under environmental stress and the flight ability of irradiated adults would be two further quality tests that would be important to examine for *L.bryoniae*. It is important to assess the flight ability of irradiated *L.bryoniae* as it is important that sterile insects can disperse throughout the greenhouse after release to mate with the wild population. Irradiation 24 to 48 hours prior to eclosion reduces damage to the flight muscles as the wings and flight muscles are already fully formed once the pupa has become a pharate. In the chrysanthemum leafminer, *Liriomyza trifolii* (Burgess), both the proportion of adult emergence and the flight ability of males irradiated with a dose of 170 Gy were not different to those of un-irradiated adults (Kaspi & Parrella 2003). As such, since irradiation at 160 Gy did not have any detrimental effects on adult eclosion, it can be assumed that irradiation with 160 Gy would not have a detrimental effect on the flight ability of *L.bryoniae* males. Assessment of flight ability is still an important requirement to measure male fitness despite this assumption. Data on the fitness of both wild and sterile male *L.bryoniae* are required as a standard against which

future quality testing can be measured. Continued monitoring is required for the production of high quality sterile males as part of any SIT program.

Table 3.1: Quality tests used to assess fitness in Tephritid fruit flies (TFF), Tsetse flies *Glossina* spp., *Liriomyza trifolii* and *Liriomyza bryoniae* (FAO/IAEA/USDA 2003, FAO/IAEA 2006, Kaspi & Parrella 2006)

	TFF	Tsetse	<i>L.trifolii</i>	<i>L.bryoniae</i>
Pupal size	x	x		
Pupal weight	x	x		
Percentage emergence	x	x	x	x
Sex ratio	x	x		x
Timing of emergence	x			x
Flight ability	x		x	
Longevity under stress	x	x	x	x
Longevity		x	x	
Sterility test	x			
Fecundity		x		

The work in this chapter and the previous work (chapter 2) focussed on the determination of an optimum radiation dose which results in the production of high quality sterile males. The absorbed radiation dose, however, is not the only factor affecting the quality of sterile males. There are three branches of quality control: production quality control, process quality control and product quality control (Calkins & Parker 2005). Currently the work on *L.bryoniae* SIT has involved process quality control. Examining the dose-sterility relationship and measuring the emergence and fitness of irradiated *L.bryoniae* are all examples of process quality control measures (Calkins & Parker 2005).

Production quality control examines the inputs of mass-rearing insects for use in SIT (Calkins & Parker 2005). This involves examination of food quality, equipment and insect rearing methods. Small changes in rearing techniques can lead to big improvements in the quality of sterile insects and increase the cost efficiency. The feasibility of a SIT program for *L.bryoniae* is dependent on the development of an efficient mass-rearing system (Lance & McInnis 2005). Currently there is no artificial medium on which to rear *L.bryoniae*, so rearing high

quality insects is dependent on the quality of the host plant. In this study and in previous work (Walker 2007 & chapter 2) *L.bryoniae* were reared on tomato plants (cv. Moneymaker). The density of oviposition on tomato plants, however, is not uniform. There is no density control in *L.bryoniae* oviposition. Female *L.bryoniae* continue to puncture and oviposition in leaves, even at a high density where the subsequent developing larvae will die through a lack of available photosynthetic material with which to feed on due to larval overcrowding. In the chrysanthemum leafminer, *L.trifolii*, females feed from leaves to assess leaf quality and nitrogen content prior to oviposition, as such females display plant preference and it is assumed that this is also true in *L.bryoniae* (Minkenberg & Fredrix 1989, Minkenberg & Ottenheim 1990). Leaves with a high nitrogen content increase the development and survival rate of larvae and consequently results in improved fitness and fecundity as adults (Minkenberg & Fredrix 1989, Minkenberg & Ottenheim 1990). *Liriomyza bryoniae* is a polyphagous insect (Spencer 1973) and has the potential to be reared on other plant species aside from tomato. Worldwide *L.bryoniae* has been observed on cucumber *Cucumis sativus* L., melon *Cucumis melo* L., kidney bean *Phaseolus vulgaris* L., cabbage *Brassica oleracea* var. *capitata* L., lettuce *Lactuca sativa* L., courgette *Curcubita pepo* L., watermelon *Citrullus lanatus* (Thunb.) and aubergine *Solanum melongena* L. as well as in tomato crops (Spencer 1973, Tokumaru & Abe 2005, Tokumaru *et al.* 2007).

The development of an artificial diet would allow for easier, more efficient *L.bryoniae* mass-rearing than is currently possible. Artificial diets have been developed for Tephritid fruit flies and New World Screwworms as part of SIT programs (Melvin & Bushland 1936, Klassen & Curtis 2005). A method of egg collection has recently been developed for *Liriomyza* leafminers (Mizoguchi *et al.* 2011). Females lay eggs into 5-10% glucose solution between two stretched layers of paraffin film (Mizoguchi *et al.* 2011). The egg hatch rate using this method is 80% (Mizoguchi *et al.* 2011). If developed further, eggs collected from between the paraffin film could be transplanted into either an artificial diet, or into host plants to achieve a uniform density for larval development.

Insect strain renewal is important in mass-rearing insects to ensure genetic diversity. Changes in the genetic diversity of an insect colony can be presented as phenotypic changes, such as changes in longevity and sex ratio (Caprio 2009). Insect colonies experience

selection pressure due to colonisation and adaptation to a novel environment. The removal of insects from a colony, for use in studies, results in genetic drift causing random changes in allele frequency within the colony. The decline in heterozygosity can result in a colony with a reduced fitness in comparison to a wild population (Caprio 2009). Maintaining a colony with a large effective population size and infusing the colony with individuals from a wild population can reduce the decline in heterozygosity and ameliorate the effects of genetic drift (Caprio 2009). Approximately four thousand *L.bryoniae* pupae were collected in late April to establish the colony for this study. In late June the population size had declined, partly through sampling for the study, so a further two thousand pupae were collected to increase the size and the heterozygosity of the colony. At present, due to *L.bryoniae* overwintering (van der Linden 1993), a new colony has to be established annually from naturally occurring infestations. It is important for any mass-rearing to ensure measures are taken to ensure the genetic diversity of the *L.bryoniae* colony in order to produce high quality insects.

Product quality control evaluates the efficacy of sterile releases. So far, the development of SIT for *L.bryoniae* has focussed on determining the optimum radiation dose to produce high quality insects for release. The next question that needs to be addressed is to what extent sterile *L.bryoniae* releases suppress an established *L.bryoniae* population. Ideally SIT against *L.bryoniae* would consist of male only releases. Since there is currently no method for pupal sex separation, however, bi-sex releases need to be investigated also. SIT has been proposed as an additional method of *L.bryoniae* suppression that could be used in conjunction with current pest management methods. The release of sterile male *L.bryoniae* and existing pest management methods, such as the inundative release of parasitoid wasps, need to be examined to determine both their compatibility and their relative efficacy.

3.6 Conclusions

Irradiation of late stage *L.bryoniae* pupae with an absorbed dose of 160 Gy does not have a detrimental effect on either adult eclosion proportions or the sex ratio of the emerged adults. Male *L.bryoniae* irradiated with 160 Gy exhibit a high level of sterility and demonstrate male longevity under stress comparable to wild males. This suggests that 160 Gy is a suitable dose of gamma radiation to produce high quality sterile males that are of

comparable fitness to wild males. Future work, examining the efficacy of sterile *L.bryoniae* to suppress a pest population and their compatibility with current pest management methods, can reliably use sterile adults irradiated with 160 Gy.

4. Cage Experiment: Release of sterile leafminers *Liriomyza bryoniae* (Diptera: Agromyzidae) and parasitoids *Diglyphus isaea* (Hymenoptera: Eulophidae)

4.1 Abstract

The tomato leafminer *Liriomyza bryoniae* (Kaltenbach) (Diptera: Agromyzidae) is a primary pest of greenhouse tomato crops in the UK. Sterile insect technique (SIT) is a possible method for *L.bryoniae* suppression that could be used in conjunction with other methods, such as the inundative release of the ectoparasitoid *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae), to achieve greater levels of pest reduction. In a large cage experiment different SIT and *D.isaea* treatments were used to assess the effect of release on an established *L.bryoniae* population. The objective of this study was to compare the effects of male only and bi-sex releases of sterile insects to determine the feasibility of releasing sterile females alongside sterile males. The study also compared the use of *D.isaea* with sterile male releases both separately and concurrently. This determined whether these two methods could successfully be used together. *Diglyphus isaea* was the most successful at reducing mine damage in relation to the control cages. Bi-sex releases of sterile *L.bryoniae* also reduced mine damage; but this was not statistically significant. This, however, suggests that females do not have a significant detrimental effect and that bi-sex releases could be used as a feasible option. A synergistic interaction was not observed when *D.isaea* and sterile males were released concurrently; but this could be attributed to low experimental replication. The high greenhouse temperatures and high pest density in the cages favoured optimal conditions for *D.isaea* oviposition and development. However the possibility of using SIT during the early growing season, when conditions are unsuitable for *D.isaea*, is discussed.

4.2 Introduction

Liriomyza bryoniae (Kaltenbach) is a polyphagous agromyzid leafminer. Originating from Southern Europe (Spencer 1973) it is now a cosmopolitan species and can be found in protected crop greenhouses across Japan, Russia and Northern Europe, including the UK. Historically, *L. bryoniae* was classed as a secondary pest of tomatoes. Since 1985, following a reduction in the use of pesticides and soil sterilisation techniques (Ledieu & Helyer 1985, European Parliament 1991, 2005) and an uptake in the use of biological controls such as predators and parasitoids for integrated pest management (IPM), *L. bryoniae* has emerged as one of the primary pests in protected tomato cultivation in the UK. Without treatment, *L. bryoniae* damage of 30 mines per leaf in the leaves adjacent to the developing tomato truss can result in an associated 10% loss in yield (Ledieu & Helyer 1985).

One of the main methods for *L. bryoniae* control is the inundative release of the parasitoid *Diglyphus isaea* (Walker) (Minkenberg & Van Lenteren 1987, Ozawa *et al.* 1999). The threshold for *D. isaea* release in commercial tomato greenhouses is set at one new mine per plant per week (Sampson & Walker 1998). Under experimental conditions it was noted that *D. isaea* was able to parasitise 95.1% of the available leafminer larvae (Ozawa *et al.* 1999). However, in commercial greenhouse conditions *D. isaea* releases are unable to suppress the pest population to a level that is satisfactory for growers (Jacobson pers. comm) despite its high cost. Releases of *D. isaea* can cost tomato growers up to £3000 per hectare per annum. The rate of *D. isaea* efficiency is reduced during the early tomato growing season due to low *L. bryoniae* population density and mild temperatures (Barclay 1987, Minkenberg 1989, Bazzocchi *et al.* 2003). At 15°C the development time of female *D. isaea* is 25.5 days, which is more than twice the development time of 9.8 days at 25°C (Minkenberg 1989). *Diglyphus isaea* releases are also most effective at high pest densities (Barclay 1987). At low pest densities the reproductive capacity of the female *D. isaea* is limited by their ability to find hosts, rather than by their oviposition rate (Minkenberg 1989, Kaspi & Parrella 2006).

The development of sterile insect technique (SIT) against *L. bryoniae* has been proposed as a novel, additional method of pest suppression that could be used in conjunction with the inundative release of parasitoids such as *D. isaea* (Minkenberg & Van Lenteren 1987, Minkenberg 1989, Ozawa *et al.* 1999). SIT is the release of sterile insects in order to reduce

the reproductive potential of a pest population of the same species (Knipling 1955). Successive releases of sterile insects can suppress the pest population and in some cases even eradicate the pest. In the past SIT programs were designed for areas, such as islands, where it was possible to maintain a barrier to prevent re-infestation through immigration after the pest had been eradicated (Baumhover *et al.* 1955, Knipling 1955, Lindquist 1955). In recent years, it has been suggested that SIT could be developed as a viable option for use in greenhouse protected crops because a greenhouse is an isolated habitat with little or no immigration (Kaspi & Parrella 2003). SIT has already been developed, but not yet used on a commercial scale, for the closely related species the chrysanthemum leafminer *Liriomyza trifolii* (Burgess) (Kaspi & Parrella 2003, 2006).

Previous work developing SIT against *L.bryoniae* has focussed on the production of high quality, sterile males. Sterility was observed in male *L.bryoniae* irradiated with doses of 120 Gy and above (chapter 2). Males irradiated with 150 Gy produced viable offspring in crosses with wild males; whereas mines were not observed when males were irradiated with 160 Gy (chapter 2). Males irradiated with 160 Gy were found to be sterile (chapter 2) and displayed comparable fitness to wild type males, as measured by their longevity under stress (chapter 3). Adult eclosion rates and the sex ratio of the emerging adults were unaffected by the irradiation of pupae at 160 Gy, in comparison to adults from un-irradiated pupae (chapter 3). At 160 Gy irradiated females were also unable to produce viable offspring after mating with wild type male (chapter 2).

Currently there is no known method to separate *L.bryoniae* pupae by sex. The effect of bi-sex releases, in comparison to male only releases, requires investigation. It is not known in bi-sex releases whether sterile females would be able to contribute to population reduction by mating with wild males; or whether the sterile females would reduce the effective sterile to wild male ratio by mating with the sterile males. Models of bi-sex releases suggest that females should not have a detrimental effect as long as they are also sterile (Ailam & Galun 1967, Lawson 1967, Barclay 2005). In species where the female acts as a vector or can cause extensive damage through feeding it is not possible to have bi-sex releases (Lance & McInnis 2005). In *L.bryoniae* sterile females puncture the leaves to feed and lay eggs (Spencer 1973, Benthke & Parrella 1985); but this damage is minimal in comparison to the mine damage

caused by the developing offspring of fertile females (chapter 2). At present the feasibility of an SIT program for *L.bryoniae* depends upon the effect of bi-sex releases.

To evaluate this novel method of *L.bryoniae* pest management SIT must be compared to existing methods under the same conditions. It has been proposed that SIT could be used in conjunction with augmentative releases of biocontrol parasitoids (Knipling 1955, Barclay 1987, Kaspi & Parrella 2006). This is in part due to the different life stages that the two methods target. SIT reduces the reproductive potential of the adult females by preventing the development of the eggs laid by the females (Knipling 1955). *Diglyphus isaea* is an ectoparasitoid that targets late larval instars (L3) of the leafminers. The parasitoid paralyses the L3 larva and then oviposits a single egg next to the paralysed larva. In this study the release of sterile males is evaluated in comparison to the use of *D.isaea*; both as a direct comparison between the two methods and also as a concurrent release of the two methods to assess compatibility. In the chrysanthemum leafminer *L.trifolii* the release of sterile males and *D.isaea* together resulted in a synergistic interaction effect, whereby the reduction in leafminer damage was greater than the sum of the damage reduction for the two methods separately (Kaspi & Parrella 2006).

The aim of this study was to carry out a cage experiment to compare the effects of different treatments against an existing *L.bryoniae* population under the same greenhouse conditions. Building on previous work (chapters 2 and 3) the study was scaled up in size to attempt to replicate the conditions found in a commercial greenhouse, whilst taking into account the limitations of both the available space and the seasonal nature of *L.bryoniae*. All of the experimental treatments were examined in comparison with control populations that received no pest management. This study allowed direct comparison between male only releases and bi-sex releases of sterile insects. There was also a direct comparison between the use of SIT and the use of *D.isaea* as a biocontrol and a comparison with the concurrent release of both sterile males and *D.isaea*. This multi-treatment experiment investigated the compatibility of these two methods and determined whether they have detrimental effects when released together; or whether there is any evidence of a beneficial synergistic interaction, as observed in *L.trifolii* (Kaspi & Parrella 2006).

4.3 Methods

4.3.1 Experimental set up

The experiment was conducted in a greenhouse at Silwood Park Campus, Ascot, UK (51.507N, 0.640W). The experiment was conducted from April to August 2012. The greenhouse was set to 20 – 30°C and automatic overhead shading was used during high light intensity periods to prevent leaf scalding. Humidity was not controlled and natural lighting was used. A culture of *L.bryoniae* was established from a commercial tomato greenhouse with naturally occurring infestations (Wight Salads Ltd, Isle of Wight) by pupae collection in mid April and replenished, to ensure genetic diversity, in mid June. *L.bryoniae* were reared on pest-free ten to twelve week old tomato plants, *Lycopersicum esculentum* (cv. Moneymaker) under greenhouse conditions. Tomato plants were exposed to adult *L.bryoniae* for 48 hours to allow oviposition in a controlled temperature room (20 ± 5°C, 70 ± 5% RH and 16:8 LD) and transferred to a greenhouse (20 ± 10°C) to allow leafminer development to pupal stage.

Twenty wood and insect netting cages (116 x 53 x 180 cm) were set up in a greenhouse (7.5 x 8 m) (figure 4.1). Each cage contained three tomato plants (cv. Moneymaker) that were transplanted into one 25 litre growbag at ten-weeks old (figure 4.1a). The tomato plants were watered as required and fertilised using a liquid feed (N, P, K and micronutrients) fortnightly. After a further six weeks, 14 adult *L.bryoniae* (1:1 sex ratio) were released into each cage (figure 4.1b). The leafminers were pupated in individual wells (7 mm diameter) in 12-well plates on damp filter paper (5 mm diameter) to ensure virginity and were sexed and released into the cage within a few hours of emergence. A further three weeks later the treatments were simultaneously released into the cages.



Figure 4.1: Cage set up a) cages with ten week old tomato plants in b) one of the cages at 16 weeks old at population establishment.

4.3.2 Experimental treatments

The twenty cages were randomly assigned a treatment ensuring four replicates of the following five treatments (figure 4.2). In IM treatments 21 irradiated males were added to achieve a 3:1 sterile male to fertile male ratio. The IM+IF treatments had 42 irradiated males and females added to achieve a 3:1 sterile to fertile insect ratio. In the *D.isaea* treatments 14 *D.isaea* were added. In the IM+*D.isaea* treatments 21 irradiated males in a 3:1 sterile male to fertile male ratio plus 14 *D.isaea* were added. The control cages received no pest management treatments.

Power analyses were used to decide the number of replicates and consequently the number of treatments. This was done to assess the probability of identifying a significant effect given the level of replication, the number of treatments and the effect size. Power analyses were carried out for two levels of effect size: high (0.4) and medium (0.25) to determine whether to have four replicates of five treatments, or five replicates of four treatments. The significance level was set at $p = 0.05$. With a high effect size the power values were 0.20

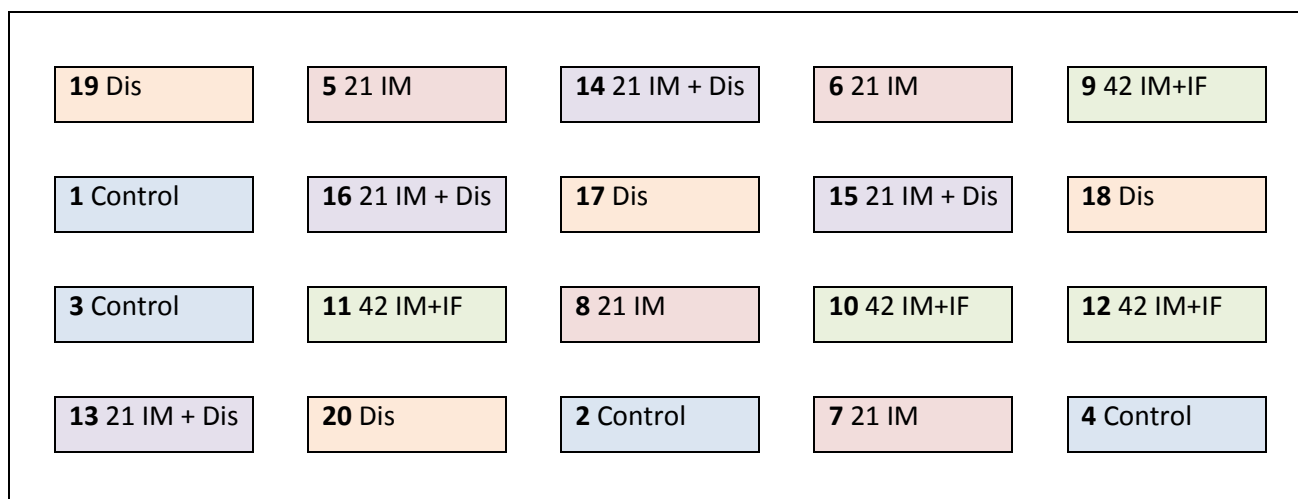


Figure 4.2: Schematic plan of cage treatments. Treatments were randomly assigned.

Treatment codes: IM = irradiated males, IF = irradiated females, Dis = *D.isaea*. Cage number in bold.

with four replicates and 0.24 with five replicates (appendix 4.1). With a medium effect size the power values were 0.10 with four replicates and 0.11 with five replicates (appendix 4.1). At both effect sizes the power values for the different replicate to treatment sizes were of negligible difference. This meant that there would be a negligible difference in choosing four replicates of five treatments over the other scenario.

The sterile adults were irradiated as pupae 24-48h prior to eclosion with 160 Gy gamma radiation using a Gammacell 3000 Elan Caesium-137 source (MDS Nordion, Ottawa, Canada) as determined by previous work on the dose required to produce high quality, sterile male and female *L.bryoniae* (chapters 2 & 3). Pupae were irradiated in individual wells (7 mm diameter) in 12-well plates on damp filter paper (5 mm diameter) and sealed with paraffin film prior to irradiation to create a hypoxic environment. The number of pupae irradiated was greater than the number of irradiated adults required with the assumption of 1:1 sex ratio and 70 % adult emergence (chapter 3). Commercially available adult *D.isaea* ("Miglyphus 250") were purchased from Koppert UK (Haverhill, Suffolk) and released on the day of receipt.

After four weeks, seven weeks since the initial population establishment, the number of leaves per cage that had no damage, puncture damage and mine damage were counted. The number of mines per leaf and the number of pupae, both alive and empty pupal cases, were also counted.

4.3.3 Statistics

All statistical analyses were performed using R (version 2.10.1, www.r-project.org). Generalised linear models (GLM) were used to investigate significant differences between the treatments (Control, IM, IM+IF, IM+D.isaea and D.isaea) and the response variables. The response variables were selected to assess the efficacy of each of the treatments. Response variables were: a count of the total number of leaves; a count of the number of leaves with no damage, with puncture damage and with mine damage; the total number of mines; and the number of alive and total pupae (alive plus empty pupal cases). To fulfil the assumptions of the statistical tests the data on the effect of treatment on the number of leaves with no damage, puncture damage, mine damage and the total number of leaves were square-root transformed. The data on the effect of treatment on the total number of mines and pupae, both alive and total pupae, were also square-root transformed and analysed using GLM. Graphical observation of the square root transformed variables ensured a normal distribution prior to statistical testing. The relationship between the number of pupae and the number of mines was analysed using linear regression, data were not transformed.

4.4 Results

There was no difference between the total numbers of leaves per cage ($p > 0.05$). The mean total leaves per cage were 3973 ± 150 (mean $\pm$ se). There was no difference in the number of leaves without *L.bryoniae* damage between all the treatments ($p > 0.05$, figure 4.3a). The mean number of leaves per cage without *L.bryoniae* damage was 2216 ± 172 leaves (mean $\pm$ se). Similarly, there was no difference in the number of leaves with puncture damage between treatments ($p > 0.05$, figure 4.3b). The mean number of leaves with puncture damage was 1444 ± 98 (mean $\pm$ se) leaves per cage.

The number of leaves with mine damage in the D.isaea treatment (1676 ± 46 , mean $\pm$ se) was reduced in comparison to the number of leaves with mines in the control treatment

(443 ± 94 , mean $\pm$ se) ($p < 0.05$, figure 4.3c). In the IM+IF treatment the number of leaves with mine damage (224 ± 52 , mean $\pm$ se) was almost half the number of leaves (49% lower) than in the control but this was not significant ($p = 0.12$, figure 4.3c). In the IM treatment the number of leaves with mines (461 ± 254 , mean $\pm$ se) was 4% higher than in the control group; but this was not statistically significant ($p > 0.05$). There was a high level of scatter in the results from the two IM replicates with 207 and 705 leaves with mine damage. This was a 53% reduction and a 59% increase in the number of leaves with mine damage respectively in comparison to the control (figure 4.3c).

The total number of mines per cage was reduced by 73% in the D.isaea treatment (219 ± 54 , mean $\pm$ se) in comparison to the control (828 ± 256 , mean $\pm$ se) ($p < 0.05$, figure 4.3d). The IM+IF treatment also showed a reduction in the number of mines (324 ± 93 , mean $\pm$ se) in comparison to the control but this was not significant ($p = 0.14$, figure 4.3d). In the IM

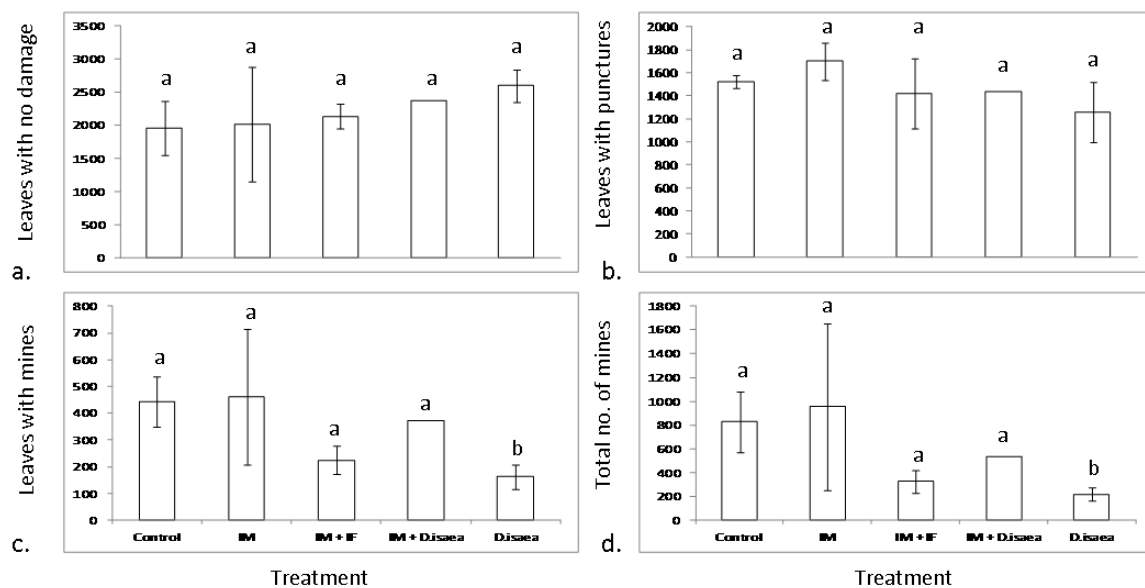


Figure 4.3: Effect of treatment on the mean number of leaves per cage with a) no leafminer damage, b) puncturing only, c) both puncture and mines; and d) the mean number of mines per cage. Error bars represent standard error. Bars labelled a are not significantly different to the control ($p > 0.05$). Bars labelled b are significantly different to the control bar ($p < 0.05$). Replication: Control = 4; IM = 2; IM+IF = 3; IM+D.isaea = 1; D.isaea = 4.

treatment the total number of mines (955 ± 703 , mean $\pm$ se) was 15% higher than in the control group (figure 4.3d); but this was not significant ($p > 0.05$). The total number of mines (535 mines) in the IM+D.isaea treatment was 35% lower than in the control treatment (figure 4.3d); but this was also not statistically significant ($p > 0.05$).

There was no difference in the number of alive pupae per cage for the treatments ($p > 0.05$) (figure 4.4a). On average there were 22 ± 16 (mean $\pm$ se) alive pupae per cage found in the control treatment (figure 4.4a). The mean number of alive pupae in the IM+IF, IM+D.isaea and D.isaea treatments were reduced by 94%, 81% and 86% respectively in comparison to the control treatment (figure 4.4a). The mean number of alive pupae in the IM treatment (49 ± 46 , mean $\pm$ se) was more than double that of the control treatment (22 ± 16 , mean $\pm$ se); but the range of the IM treatment was greater (3 to 95 pupae) than that of the control treatment (1 to 69 pupae) (figure 4.4a).

Similarly, the total number of pupae (both alive pupae plus empty pupal cases) per cage was not different between treatments ($p > 0.05$) (figure 4.4b). The mean total number of pupae in the control treatment was 342 ± 120 (mean $\pm$ se) (figure 4.4b). The mean total pupae in the IM+IF, IM+D.isaea and D.isaea treatments were reduced by 62%, 51% and 78% respectively in comparison to the control treatment; however, there was no statistical difference ($p > 0.05$) due to the large range of pupae in the control treatment (133 to 621 pupae) (figure 4.4b). The mean total pupae in the IM treatment was 440 ± 315 (mean $\pm$ se); however, the number of pupae per cage ranged from 125 to 775 (figure 4.4b).

The total number of pupae counted per cage, both pupae and empty pupal cases combined, were approximately half the number of mines counted per cage with a relationship of 1 pupa to 2.3 mines (figure 4.5). The significant linear model was forced through the intercept

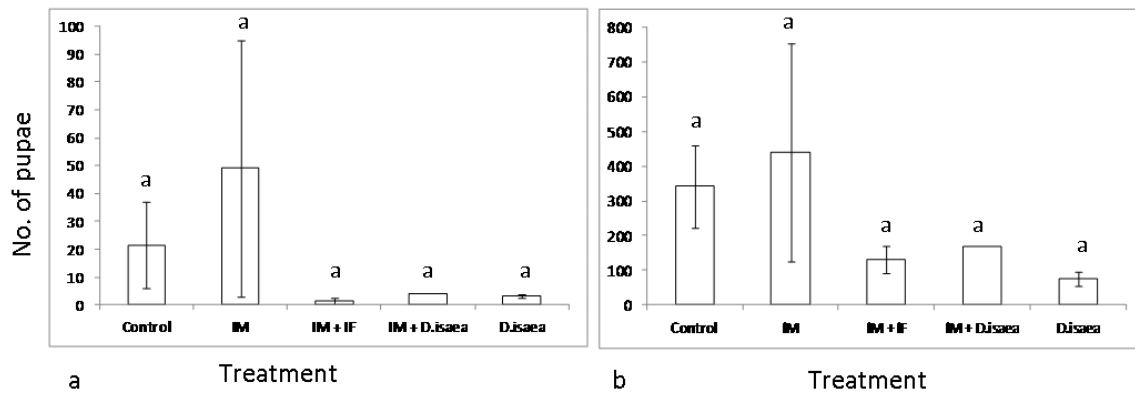


Figure 4.4: Effect of treatment on the number of a) alive pupae and b) total number of pupae counted per cage. Bars labelled a are not significantly different to each other ($p > 0.05$). Replication: Control = 4; IM = 2; IM+IF = 3; IM+D.isaea = 1; D.isaea = 4.

at 0, as no pupae would develop when mines are absent ($p < 0.001$). The highest ratio of 1 pupa to 7 mines was observed in the D.isaea treatment cage with 19 pupae developing from 145 mines (figure 4.5). The lowest ratio of 1 pupa to 1.9 mines was observed in a control cage with 879 mines producing 463 pupae (figure 4.5).

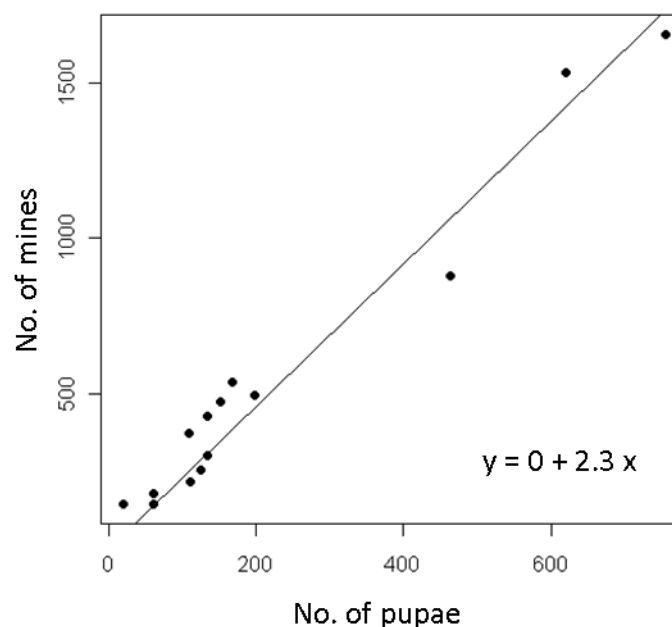


Figure 4.5: Relationship between the total number of pupae counted and the number of mines. ($p < 0.001$, $f = 680$, $df = 13$, $r^2 = 0.98$, $y = 0 + 2.3 x$).

4.5 Discussion

The different life stage targets of SIT and *D.isaea* suggest that these two methods are compatible and could be used simultaneously to suppress an infestation of *L.bryoniae*. SIT targets the reproductive potential of the population; whereas the inundative release of parasitoids, such as *D.isaea*, targets the late larval stages of *L.bryoniae*. In theory the simultaneous use of these two compatible methods for pest suppression should lead to greater pest population reduction than by any one method only (Barclay 1987). This is also because parasitoids are the most effective at high pest population densities, whereas SIT is inversely-density dependent and has increased efficacy at low pest densities (Knipling 1955, Barclay 1987).

One of the issues with using *D.isaea* as a biocontrol method against *L.bryoniae* in tomato greenhouses is that at low pest infestations female *D.isaea* reproductive potential is limited by the searching efficiency of the females, rather than by the rate of oviposition (Minkenberg 1989, Kaspi & Parrella 2006). The inundative release of parasitoids for biocontrol is more effective in high density pest infestations (Barclay 1987). However, the data presented in this study showed that the release of *D.isaea* was the most successful treatment in reducing the *L.bryoniae* damage in comparison to the other treatments, at the rates used in this study. This may be because the cages used provided a finite area and in doing so the population established was of relatively high density in a reduced area for host searching for female *D.isaea*.

Another reported issue with using *D.isaea* is the low level of population suppression at lower temperatures, such as 15 °C, especially during the winter and early spring (Minkenberg 1989, Kaspi & Parrella 2006). The rates of *D.isaea* development and oviposition increase with temperature (Minkenberg 1989). The mean female development time on *L.bryoniae* at 15 °C of 25.5 days is more than double the development time at 25 °C of only 9.8 days (Minkenberg 1989). The optimum temperature for *D.isaea* development on *L.trifolii* is 32.8°C (Bazzocchi *et al.* 2003). In this study the temperature of the greenhouse used was set to be between 20 to 30°C. For the majority of the study period the greenhouse temperature was towards the upper end of this temperature range and frequently

exceeded 30 °C, close to the optimum temperature for *D.isaea* development. In this study, temperature was not a limiting factor in *D.isaea* suppression of *L.bryoniae*.

Bi-sex sterile adult releases reduced both the number of leaves with mines and the total number of mines more than sterile males only and sterile males combined with *D.isaea*. However, even though there was a reduction in mine damage it was not statistically different from mine damage in the control cages. The release of sterile females did not increase the observed amount of pest damage. There was no difference in the number of leaves with puncturing when sterile females were added. Most existing SIT programs use male only releases as bi-sex releases are thought to reduce the efficiency of SIT (Knipling 1955, Barclay 2005). Models show, however, that females may not be detrimental to the SIT program if they are fully sterile and do not add to the damage through extensive feeding (Ailam & Galun 1967, Lawson 1967). If the female adults of a pest species are added to the pest problem or are a vector, then male only releases are the only possibility (Lance & McInnis 2005). It has been shown that female *L.bryoniae* can be sterilised using the same dose as the males (160 Gy) and do not produce offspring when crossed with wild-type males (chapter 2). Currently there are no known methods to separate *L.bryoniae* pupae by sex and, as such, bi-sex releases are the only feasible option for SIT for this species at present.

In the case of the chrysanthemum leafminer *L.trifolii* simultaneous releases of sterile male leafminers and *D.isaea* were shown to have a synergistic effect; in other words, together the treatments reduced leafminer damage by more than the sum of the individual treatments (Kaspi & Parrella 2006). This was not the case in the *L.bryoniae* and the number of leaves with mine damage and the total number of mines were not reduced in comparison to the control cages. There was, however, only one replicate of this treatment and as such it is impossible to make absolute judgements on the presence or absence of synergism. The *D.isaea* parasitoids were not exposed to *L.bryoniae* prior to release.

The sterile male only releases resulted in higher damage levels than that of the control cages. There was low replication for this treatment with only two cages, rather than the designed four. One of these cages had unexpectedly high levels of *L.bryoniae* damage, with more damage seen in this cage, 1658 mines in total, than in any of the control cages, which

ranged from 427 to 1533 mines. The other cage in this treatment had much less damage with only 252 mines in total; but this reduction in *L.bryoniae* damage was obscured by the high damage of the other replicate cage.

Using the data from previous work (chapter 3) the mean sex ratio was determined as 1:1 and the mean percentage adult emergence was 75.7%. The number of pupae irradiated for this study was based upon these determinations a 1:1 sex ratio and 70% emergence were used as a guideline. The mean adult emergence was much lower in this study than the mean of 75.7% and the sex ratio was skewed towards favouring females. As such there were fewer sterile adult males than anticipated and this resulted in lower replication than was planned. This has shown that in planning for future studies the number of pupae irradiated must be much greater than the number of adults required; and that the adult emergence rate estimation must reflect the low end of the range with 50% emergence, rather than the mean, of the data collected previously (chapter 3). The number of pupae irradiated should therefore be double the number of adults required to insure protect against low emergence and a skewed sex ratio.

The number of pupae (both alive pupae and empty pupae cases) counted was approximately half that of the number of mines (1 pupa to 2.3 mines). This is the same ratio that was observed in the male sterility-dose relationship study (chapter 2). The pupae counted were still attached to the leaves; it was not possible to count any pupae that had fallen into the soil for pupation. A proportion of the mines counted had reached maturity and the larva from that mine had developed into a pupa; however not all of the mines counted had reached maturity and either contained alive larvae, or in the *D.isaea* treatments some of the late stage larvae had been paralysed by the female *D.isaea*. Female adult *D.isaea* sting and paralyse L3 leafminer larvae to feed from and also for oviposition (Minkenberg 1989). Due to the large numbers of leaves and mines involved it was not feasible to examine each mine individually to determine the development stage of the larva. However, this data shows that counting the pupae still attached to the leaves could be a useful indicator of population size and, in particular, the size of the next generation.

It is important to use the results presented in this study to determine the feasibility of a SIT program against *L.bryoniae*. Whilst it has been shown that bi-sex releases can reduce *L.bryoniae* damage, the release of *D.isaea* was the most effective method for reducing the pest infestation in this study. However, it is important to note that the use of both of these methods together did not have a detrimental effect and there is some evidence which suggests that that these methods could be used in conjunction to provide a more efficient strategy for *L.bryoniae* suppression.

Since the reduction in soil sterilisation in tomato greenhouses and the consequent survival of overwintering *L.bryoniae* pupae in the soil (Ledieu & Helyer 1985, van der Linden 1993) leafminer populations can be observed in greenhouses early in the growing season at low densities. Currently, the threshold for *D.isaea* release is set at one mine per plant (Jacobson pers. comm.). However, early in the growing season *L.bryoniae* have discrete generations that do not overlap for the first two to three generations (Minkenberg 1989, Boot *et al.* 1992). During this time there is a week where there are no late larval instars available for *D.isaea* to develop on and as a result they are unable to survive between these discrete leafminer generations (Minkenberg 1989, Boot *et al.* 1992).

SIT displays inverse density dependence and is most efficient at low population densities. If the number of sterile males released is the same as at higher pest densities the ratio of sterile to wild males increases resulting in a higher chance a wild female will mate with a sterile male, rather than a wild male (Knipling 1955). Also sterile males actively seek out matings with wild female even at low densities. As such SIT could be used as an early season method for *L.bryoniae* population suppression. In greenhouses that frequently suffer from *L.bryoniae* infestation, particularly those in which the tomato cultivars most susceptible to *L.bryoniae* damage are being grown, sterile adults could be released as a preventative measure against pest establishment. If bi-sex releases were used there would be a small amount of puncture damage due to the sterile females; but as a trade-off to reducing mine damage overall this may be acceptable.

Later in the growing season, when temperatures are higher, inundative releases of *D.isaea* will have a greater effect on pest suppression (Minkenberg 1989). The use of parasitoids

such as *D.isaea* and *Dacnusa sibirica* (Telenga) (Hymenoptera: Brachonidae) could be used in conjunction with the release of sterile adults as part of an IPM strategy to exert greater pressure on a pest infestation by targeting different life stages of *L.bryoniae*. The more targeted, combined use of SIT and parasitoids should provide a more efficacious pest suppression strategy, especially if the pest infestation can be suppressed to below economic thresholds early in the growing season. The frequency of SIT releases and the combination with parasitoid release would however be dependent on the cost. The expense of producing high quality sterile adults is currently unknown, but more work is required to develop mass-rearing techniques for *L.bryoniae*. For SIT to be a competitive pest management option against *L.bryoniae* it would have to provide pest suppression at less than the £3000 per hectare per annum cost of the current biocontrols. Currently there is no efficient method to mass-rear *L.bryoniae* on the scale that would be required for release in commercial greenhouses.

5. Grower questionnaire

5.1 Introduction

To develop a successful sterile insect technique, SIT, program against the tomato leafminer *Liriomyza bryoniae* (Kaltenbach) (Diptera: Agromyzidae) knowledge must be acquired from growers on the frequency of leafminer infestation, their perceived problems and the current phytosanitary and pest management methods used to suppress pest populations. The use of a questionnaire survey is a method that has previously been used to collect information from growers on pest problems (Mexia 1990). Detailed knowledge of the market for a SIT product is a key requirement in the development of a financially viable SIT programme (Quinlan *et al.* 2008). Whilst some of the questions regarding the market issues (Quinlan *et al.* 2008) have been answered to some extent by previous work (chapters 2, 3 & 4), questions on pest frequency and damage level and current control costs have yet to be addressed.

The use of a grower survey is often regarded as a necessity in understanding current pest management practices and for the development of novel pest management methods (Mexia 1990). The advantages of a grower survey at the early development stages of a novel IPM strategy allow for the definition of growers' needs, perceptions, objectives and resource constraints (Reichelderfer *et al.* 1984, Mexia 1990). It can also be used to identify research gaps as perceived by the growers (Reichelderfer *et al.* 1984, Mexia 1990). Semi-structured grower questionnaires have been used to collect information about control methods for IPM in tomato crops in Spain (Guillén *et al.* 2008). Information was collected on the different methods used and also the reasons why farmers had, or had not, adopted the different pest control methods (Guillén *et al.* 2008).

The aim of this chapter is to design and circulate a questionnaire to tomato growers in the UK to collect data on the frequency and severity of *L.bryoniae* infestations in greenhouse tomato crops. Data will also be collected on the tomato crops: the cultivars grown, time of tomato transplant and removal and any phytosanitary measures used. This data may help identify any areas which could be used to explain the frequency or severity of *L.bryoniae*

infestations. The aim of this questionnaire is to collect data that can be used to understand the market and make decisions on the financial feasibility of SIT against *L.bryoniae*.

5.2 Methods

The questionnaire was divided into two main sections: the greenhouse environment and the tomato leafminer (appendix 5.1). The first section focussed on the greenhouse environment: the area under glass, cultivars grown, planting and removal dates and greenhouse phytosanitary measures. This section consisted of ten questions. Three required a single word or number answer (Q. 1, 2 & 9). Four of the questions were dichotomous format questions requiring a yes/no answer or the selection of one of two options (Q. 6, 7, 8 & 10). Two questions were multiple choice questions with tick boxes (Q. 4 & 5). Question 3 was a table to list the cultivars of tomato grown and the conventional or organic area grown.

The second section focussed on *L.bryoniae* infestations and consisted of eight questions. The first question of this section (Q. 11) was a dichotomous question so that growers that never experience *L.bryoniae* problems did not have to complete the rest of the questionnaire. Four of the questions in this section were short answer questions requiring a number or year (Q. 12, 13, 14 & 16). One question on infestation frequency was a nominal response question with multiple choice tick boxes (Q. 15). The question (Q. 17) on tomato susceptibility to *L.bryoniae* was a table which required respondents to rank different tomato categories on a scale of 1 to 5 as a multiple ordinal response question. This was followed up with an open ended question to allow the respondent to comment more freely on their perceived cultivar susceptibility (Q. 18).

Short answer questions and tick boxes were used to ease answering to encourage the respondent to answer. Easy, general questions about the greenhouse were put in at the start and more complex questions on dates and perception were at the end of the questionnaire (Milner-Gulland & Rowcliffe 2007). The questions were worded to be short and simple to be clear and easily understood (Milner-Gulland & Rowcliffe 2007). Jargon words were avoided. The tomato leafminer *Liriomyza bryoniae* and the damage caused by it were explained in a cover letter (appendix 5.2). This was to avoid confusion between

L.bryoniae and *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), an invasive leafminer moth species of tomato. At the beginning of section B “the tomato leafminer *Liriomyza bryoniae*” was referred to in full (appendix 5.1). After that it was referred to as “the tomato leafminer” for simplification.

A preliminary version of the questionnaire was discussed separately with a pest management consultant and with a tomato grower. Changes were made to the structure of the questionnaire following both of these consultations to encourage responses.

A cover letter was written and distributed to accompany the questionnaire (appendix 5.2). The cover letter was used as a means of getting informed consent from the respondent (de Vaus 2002). The cover letter explained about the development of SIT against *L.bryoniae* and the rationale behind the questionnaire. Information was provided about the researcher involved and the funder of the work, the Horticultural Development Company, HDC. An offer to answer any questions that the respondent had about the work was made. The responses were guaranteed to remain anonymous. By reading the cover letter and responding to the questionnaire the respondent gave informed consent for their responses to be used in this work, with the condition of anonymity, to conform with the conditions explained in the cover letter (de Vaus 2002).

The questionnaire and cover letter were initially distributed at a Tomato Growers’ Association, TGA, meeting on the 15/02/2012 by Becky Turner, a research manager at the HDC. The questionnaire and cover letter were circulated electronically prior to the meeting and paper copies were also available on the day. The questionnaire was then mentioned in the HDC weekly email on the 17/02/2012 under the section on Protected Edibles. A reminder email was sent out to industry members on the 13/04/2012 by the HDC, as shown in appendix 5.3. Growers were also reminded about it at the TGA meeting in June 2012. Responses were emailed or posted to Louise Arculus the communications administrator at the HDC and forwarded on for collation and analysis.

5.3 Results and discussion

Unfortunately there were only eight responses to the questionnaire. As such only descriptive statistics can be used; analytical statistics cannot be used as the number of responses was too low. This is especially true for some questions where responders did not provide a response. The responses were randomly assigned a number for analysis only to preserve the anonymity of the respondent to conform to the conditions of informed consent (de Vaus 2002).

5.3.1 Greenhouse environment

To preserve the anonymity of the responding growers, particularly due to the low response ($n=8$), the county in which the greenhouse is based was changed into a region of the UK. Two growers were from the South of England, two from North East England, three from North West England and one from Scotland (table 5.1). The mean area under glass was 3.71 ha; but this varied greatly with one grower having 14.5 ha and one having only 0.09 ha under glass (table 5.1). Out of the total area of 29.64 ha included in this survey only 0.66 ha were designated as “organic” tomato production, rather than “conventional”. The total area of tomato production under glass in the UK is estimated at 178 ha in 2011 (Source: Tomato Growers’ Association Statistics). This means that only 17% of the UK growing area is represented by the responses from the questionnaire. Respondents reported growing 18 different cultivar varieties (table 5.2). Classic varieties had the highest area under glass with 5.62 ha (table 5.2). Cherry cultivars had the second highest area with 4.29 ha under glass. Midi plum and cocktail cultivars had the smallest area under glass with 0.307 ha and 0.627 ha, respectively (table 5.2). One respondent declined to say which cultivars they produced but listed the tomato category instead; another declined to give areas.

Table 5.1: Data on glasshouse environment, tomato cultivars, growing season and greenhouse phytosanitation.

Grower	Region of UK	Area under glass (ha)	Date of transplant	Date tomatoes removed	Months in greenhouse
1	South	14.5	Beginning December	End November	12
2	North East	4.6	Beginning January	Mid November	10.5
3	North East	2	Beginning March	End November	9
4	North West	0.45	Beginning January	End November	11
5	South	0.2	Beginning January	Beginning November	10
6	North West	0.2	Mid January	Mid October	9
7	Scotland	0.09	End March	Mid November	7.5
8	North West	7.6	Beginning January	Mid November	10.5

Grower	Plant debris removed?	Plastic or Mypex?	Plastic removed annually?	How many seasons Mypex left in place?	Propagation method
1	Yes	Both	Yes	6	Commercial
2	Yes	Plastic	Yes	-	Commercial
3	Yes	Plastic	Yes	-	Commercial
4	Yes	Mypex	-	5	Commercial
5	Yes	Plastic	Yes	-	Commercial
6	Yes	Mypex	-	5	Commercial
7	Yes	Plastic	No	-	On Site
8	Yes	Mypex	-	7	Commercial

Category	Cultivar	Area (ha)	Total area (ha)
Cherry	Claree	0.02	4.29
	Conchita	0.015	
	Florino	0.005	
	Piccolo	4	
	-	0.25	
Baby plum	Golden angelle	0.22	0.78
	Lipso	0.34	
	Sweetelle	0.22	
Midi plum	Sunstream	0.307	0.307
Classic	Carousel	-	5.62
	Encore	4.92	
	Mecano	0.66	
	Octavio	0.04	
Large vine Cocktail	Elegance	-	0.627
	Prolyco	0.12	
	RZ 72-144	0.307	
	-	0.2	
Beef	Rojito	1.27	1.27
Novelty	Green Tiger	0.205	0.815
	Jester	0.61	

Table 5.2: The areas of tomato cultivars grown in the UK by respondents (n = 8).

Five of the eight growers transplanted their tomato plants into the greenhouses at some point in January (figure 5.1, table 5.1). One grower transplanted earlier than this at the beginning of December. The other two growers transplanted later in March. Seven of the growers removed the tomato plants from the greenhouse in November; but one grower removed the plants earlier in mid October (figure 5.1, table 5.1). The mean length of the growing season for tomatoes in the greenhouse was 9.9 months (table 5.1). The longest growing season was almost 12 months with plants transplanted at the beginning of December and removed at the end of the following November (figure 5.1). The shortest growing season was in Scotland where the tomatoes were transplanted at the end of March and removed 7.5 months later in mid November (figure 5.1). This grower was also the one that propagated new season tomato plants on site (table 5.1). The other seven growers sourced the new season tomato plants from commercial propagators (table 5.1). At the end

of the growing season all of the growers removed plant debris from the greenhouse (table 5.1).

Four of the growers used plastic to cover the floor of the greenhouse and three used Mypex (table 5.1). One of the growers used a combination of both plastic and Mypex. It is not known whether they are used together or, since the grower in question had 14.5 ha under glass, whether some of the greenhouses on site used plastic and others Mypex. The growers that used plastic removed and changed it annually, apart from the grower in Scotland with only 0.09 ha under glass (table 5.1). Greenhouses using Mypex left it there for multiple seasons and it was changed every 5.75 years on average (table 5.1).

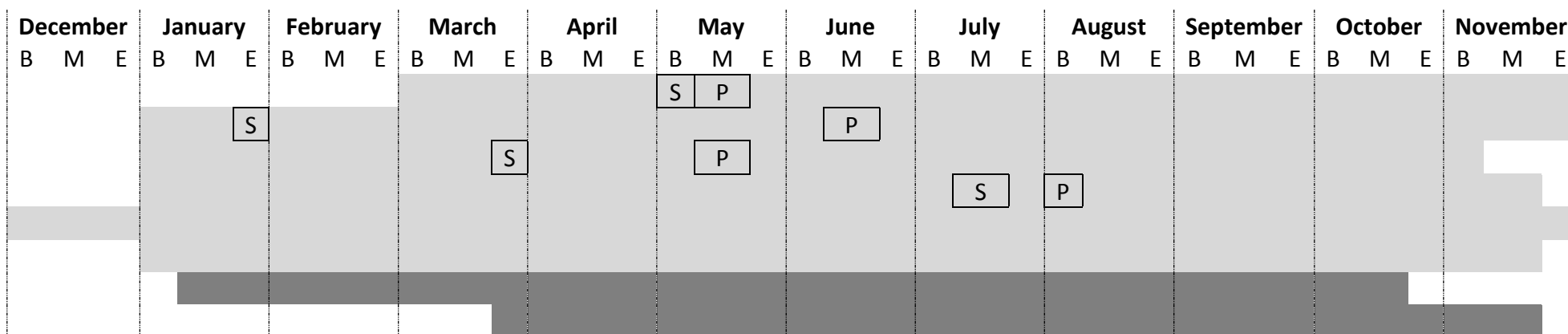
5.3.2 The tomato leafminer *Liriomyza bryoniae*

Two of the respondents stated that their greenhouses had never had an infestation of *L.bryoniae* (table 5.3). Out of the six respondents that stated their greenhouses had experienced *L.bryoniae*, five of them said that their greenhouses had *L.bryoniae* infestations during the previous growing season in 2011 (table 5.3). The other grower last had a *L.bryoniae* infestation in 2008 (table 5.3). Three of the respondents said that the frequency of *L.bryoniae* infestation was every year (table 5.3). One grower said the infestation frequency was once every five years and another said once every ten years (table 5.3). One respondent declined to answer this question.

The number of weeks after transplant when the *L.bryoniae* infestation was spotted varied greatly, ranging from 3 to 29 weeks after transplant (figure 5.1, table 5.3). One infestation was seen at the end of January, whereas another infestation was not noted until mid July (figure 5.1). The peak of *L.bryoniae* infestation also varied but was estimated to be between the middle of May and the beginning of August (figure 5.1). The estimated percentage of the crop affected varied greatly from 3% up to 100% with a mean of 27% (table 5.3). The wide range of percentages probably shows that not only are some cultivars more

Table 5.3: Tomato leafminer *Liriomyza bryoniae* infestation frequency and timing during the growing season. The time at which *L.bryoniae* were spotted and the peak of infestation were measured in weeks since tomato transplant into greenhouse. the end point of infestation was not noted. The frequency of infestations is the number of years estimated between infestations. 1 is annually and 5 is once every 5 years.

Grower	<i>L.bryoniae</i> infestation?	Year of last infestation?	<i>L.bryoniae</i> spotted (Weeks)	Peak of infestation (Weeks)	Frequency of infestations	Estimated % crop affected
1	Yes	2008	Mid summer	-	5	10
2	Yes	2011	-	-	-	5
3	Yes	2011	10	12	1	3
4	Yes	2011	3	24	1	25
5	Yes	2011	12	20	1	100
6	No	-	-	-	-	-
7	No	-	-	-	-	-
8	Yes	2011	29	33	10	20



- Growing season - crops infested with *L. bryoniae*
- Growing season - crops never infested with *L. bryoniae*
- S Point at which *L. bryoniae* infestation identified in crop
- P Estimated peak of *L. bryoniae* infestation

Figure 5.1: Calendar of greenhouse tomato growing season of respondents. Two growers never experience *Liriomyza bryoniae* infestations, as shown in dark grey. The other growers experience *L. bryoniae* infestations at some point in the growing season. “S” marks the point at which *L. bryoniae* is identified in the crop and “P” marks the estimated time of the peak of the infestation. The end point of the infestation was not reported. B, M and E represent the beginning, middle and end of the month, respectively.

susceptible than other; but also that grower perception is also important in pest estimation. For some of the smaller growers it is possible that *L.bryoniae* can move between all the cultivars easily and infest them all. The grower that estimated that 100% of their crop was infested with *L.bryoniae* had 0.2 ha under glass and grew three cultivars of cherry tomato and one classic cultivar.

When asked to rate the susceptibility, on a scale of 1 to 5, with 1 having the least amount of yield loss and 5 the most amount of yield loss, of the different categories of tomato to *L.bryoniae* infestation only three respondents answered. One grower rated them all as unsusceptible to yield loss (1) stating that the “infestation needs to be gigantic to have yield loss”. Another grower rated classic at 2 and cherry tomatoes at 3. The other grower rated cherry, loose cocktail and vine cocktail as having the least yield loss (1). The cultivars that growers believed to be the most susceptible to *L.bryoniae* infestation were named as Conchita and Piccolo, both cherry cultivars, and Santa, a baby plum cultivar. This shows that the perception of growers agrees with previous observations that certain cherry, cocktail and campari cultivars such as Piccolo and Conchita are susceptible to foliar damage by *L.bryoniae* (Jacobson 2008). One grower suggested that with the Conchita cultivar there was a knock on effect with *Macrolophus caliginosus* (Wagner) (Heteroptera: Miridae); but it is not known to what extent this happens.

5.4 Conclusions

Due to the low number of responses (n = 8) no real conclusions can be made from the data collected about the problem of *L.bryoniae* in the UK. Only a small proportion of the UK tomato growers were represented by the results of this questionnaire and as such the results were not representative. The respondents represented only 17% of the tomato production area under glass in the UK and only an estimated 29% of the number of TGA members (Source: Tomato Growers' Association Statistics). The results, however, did show that not all tomato growers in the UK do have *L.bryoniae* infestations. Of those that did some experience infestations annually, while others experience them less frequently. The time when *L.bryoniae* populations are first noticed in greenhouses also varies. The variation in frequency and timing of occurrence makes the market for *L.bryoniae* SIT difficult to predict on this data alone. The need for sterile insects would fluctuate depending on the

year. Preventative releases of sterile *L.bryoniae* early in the growing season in combination with greater phytosanitary measures to prevent mid-season immigration may help to reduce infestations. To understand the market better and to make predictions on the need for SIT more detailed research needs to be undertaken with extra effort to ensure that a greater proportion of the tomato growers in the UK are represented. In order to better understand the market, research is required to provide answers to the following questions:

- How much is spent in a typical year to control insect and mite pests?
- Which pest management methods are currently used to control tomato leafminer outbreaks?
- Are soil sterilisation methods employed?
- What are the costs, such as labour costs, associated with pest management?

The use of semi-structured interviews conducted on a one to one basis, such as those used by Guillén *et al.* (2008) on IPM in tomatoes in Spain, may prove more conducive to collecting detailed market data on the perceptions of *L.bryoniae* problems and the current pest management methods. Semi-structured interviews were used in The Netherlands to collect data from 95 horticultural growers on energy use policy and energy saving investments (Verstegen *et al.* 2004). Questionnaires and semi-structured interviews were also used in Brazil to understand the use of technology available to organic vegetable producers (Tereso *et al.* 2012). A sample of 20 respondents would provide a better representation, as this would represent more than 70% of the tomato growers in the UK.

6. General Discussion

6.1 Overview of findings

The aim of this thesis was to determine whether sterile insect technique, SIT, is a feasible method of pest management against the tomato leafminer *Liriomyza bryoniae* (Kaltenbach) to reduce damage to below an economic damage threshold. At high pest infestations, leaf puncturing and mine damage can cause a loss in yield of up to 17% and reduction in the quality of tomatoes produced (Helyer & Ledieu 1985). The combined loss of quality and yield can result in up to a 20% reduction in the monetary value of the tomato crop at high infestation levels (Helyer & Ledieu 1985). Whilst not all tomato growers in the UK experience *L. bryoniae* infestations every year (chapter 5), in a market producing 70,540 tonnes of tomatoes with a value of around £175 million (Jacobson & Morley 2007), losses of up to 17% would be substantial. This is particularly significant for growers that focus on the production of specialist and cherry varieties, such as the Piccolo cultivar, which are the most susceptible to *L. bryoniae* damage (Jacobson & Morley 2007).

One of the main aims of this thesis was to understand the relationship between dose, sterility and fitness in male *L. bryoniae*. SIT is dependent on the production of high quality males that are sterile and exhibit comparable fitness to wild males so that they can compete and mate successfully with wild females. High levels of sterility were observed in male *L. bryoniae* after irradiation of the pupal stage with doses of 120 Gy up to 160 Gy (chapter 2). Further work showed that no viable offspring were produced from crosses between wild females and males irradiated with 160 Gy, whereas some offspring did develop from crosses with males irradiated with 150 Gy and this dose was therefore excluded (chapter 2). In most SIT programs 96 - 99% sterility is required to achieve population suppression. Due to the low replication in the study and the presence of viable offspring at 150 Gy it was assumed that the actual sterility level at 160 Gy was likely to be lower than the 100% sterility that was observed at that dose in the available sample. The irradiation of late stage *L. bryoniae* pupae with 160 Gy did not affect the rate of adult emergence after pupation; nor did irradiation skew the sex ratio with adults emerging, with a sex ratio of one male to one female (chapter 3). The longevity of males deprived of food and water was also not affected by irradiation

with 160 Gy (chapter 3). The work in chapters 2 and 3 established that the irradiation of *L.bryoniae* pupae 24 to 48 hours prior to eclosion with a dose of 160 Gy produced sterile males with comparable fitness to that of un-irradiated adults.

Another important objective of this thesis was to examine the efficacy of sterile male releases in comparison to existing biocontrol methods against *L.bryoniae*. It was also important to determine whether sterile male releases could be used in conjunction with the existing biocontrols, as an additional method to be used in an IPM strategy against *L.bryoniae*. Using a multi-treatment cage experiment, the efficacy of sterile male releases was examined as a direct comparison, and in conjunction with, inundative releases of the parasitoid *Diglyphus isaea* (Walker) against an established *L.bryoniae* infestation. *Diglyphus isaea* was the most successful treatment at reducing mine damage in relation to the control cages, probably due to the high temperatures and high pest density favouring *D.isaea* oviposition and development (chapter 4). Whilst a synergistic interaction was not observed with concurrent releases of *D.isaea* and sterile males, the two methods were shown to be compatible and target different life stages of *L.bryoniae*. The success of a SIT programme against *L.bryoniae* depends not only on the ability of sterile insects to suppress a population; but also on its compatibility with existing pest management methods.

6.2 Current limitations

One of the major issues with developing SIT for *L.bryoniae* is the current inability to separate pupae by sex. The majority of SIT programs use male-only releases. The use of bi-sex releases reduces the efficiency and the release ratio has to be increased to compensate for the presence of females that may distract sterile males from mating with wild females; this in turn increases the cost of production. The release of females cannot be considered if they are the main damaging stage of the pest and add to the pest problem, or are a vector of a virus or parasite (Lance & McInnis 2005). Female *L.bryoniae* were found to be sterile after irradiation with 160 Gy, producing no viable offspring after mating with un-irradiated males (chapter 2). When the efficacy of bi-sex releases were compared to male-only releases against an established pest population, as part of the multi-treatment cage experiment (chapter 4), it was shown that sterile females did not have a significant

detrimental effect. This suggests that bi-sex releases are feasible; but that the ratio of sterile males to wild females may have to be adjusted to compensate for the presence of females.

A current limitation in the use of SIT against *L.bryoniae* is the mass-rearing process.

Currently, the mass-rearing of *L.bryoniae* is time consuming and inefficient. This could be overcome with the development of an artificial medium on which to rear *L.bryoniae*.

Recently a method for egg collection has been developed for *Liriomyza* spp. (Mizoguchi *et al.* 2011). Females lay eggs into two layers of stretched paraffin film containing a 5 - 10% glucose solution (Mizoguchi *et al.* 2011). The eggs laid in this manner hatch at a rate of 80%. Further development is still required for this method to allow for the development of the larvae through to pupation.

6.3 The future of SIT against *Liriomyza bryoniae*

In the past, *L.bryoniae* was seen as a secondary pest of tomato greenhouse crops (Helyer & Ledieu 1985). Over the last 25 years, whilst not all growers experience infestations of the same frequency or severity, *L.bryoniae* has become a primary pest of greenhouse tomato. This increase in pest status is, in part, due to an increase in the uptake of biocontrols for pest management and the associated shift away from the use of pesticides, particularly broad spectrum pesticides (Helyer & Ledieu 1985). There has also been a reduction in the use of soil sterilisation using steam (Helyer & Ledieu 1985). Soil sterilisation kills *L.bryoniae* pupae that are overwintering in the soil in the greenhouse. Re-introducing soil sterilisation, or steam sterilisation of the greenhouse between growing seasons may help to eliminate overwintering pupae, or any residual pupae in the greenhouse, before the new season tomato plants are transplanted. In greenhouses that frequently experience infestations the preventative release of sterile *L.bryoniae* in conjunction with increased phytosanitary measures, such as steam sterilisation, may help to prevent the establishment of *L.bryoniae* pest populations.

The development of SIT against *L.bryoniae* still requires further work to become a viable business and pest management option. Currently, some of tomato growers experience only infrequent *L.bryoniae* infestations, whilst others experience severe infestations every year. This makes the market for *L.bryoniae* SIT difficult to predict on a yearly basis. Also the

requirement for sterile insects would be intermittent, with a higher need for sterile releases early in the growing season than later. A possibility to overcome this would be to utilise the ability of *L.bryoniae* to overwinter to stockpile pupae. Dormancy and cryopreservation are two methods frequently used to stockpile and store insects to extend the shelf-life and maintain quality prior to use (Leopold 2007). The codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) is stored in diapause to stockpile for when the pest population is unexpectedly high and as a reserve population for mass-rearing (Bloem *et al.* 2000, Dyck 2010). *Cydia pomonella* stored in a state of diapause were shown to be of high quality in comparison to non-diapause moths, especially when released during low spring temperatures (Dyck 2010). The production of *C.pomonella* during the autumn and winter to stockpile also allows for more cost efficient use of the production facility (Bloem *et al.* 2000). *Liriomyza bryoniae* overwinters in an extended pupation (van der Linden 1993). Whilst it is not known for sure, it is thought that extended pupation of *L.bryoniae* is triggered through a combination of low temperature and short day length (van der Linden 1993). Overwintering pupae, however, were observed developing in controlled environment rooms after early instar larvae development in greenhouses with natural lighting. This suggests that photoperiod and temperature cues throughout the larval development are triggers for the onset of overwintering. Further understanding of the onset of overwintering could be harnessed to induce extended pupation into irradiated pupae. When required, pupae could be re-activated from extended pupation and released into greenhouses.

Another option to make *L.bryoniae* SIT a financially feasible business is to combine it with SIT for other greenhouse pest species to increase the market size. The combination of providing SIT against more than one greenhouse pest would add increased financial stability, particularly if it possible to stockpile sterile insects. SIT has already been developed, but not used commercially, against greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyroididae) which is an economically important pest of protected crops (Calvitti *et al.* 1998). Using gamma irradiation, males can be sterilised using a dose of 50 Gy and females with 60 Gy (Calvitti *et al.* 1998). The western flower thrip *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and fungus gnats (Diptera: Sciaridae & Mycetophilidae) are possible candidates for SIT development. *Frankliniella occidentalis* produces haploid males using arrhenotokous parthenogenesis; but

fertilises eggs to produce diploid females. SIT could not be used to suppress the production of males; but it could be used to reduce the female thrips population. A mass-rearing method has already been developed for thrips (Murai & Loomans 2001). Eggs are laid through paraffin film into water and larvae feed on pollen and germinated broad bean seeds (Murai & Loomans 2001). The costs of rearing more than one species at the same site would increase. The benefits from sharing an irradiator (either gamma or X-ray), the increased market size and a more stable income would hopefully balance the increased costs.

6.4 Alternatives to SIT using gamma irradiation

The majority of biocontrols used have been developed for greenhouse pests, particularly in greenhouse vegetable production. There has been a shift towards using pesticides against novel pest species for which there is no biocontrol agent, however, meaning that the affected greenhouse can no longer rely on biological controls for pest management (Parrella 2008). This is particularly important for emerging pests such as *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae). The development and implementation of SIT should be examined as a viable option for pest management in greenhouse crops to control both existing and emerging pests, as an alternative to pesticides that can be used without upsetting the balance of biocontrol.

To date, the majority of SIT programs use ¹³⁷Caesium or ⁶⁰Cobalt gamma irradiators to sterilise the insects. However, increased security concerns over the use of radioactive isotopes for terrorism have led to greater restrictions and regulations regarding the transboundary shipment of radioactive material (IAEA 2007). The company responsible for the manufacture of the Gammacell irradiators usually used in SIT programmes has also ceased production of these irradiators (IAEA 2007). As such, alternatives such as X-ray irradiators and RIDL technology are being used to replace the gamma irradiators, as described below.

X-ray irradiators are a good alternative to gamma irradiators. New generation self-shielded X-ray irradiators are being trialled in conjunction with the IAEA as a direct replacement for gamma irradiators in SIT programmes (IAEA 2007, Mastrangelo *et al.* 2010, Mehta & Parker 2011). X-ray radiation is directly comparable to gamma radiation and the relative biological

effectiveness can be easily determined (Mastrangelo *et al.* 2010). In the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) 99% sterility can be induced in males with 124.9 Gy gamma radiation or 91.2 Gy X-rays (Mastrangelo *et al.* 2010). The new generation X-ray irradiators run on electricity and the X-ray beam can be turned off when not in use (IAEA 2007). One of the advantages of X-ray irradiators to gamma irradiators is the lack of security issues. There are no difficulties involved with transboundary shipments of the X-ray irradiator and as such the cost of transport is greatly reduced. The cost of transporting an X-ray irradiator from the manufacturer to the SIT production facility is US \$5000, ten times less than the cost for a gamma irradiator (IAEA 2007). X-ray irradiators provide an easy replacement for gamma irradiators in SIT programmes, not only for SIT of a new pest species; but also for existing SIT programmes that are starting production in a new location.

RIDL is the 'release of insects carrying dominant lethal' genes (Black *et al.* 2011). RIDL insects are genetically modified by the insertion of a lethal mutation gene (Black *et al.* 2011). The lethal mutation is female-specific and causes the production of a cytotoxin in the absence of an additive in the diet. Females reared in the lab on media containing this additive are viable; but once the additive is removed from the diet the gene inserted into the female insects' expresses a cytotoxin (Black *et al.* 2011). RIDL males that mate with wild females do not produce female offspring that contain lethal mutations and die as a result; but are able to produce viable male offspring that also contain the RIDL lethal mutation. RIDL technology has a similar action on the population as with SIT (Black *et al.* 2011). Successive releases of RIDL males can suppress and even eradicate a pest population. RIDL technology has been developed for the *Aedes aegypti* L. (Diptera: Culicidae), which is the mosquito vector of dengue fever, and for agricultural pests including the pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), a pest of cotton (Oxitec 2002-2012). RIDL technology provides an alternative to traditional SIT as it can be developed for species that are not suitable for sterilisation using gamma radiation, that can repair mutations caused by irradiation and have complex mating systems (Tothová & Marec 2001, Lance & McInnis 2005). A current disadvantage to RIDL technology is that the release of genetically modified (GM) organisms is, at present, not allowed in the European Union (EU). The European Food safety Authority (EFSA) has recently released a draft document of

guidance for the environmental risk assessment of the release of GM animals, including insects, into the environment (EFSA 2012). The guidance is to assess the potential adverse effects of GM animals, characterise the hazards and exposure and to develop strategies to manage the risks (EFSA 2012). Until the release of GM insects has been approved in the EU, RIDL cannot provide an alternative method of pest suppression to gamma or X-ray induced SIT.

6.5 Conclusions

The determination of a radiation dose (160 Gy) to produce high quality insects that exhibit a high proportion of sterility and comparable fitness to wild *L.bryoniae*, that show some level of population suppression are important, promising steps in the development of SIT as a viable option for *L.bryoniae* suppression. To develop a financially viable SIT programme the current limitations must be addressed. Further work must be undertaken to increase the ease and cost efficiency of the mass-rearing process. Knowledge is also required to understand the triggers that cause pupae to enter and leave extended pupation for overwintering. The release of sterile *L.bryoniae* needs to be examined on a larger scale, than the cage study, to determine the sterile to wild release ratio and the release frequency required to suppress a *L.bryoniae* infestation. Ideally this would be carried out during the early growing season when the greenhouse conditions are optimal for *L.bryoniae* development and oviposition. To develop *L.bryoniae* SIT to this stage a cost-benefit analysis is required to determine the financial feasibility of this method. More detailed knowledge of the market will allow for an accurate cost-benefit analysis. Financial investment is required to develop *L.bryoniae* SIT into a fully functioning SIT programme; but this is only possible if the SIT programme can be developed as a financially viable business.

The overarching aim of this thesis was to enable the production of high quality tomatoes with pest damage, specifically *L.bryoniae* damage, reduced below economic thresholds. *Liriomyza bryoniae* SIT in conjunction with the release of compatible biocontrols *Diglyphus isaea* and *Dacnusa sibirica*, with careful consideration of the timing of releases and proactive SIT releases during the early growing season, could reduce the level of *L.bryoniae* damage to below economic thresholds.

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8. Appendix

Appendix 4.1: Summary of power analyses used to determine the appropriate number of replicates and treatments. The significance level for all power analyses was $p = 0.05$.

Effect size	Replicates	Treatments	Power Value
High 0.4	4	5	0.199
	5	4	0.235
Medium 0.25	4	5	0.102
	5	4	0.115

Section A: Glasshouse Environment

1. In what county of the United Kingdom are you based?

2. What is the total area you have under glass? _____ Hectares

3. What cultivars of tomato do you grow and what is the area grown?

Cultivar	Conventional Area (Ha)	Organic Area (Ha)

4. On what date do you typically transplant the tomato plants into the glasshouse? Please tick

Beginning ☐ Middle ☐ End ☐

December ☐

January ☐

February ☐

March ☐

5. On what date do you typically remove the tomato plants from the glasshouse? Please tick

Beginning ☐ Middle ☐ End ☐

September ☐

October ☐

November ☐

December ☐

6. At the end of the growing season do you remove plant debris from the greenhouse? Please tick

YES ☐

NO ☐

If not, please explain why _____

7. Do you cover the floor with plastic or Mypex? Please tick

Plastic ☐ → Go to Question 8

Mypex ☐ → Go to Question 9

8. Do you remove the plastic annually? Please tick

YES ☐

NO ☐

9. How many seasons do you leave the Mypex in place?

_____ Seasons

10. Where do you source the new season tomato plants from? Please tick

Propagation on site ☐

Plants from a commercial propagator ☐

Section B: The Tomato Leafminer *Liriomyza bryoniae*

11. Have you ever had an infestation of the tomato leafminer *Liriomyza bryoniae*?

Delete as applicable

YES

→ Continue to Question 12

NO

→ Go to Question 19

12. If so, in which year did you last have an infestation of tomato leafminer *Liriomyza bryoniae*?

13. In the year above, how many weeks after transplant was the tomato leafminer infestation first spotted?

_____ Weeks

14. In the year above, how many weeks after transplant do you estimate the infestation reached its peak?

_____ Weeks

15. How often do you have an infestation of tomato leafminer? Please tick one box only:

Yearly

☐

Once every 2-3 years

☐

Once every 5 years

☐

Once every 10 years

☐

16. What percentage of your tomato crop was affected by the leafminer during the last infestation?

_____ %

17. How susceptible do you rate these categories of tomato to yield loss from leafminer infestation on a scale of 1 to 5, with 1 having the *least* amount of yield loss and 5 having the *most* yield loss? Please tick

Category	Susceptibility (1-5)				
	1	2	3	4	5
Cherry					
Baby cherry					
Santa; Baby plum					
Baby plum; yellow					
Baby plum					
Midi-plum					
Midi-plum; yellow					
Midi-plum; vine					
Classic					
Classic vine					
Cocktail loose					
Cocktail vine large					
Cocktail vine					
Beef					
Novelty					

18. Out of the category with the highest yield loss above, which cultivars do you believe to be the *most* susceptible to leafminer infestation?

19. If you are happy to be contacted for a follow-up interview, about the pest management methods you employ to control leafminer infestation, please could you leave a name and contact details below. All the information in the questionnaire will still be treated in confidence. Thank you.

Name _____

Email address _____

Phone number _____

Appendix 5.2: Cover letter for questionnaire

Tomato Leafminer (*Liriomyza bryoniae*) Questionnaire

As part of an HDC funded PhD on developing sterile insect technique to control the tomato leafminer *Liriomyza bryoniae* I am conducting a questionnaire about tomato leafminer problems.

Sterile insect technique is the large scale release of sterile leafminer males to over-flood the infestation. Females that mate with the sterile males lay eggs which are unable to develop into larvae and therefore stop the mine damage. Successive releases of sterile males can lead to a reduction in the pest population.

So far this technique has been developed to the stage where both males and females can be sterilised and used to suppress a small cage population of tomato leafminers. In order to scale this up it is important to understand the behaviour of the leafminer in a glasshouse environment. It is also necessary to understand the extent to which it is a problem for growers and the severity of infestations.

All the information collected in the questionnaire will remain anonymous. The information collected will be analysed to determine the prevalence of *Liriomyza bryoniae* infestations in the UK and be used to develop more effective and more targeted control methods. If you would be interested in answering further questions on the methods you currently you employ to control tomato leafminers then I would be very grateful.

Please feel free to contact me if you have any questions at catherine.walker06@imperial.ac.uk.

Thank you very much for your time.

Catherine Walker

PhD student
Silwood Park Campus
Imperial College London

Appendix 5.3: Section of reminder email sent out by HDC about questionnaire on
13/04/2012

	
Tomato Leafminer (<i>Liriomyza bryoniae</i>) Questionnaire	
As part of HDC project CP 53 'The use of Sterile Insect Technique (SIT) to increase the success of IPM in horticultural crops' Catherine Walker (HDC, PhD student) compiled the attached questionnaire to collect data to help her determine the prevalence of the tomato leafminer (<i>Liriomyza bryoniae</i>) in glasshouse tomato and cucumber crops. Catherine needs to understand the behaviour of the tomato leafminer in glasshouse environments and the extent to which the pest is a problem for growers. All collected data will remain anonymous. We would be grateful if you could fill in the attached to help Catherine with her studies. Please complete the attached PDF document and press the submit button or alternatively you can send it to Catherine at catherine.walker06@imperial.ac.uk or post it to AHDB, HDC, Stoneleigh Park, Kenilworth, Warwickshire, CV8 2TL. Thank you	