PART I Dispersal

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**CHAPTER 2. Marking with Fluorescent Pigments:**
Pupal Emergence, Adult Mortality, and Visibility and Persistence of Marks


### 2.1. Summary

A self-marking technique and fluorescent pigments were used to assess the effect of ptinal pigment marks on pupal emergence, adult mortality, and marker visibility and persistence in Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). Marking of *B. tryoni* with fluorescent pigment powders resulted in lower emergence caused by a higher proportion of newly-emerged adults being unable to escape the emergence tray. Mortality of marked adults that emerged successfully was similar to that of unmarked adults. Visibility of pigment marks varied with pigment colour and the wavelength of light used to view the marks. Most pigments were very durable, persisting throughout the experiment. Several fluorescent pigment powders are suggested as candidates for use in mark-release-recapture studies on *B. tryoni*.
2.2. Introduction

Fluorescent pigments offer a simple, inexpensive marking technique that does not require elaborate protocols or expensive equipment, and enables marking of large numbers of insects (Reinecke 1990; Fleishman et al. 1993; Garcia-Salazar & Landis 1997). The pigment can be applied as a dust or suspended in solution and applied directly to the body of the insect, or self-marking may be possible (Turchin 1998). Marking with fluorescent pigments is commonly used to discriminate between insects in dispersal studies employing mark-release-recapture/resample (MRR) methods. Fluorescent pigment marking has been used to monitor dispersal in grasshoppers (Narisu et al. 1999), leafhoppers (Bottenberg & Litsinger 1989), grain borers (Reinecke 1990; Dowdy & Mcgaughey 1992), mosquitoes (Meek et al. 1987; Meek et al. 1988; Fryer & Meek 1989; Niebylski & Meek 1989), and moths (Kipp & Lonergan 1992).

Queensland fruit fly, Bactrocera tryoni (Froggatt) (Diptera: Tephritidae) is a serious horticultural pest in eastern Australia. Marking of B. tryoni using fluorescent pigments has been used extensively in association with sterile insect technique (SIT) (MacFarlane et al. 1987; James 1992; Horwood & Keenan 1994; Dominiak & Webster 1998; Dominiak et al. 2000b; Dominiak et al. 2003), with the recognition that fluorescent pigment marks provide an efficient method of quickly identifying and separating sterile flies from wild flies caught in monitoring traps (Dominiak et al. 2000c). B. tryoni and other tephritid fruit flies are marked using a mass-marking method modified from that developed by Norris (1957), and later by Steiner (1965), where pupae are coated in fluorescent pigment which is retained in the ptilinum of the adult after emergence. Flies are examined with a dissecting microscope under an ultra-violet or blue light source for traces of fluorescent pigment (Holbrook et al. 1970; Dominiak et al. 2000c). Some studies have reported that using this self-marking technique to mark B. tryoni leads to decreased rates of adult emergence from the puparium (Dominiak et al. 2000c;
Dominiak et al. 2003), although these trials lacked adequate controls and results were variable.

Numerous fluorescent colours have been assessed and used for marking *B. tryoni* based on the relative visibility of the pigment mark (see Dominiak et al. 2000c). In a recent study on dispersal of sterile *B. tryoni* in Sydney (Dominiak et al. 2003), recapture rates of flies marked with blue, green and pink fluorescent pigment were lower than that of flies with orange marks in Lynfield traps (Cowley et al. 1990) baited with cuelure. One possible explanation for this observation was that blue, green and pink fluorescent pigment colours were toxic to adult *B. tryoni*, resulting in increased mortality. A fundamental requirement for any successful marking technique is that it should not affect the competitiveness, survival, longevity or behaviour of the treated animal (Southwood 1978). Another possibility is that the pigment marks may have disappeared with time. Fluorescent ptilinal marks in the house fly, *Musca domestica* L. (Diptera: Muscidae) decreased in intensity over a period of five days, although no individuals were unmarked (Beck & Turner 1984). Over longer periods of time, it is possible that ptilinal fluorescent marks may fade considerably, although this assumption has not been tested. If increased mortality does result from marking with particular fluorescent pigment colour, then this marking technique would require reassessment for their use in *B. tryoni* release programs, or need to be recognised when analysing recapture data from monitoring traps. Similarly, if permanence after release was shown to be questionable, then this finding would also result in a re-evaluation of these dyes for marking flies.

This study examined the effect of fluorescent pigment marking, using a self-marking technique, on adult emergence of *B. tryoni*. The effect of ptilinal fluorescent pigment marking and fluorescent pigment colour on mortality of adults in the laboratory was observed. Visibility and permanence of ptilinal fluorescent marks of various colours was assessed in the laboratory over a period of five weeks.
2.3. Materials and Methods

2.3.1. Cultures and pigment application

*Bactrocera tryoni* were obtained from a laboratory colony maintained at the University of Sydney. The colony had been maintained under laboratory conditions for more than 15 generations and continuously selected to increase longevity of adult flies by using only eggs from females greater than 16 weeks of age.

Nine different fluorescent pigments produced by two manufacturers were assessed: Deep Green (JST-31), Chartreuse (RS-10), Orange (RS-13), Cerise (PS-16) and Lilac (PC-98) manufactured by Radglo Color, N.V.; and Aurora Pink (HM-11), Rocket Red (HM-13), Arc Yellow (HM-16) and Strong Corona Magenta (HMS-30) from Dayglo Color Corp.. According to materials safety data sheets produced by each pigment manufacturer, Radglo pigments were dye solutions in thermoplastic resins with average particle size of 4 - 5 µm, and Dayglo pigments were solid dye solutions in thermoplastic resins described as ‘granules’ with particle size greater than 60 µm. For each pigment treatment, approximately 100 pupae were placed in 40 mL plastic pill cups (emergence trays) to which 0.2 g of pigment was added. Pupae were gently agitated by hand for up to 10 seconds to coat the pupae with pigment. Pigment-coated pupae were added to an equal quantity (by volume) of sawdust and agitated gently for approximately 10 seconds. The control consisted of approximately 100 pupae that were agitated and covered with sawdust in the same manner, but with no fluorescent pigment added. Three replicates of each treatment were performed.

2.3.2. Emergence and mortality

Pupae combined with pigment were placed in sleeve cages (45 x 33 x 34 cm), with one pigment treatment per cage. Cages were kept near a window that
provided natural light-dark conditions, and maintained at 23 ± 2°C and 41 – 65 % RH (Plate 2-1). Cages contained a sealed, plastic water container with a cotton dental roll wick, and an open plastic container filled with granulated sucrose to provide emerged flies with water and food ad libitum. Four days after adult flies first emerged, emergence trays were removed from the cages. The number of pupae that failed to emerge, partially emerged flies, and emerged flies that failed to escape the emergence tray were recorded. Analysis of variance was performed to identify differences between the results for the pigment treatments. Variables were analysed as proportions to standardise for differences in the number of pupae. No variables conformed to the assumptions of ANOVA, so the arcsine square root transformation, routinely used on proportion data (Underwood 1997), was applied prior to analysis. Tukey multiple comparison tests were conducted to identify homogeneous groups within the fluorescent pigment treatments. Numbers of successfully emerged flies were not analysed due to non-independence of data.

After adult emergence, in addition to water and granulated sucrose already provided, a paste of yeast autolysate paste, sucrose and water was applied to the walls of sleeve cages to provide a source of protein which is required by female *B. tryoni* to become sexually mature (Drew *et al.* 1983; Drew 1987). Cages were inspected at 4, 7, 14, 21, 28 and 35 days after adult emergence for mortalities in each cage, and dead flies were removed. The number and sex of dead flies in each treatment was recorded (total mortality). The cages were then placed in an incubator at 70°C for 30 minutes to kill remaining flies. The total number of successfully emerged flies was recorded. Mortality rate was calculated for each week in each pigment treatment. Two-way ANOVA was used to compare total mortality and mean weekly mortality rates in males and females for different pigment colours. Total mortality did not conform to the assumption of homogeneity of variances and was arcsine square root-transformed prior to analysis.
Plate 2-1. Standard laboratory sleeve cages used to test emergence, mortality, and visibility and persistence of marks on *B. tryoni* treated with different fluorescent pigment colours. Cages were installed near a window in a room maintained at 23 ± 2°C and 41 – 65 % RH.
2.3.3. Marker visibility and persistence

Four days after adults first began emerging, five male and five female flies were chosen at random from each pigment treatment. Flies were examined in glass vials under four light wavelengths for fluorescent pigment marks on the ptilinal suture: blue (467 nm), green (511 nm), yellow (563 nm), and red (625 nm). Flies were not anaesthetised during observation. The numbers of males and females with marks visible on the ptilinal suture were recorded. If pigment marks were present on the ptilinal suture, but did not fluoresce, they were still counted as visible. After observation, flies were returned to the cage. This procedure was repeated at 7, 14, 21, 28, and 35 days to give an indication of mark persistence.

Marker visibility for each treatment under different light wavelengths over time was analysed using three-way ANOVA. Data did not conform to the assumptions of ANOVA. Square root-transformation of data \((\sqrt{x} + 1)\) reduced heterogeneity of variances but did not equalise them. In large experiments, ANOVA is robust to departures from the assumption of homogeneity of variances (Underwood 1997) and analysis was continued using square root-transformed data. Significant interactions between pigment treatment and time were of particular interest for assessing mark persistence. Tukey multiple comparison tests were conducted to identify homogeneous groups within light wavelengths and fluorescent pigment treatments.

2.4. Results

2.4.1. Emergence and mortality

On average, 14.4 ± 2.9% of pupae failed to emerge during the study. The proportion of pupae that failed to emerge was similar across all pigment treatments \((F = 0.154, \text{df} = 9, 20, P > 0.05; \text{Figure 2-1})\). Similarly, the proportion
of partially-emerged pupae did not differ between pigment treatments ($F = 0.956$, $df = 9,20$, $P > 0.05$; Figure 2-1), with an average of $2.9 \pm 0.3\%$ during the study. An average of $14.5 \pm 2.0\%$ flies emerged from the puparium, but died before escaping from the emergence tray. There was a significant difference in the proportion of flies that had emerged but failed to escape from the emergence tray ($F = 16.511$, $df = 9,20$, $P < 0.001$). Mortality of emerged flies in the emergence tray was not observed in the control, and was low in Arc Yellow, Aurora Pink and Strong Corona Magenta treatments (Figure 2-1). The average emergence success of pupae during the experiment was $68.2 \pm 3.8\%$.

Average mortality rate during the study was $2.1 \pm 0.1\%$ per week, and total mortality was $11.4 \pm 1\%$. Fluorescent pigment marks had no effect on mortality rate ($F = 1.213$, $df = 9,40$, $P > 0.05$; Figure 2-2a) or total mortality ($F = 1.263$, $df = 9,40$, $P > 0.05$; Figure 2-2b) between treatments. Mortality rates between males and females were similar ($F = 1.884$, $df = 1,40$, $P > 0.05$) or total mortality ($F = 3.310$, $df = 1,40$, $P > 0.05$), and there was no significant interaction between pigment treatment and sex in either mortality rate ($F = 0.297$, $df = 9,40$, $P > 0.05$) or total mortality ($F = 0.295$, $df = 9,40$, $P > 0.05$).
**Figure 2-1.** Emergence success of *B. tryoni* pupae treated with fluorescent pigment powders of different colour (mean ± 1 SE). No pigment was applied to control pupae. Pupae that failed to emerge: white bars; partially emerged flies: grey bars; emerged flies that failed to escape the emergence tray: black bars.
**Figure 2-2.** Mortality of *B. tryoni* adults marked with fluorescent pigment powders of different colour (mean ± SE). Control flies were not treated with pigment during emergence. (a) Mortality rate (proportion/week). (b) Total mortality. Male: black bars; Female: white bars.
2.4.2. Marker visibility and persistence

Overall, fluorescent pigment mark visibility did not change over a period of 35 days ($F = 1.637$, df = 5,480, $P > 0.05$). Mark visibility was significantly different when observed under different light wavelengths ($F = 3494.431$, df = 3,480, $P < 0.001$), with fluorescent marks not visible under red light (625 nm) and most visible under blue light (467 nm) (Figure 2-3). There was no interaction between mark visibility over time and light wavelength ($F = 0.524$, df = 15,480, $P > 0.05$).

There was a significant difference between visibility of different fluorescent pigment treatments ($F = 737.686$, df = 9,480, $P < 0.001$). Orange, Cerise, Aurora Pink and Rocket Red were the most visible pigments, with Deep Green and Chartreuse being the least visible. However, there was a strong interaction between fluorescent pigment treatment and light wavelength ($F = 187.622$, df = 27,480, $P < 0.001$). Deep Green and Chartreuse fluorescent pigment marks were highly visible under blue light (Figure 2-3a), but not visible under green light (511 nm; Figure 2-3b) or yellow light (563 nm; Figure 2-3c). Lilac was less visible when viewed under blue light than under green or yellow light. Visibility of different pigment treatments also varied with time ($F = 1.490$, df = 45,480, $P < 0.05$). Visibility of Strong Corona Magenta decreased during the period of the study (Figure 2-3).
Figure 2-3. Visibility of ptilinal fluorescent pigment marks on adult *B. tryoni* maintained in a laboratory over a period of 35 days. Visibility of different pigment colours was assessed under different light wavelengths. (a) Blue light (467 nm). (b) Green light (511 nm). (c) Yellow light (563 nm). Control: black diamond; Deep Green: black square; Chartreuse: black triangle; Orange: black cross; Cerise: black asterisk; Lilac: grey diamond; Arc Yellow: grey square; Rocket Red: grey triangle; Aurora Pink: grey cross; Strong Corona Magenta: grey asterisk.
2.5. Discussion

Radglo fluorescent pigment colours Deep Green, Chartreuse, Orange, Cerise and Lilac resulted in lower rates of successful pupal emergence in *B. tryoni*. Particle size of these fluorescent pigment powders was 4 – 5 µm, compared with Arc Yellow, Rocket Red, Aurora Pink and Strong Corona Magenta that were in the form of granules larger than 60 µm. On pupal emergence, flies were completely coated in a layer of fluorescent pigment and there is a possibility that the small particle size of the Radglo pigments may have resulted in blockage of respiratory surfaces. However, this does not explain high mortality of newly-emerged adults marked with Rocket Red. Similarity between pigment treatments for the proportion of pupae that failed to emerge and partially-emerged pupae indicates that differences in emergence are caused by post-eclosion issues.

It has been recommended that an attempt should always be made to ensure that marking techniques do not affect the longevity or behaviour of the animal being studied (Southwood 1978). In this current study, mortality rate and total mortality of *B. tryoni* adults that successfully emerged in the laboratory was not affected by ptinal marking with fluorescent pigments. This is the first study to demonstrate that survival of male and female *B. tryoni* with fluorescent ptinal marks does not differ from flies that are unmarked. Observations of sterile *B. tryoni* survival marked with Astral Pink (Fiesta®, FEX Series) are similar to those found in this study (A. Meats, pers. comm.). Earlier studies on the effect of fluorescent pigment marking of various insect taxa have also shown no adverse effects on mortality (Bottenberg & Litsinger 1989; Niebylski & Meek 1989; Reinecke 1990; Dowdy & McGaughey 1992; Garcia-Salazar & Landis 1997). Fluorescent pigment may be non-toxic because the fluorescent chemicals are bound within a stable plastic that is cured and then ground to a fine powder (Reinecke 1990).

Most pigments trialled were highly visible when viewed under at least one of the light wavelengths used in this study. Inspection for fluorescent pigment
marks on *B. tryoni* under blue light (467 nm) provided visibility of the greatest range of mark colours, although visibility of Lilac was lower under blue light than under green light (511 nm) or yellow light (563 nm). Inability to detect Deep Green and Chartreuse pigment marks under green and yellow light, as well as lower fluorescence of Lilac under blue light was due to these pigments possessing a peak reflectance wavelength close to that of the transmitted light. The results of the current study may provide an explanation for poor detection of sterile *B. tryoni* marked with Comet Blue 60 (Fiesta®, FEX Series) that were observed under blue light (Dominiak *et al.* 1998; Dominiak *et al.* 2003). Current practice for identifying sterile flies released to suppress wild populations of *B. tryoni* is to examine monitoring trap captures using a binocular dissecting microscope under blue light (Dominiak *et al.* 2000b; Dominiak *et al.* 2003), although wavelength of light is not reported. This practice is satisfactory, provided that fluorescent pigments used to mark *B. tryoni* have peak reflectance wavelengths that are clearly visible when viewed under blue light.

A majority of fluorescent pigments used to mark *B. tryoni* were persistent, with no change in visibility over the period of the study in the laboratory. The least persistent fluorescent pigment trialled in this study was Strong Corona Magenta, which decreased in visibility over five weeks. It is encouraging that use of this dye colour has not been reported from field releases of *B. tryoni*, even though the majority of fly recaptures are made within 3 – 4 weeks of release (Dominiak & Webster 1998; Meats 1998b; Dominiak *et al.* 2003). However, these results do not guarantee durability of the pigment mark in the field (Southwood 1978). There is a theoretical concern that fluorescent pigments may lose their fluorescent properties following extended exposure to sunlight, although this is unlikely to be a problem in studies on dispersal of *B. tryoni* lasting no more than a few weeks. Moreover, sterile flies marked with green fluorescent pigment (manufacturer unknown) have been recaptured and identified with pigment marks up to 26 weeks after release (Dominiak *et al.* 2000b). Despite this, it is advisable that further studies be conducted in the field to examine the persistence of ptilinal pigment marks under natural conditions.
Aurora Pink was the best pigment trialled for fluorescent ptinal marking due to low mortality immediately after emergence, and high visibility and persistence of the mark. Arc Yellow was also a good candidate for use in mark-release-recapture (MRR) studies with low mortality immediately after emergence, reasonable visibility and high mark persistence. Often it can be useful to be able to distinguish between marker colours when observing trap recaptures. This provides a means to compare two groups of otherwise identical flies that have been released under identical field conditions but have been subjected to different experimental treatments. If sorting flies under blue light, which is the current practice for identifying recaptured sterile flies released in the field, ptinal marking with Deep Green and Chartreuse allows for MRR studies with multiple or overlapping releases because they are distinctive from other fluorescent pigments. However, it would be necessary to compensate for lower emergence success when releasing flies marked with these colours. Strong Corona Magenta is the least suitable pigment for ptinal marking of B. tryoni. Despite high emergence in this treatment, low mark visibility and poor persistence of marks reduces the utility of this fluorescent pigment in MRR studies.


3.1. Summary

Sticky (unbaited) trap catches of irradiated Bactrocera tryoni were approximately 12% of the size of those of pot-type traps baited with cuelure. Sticky traps painted with daylight fluorescent yellow or green were equally effective but were no better than unpainted (control) sticky traps for capturing B. tryoni. No association was identified between recaptures in sticky traps and host status of the tree in which the trap was placed. Sticky traps may be useful for detecting the location of emergence foci of endemic flies but were insufficiently sensitive to offer an alternative to current monitoring techniques.
3.2. Introduction

Queensland fruit fly, *Bactrocera tryoni* (Froggatt), is a major horticultural pest in eastern Australia. Gravid females oviposit in the fruits of over one hundred species of native and introduced plants, including citrus, stonefruits and apples (Hancock *et al.* 2000), with subsequent fruit damage by developing larvae. Control of this insect in Australia is an expensive exercise and costs exceeded $A125 million in 1991 (Yonow & Sutherst 1998). Potential losses in the absence of control, based on data collected in Adelaide, could be greater than 80%, representing a value exceeding $A800 million (Sutherst *et al.* 2000).

Earlier papers indicate that *B. tryoni* possesses high dispersal capabilities. The maximum recorded dispersal distance from a sterile fly release at Wangaratta, Victoria, was 94 km, although the average recapture distance was considerably less (MacFarlane *et al.* 1987). The release at Wangaratta involved a large number of flies (470,000) so this result is not unexpected considering that trap recaptures are density-dependant (Fletcher 1974a; Meats 1998b). Knowledge of the dispersal patterns of released sterile flies is important for effective implementation of sterile insect technique (SIT). In theory, dispersal of individuals through a homogeneous environment from a single point in space will result in a two-dimensional Gaussian distribution that can be predicted by simple diffusion (eg. see Turchin 1998). According to this principle, sterile fly density will be highest immediately around a release site, and decrease approximately exponentially with distance. In *B. tryoni* it has been found that recapture rate per trap declines as the reciprocal of the square of the distance, conforming to an ‘inverse square rule’ (Fletcher 1974b; Meats 1998a). At some distance away from the release site, the density of sterile flies will drop below a level where sterile male-wild female encounters are sufficient to control population growth. If release sites are spaced too far apart the area-dilution effect will result in voids within the target area where sterile male density is below the threshold required to limit matings by fertile males. Meats (1996) estimated that if release rates were sufficient to achieve a sterile:wild ratio
of 100:1 at the release point, the ratio would drop to only 25:1 at a distance 0.5 km and 4:1 at 1 km.

The most commonly used attractant used in traps deployed to monitor populations of *B. tryoni* is cuelure (4-(3-oxobutyl) phenyl acetate). Cuelure is highly attractive to sexually mature male *B. tryoni*, and immature females to a lesser extent (Drew 1987). Drew (1987) suggested that this response is due to its chemical similarity with a female feeding attractant and the male sex attractant, 2-butanone. However, inseminated sexually mature females, that do not require mates, are repelled by the high cuelure concentration used in cuelure traps (Hill 1986; Drew 1987; Dalby-Ball & Meats 2002) and males form the majority of trap captures (Meats *et al.* 2002a). Given that cuelure is ineffective in the field for attracting immature male and adult female *B. tryoni* it is necessary to identify a better method to monitor dispersal of these animals. This is particularly important given that undirected dispersive movement is predominantly associated with the post-teneral phase (Bateman & Sonleitner 1967; Fletcher 1973).

Performances of traps baited with alternative lures based on food odour have been compared with cuelure traps in other studies (Hill 1986; Meats *et al.* 2002a). Yeast-based food lure traps capture low numbers of male and female flies but overall are much less successful than cuelure traps for detecting fly populations. It has been found that flat sticky traps painted with daylight fluorescent colours Saturn Yellow, Lime, Blaze Orange and Emerald attract tephritid fruit flies (Hill & Hooper 1984). The most attractive colours caught significantly more males than females (Hill & Hooper 1984). The aim of this study was to examine the effectiveness of a simple, unbaited sticky trap in monitoring dispersal of irradiated *B. tryoni*. Capture rates and sex ratios of sticky traps painted with daylight fluorescent colours previously shown to be most attractive to *B. tryoni* (yellow and green) were compared with cuelure trap captures.
3.3. Materials and Methods

3.3.1. Source and treatment of flies

Irradiated sterile *B. tryoni* were obtained as pupae from the mass rearing facility at Camden, New South Wales. Between 36,000 and 60,000 pupae in plastic trays of sawdust mixed with fluorescent pigment powder (Fiesta ®, FEX Series, Astral Pink 1) were allowed to emerge in 50 L black storage bins (Nally Crates, Viscount Plastics (NSW) Ltd.) covered with mesh fabric that was secured over the top of the bin by black nylon elastic. During emergence, a small quantity of fluorescent pigment adhered to the ptilinum enabling identification of released flies when examined under blue light (475 nm). Adults were transported to Richmond in an air-conditioned vehicle and released from a single point within the grid on three occasions during the 2001/2002 fruit fly season (October 2001, March and April 2002). To ensure that human movements did not assist fly dispersal, cages were carried from the car to the release point, and flies were removed by hand from all equipment and persons before leaving the release point.

3.3.2. Site description, trap types and deployment

The study was conducted at the Hawkesbury Campus of the University of Western Sydney, near Richmond, New South Wales, Australia. The property has a mixed orchard containing various species and varieties of citrus, stonefruits, and pears, as well as ornamental trees. Management practices in the orchard are restricted to grass-mowing and pruning.

A grid of 88 cuelure traps was used to monitor dispersal of *B. tryoni* and provide a positive control for the study (Plate 3-1; Figure 3-1). Traps were placed within the canopy of host and non-host trees, sampling a distance of 3 – 465 m from the release point. The trap was a disposable pot-type lure trap made of clear plastic (Lynfield trap) (Cowley *et al.* 1990). Approximately 2 mL of a cuelure and
malathion mix (8:1), was placed on three dental rolls held in place at the centre of
the trap by a clip. The trap was suspended by a piece of wire tied onto a metal
loop incorporated with the lid. The base of the traps had four 2 mm water drainage
holes. There were four 25 mm entry holes 90° apart in the clear plastic trap body.
Cuelure traps were inspected at weekly intervals, with contents emptied into
labelled plastic vials for transport back to the laboratory. Trap captures were
examined under blue light with a dissecting microscope to identify marked
individuals.

Sticky traps consisted of 150 mm plastic petri dishes suspended within the
tree canopy by wire (Plate 3-1). The bottom surface of the dish was painted with
daylight-fluorescent yellow (chartreuse), daylight-fluorescent green (Solver ®,
Flat Acrylic Scenic paint) or left unpainted before being coated with an adhesive
(Rentokil ®, Tac-Gel Formula 3). One trap of each colour was placed in each tree
at every third lure-trap location (Figure 3-1), with positions rotated weekly within
the tree. Traps were inspected at weekly intervals for one month, and removed and
replaced when B. tryoni captures were observed. Removed traps were transported
back to the laboratory for observation under blue light to identify marked
individuals. Traps with high coverage of non-target insects were also removed and
replaced, although this was usually not necessary.
Plate 3-1. Lure and sticky traps suspended within the canopy of a tree in an orchard, Richmond, New South Wales, Australia.
3.3.3. Data analysis

Due to low recapture rates in both cuelure traps and sticky traps, weekly recapture data were pooled into monthly periods. Trap recaptures were expressed as a proportion to standardise for the number of flies released in each monthly cohort. Comparison of trap effectiveness was performed using three-way, mixed model analysis of variance. Factor 1 (fixed) was trap, with four levels (positive control = cuelure trap, yellow, green, negative control = no colour). Factor 2 (fixed) was sex, with two levels (male, female). Factor 3 (random) was cohort. This procedure was repeated with cuelure trap recaptures excluded from the analysis. Chi-squared analysis was performed to test for an association between sticky trap effectiveness and tree type (citrus, stone fruit, pomes, non-host) in which it was positioned.

3.4. Results

Cuelure traps consistently recaptured more sterile \( B. \text{tryoni} \) than sticky traps. Sterile flies were caught up to 436 m from the release point in cuelure traps. Sticky trap recaptures of both sexes were limited to sticky traps located within approximately 80m of the release point. Location and number, relative to the release point, of released sterile (red-marked) and unmarked \( B. \text{tryoni} \) is shown in Figure 3-1. Recaptures of marked \( B. \text{tryoni} \) on sticky traps were only made in the first week after release and represented an average estimated recapture rate of 0.1%. The average estimated recapture rate of cuelure traps was 1.3%. In September 2001, a large number of unmarked flies were captured on sticky traps within the vicinity of the release point one week after release. The three (yellow, green and unpainted) sticky traps at the trap location closest to the release point captured a combined total of 53 unmarked flies (Figure 3-1). The origin of these flies is probably a pupal release conducted simultaneously with the adult release that was scattered and washed free of marking powder by a storm. Captures of
Figure 3-1. Location and number of released sterile (red-marked) and unmarked *B. tryoni* relative to the release point. (a) Lure (+) and sticky trap (◊) locations relative to release point. The area indicated by the grey rectangle is enlarged in (b), (c) and (d). (b) Sticky trap captures following September 2001 release. (c) Sticky trap captures following March 2002 release. (d) Sticky trap captures following April 2002 release. Circle diameter represents the pooled number of flies captured on yellow, green and unpainted sticky traps at each trap location. Marked captures one week after release: empty grey circles; marked captures two weeks after release: filled grey circles; unmarked captures one week after release: empty black circles; unmarked captures two weeks after release: filled black circles.
small numbers of unmarked *B. tryoni*, ranging from 1 – 5 flies, were found at trap locations greater than 80 m away from the release site during the September 2001 and March 2002 releases (Figure 3-1). Chi-squared tests on pooled capture data indicated that there was no significant association between sticky trap captures and tree type ($\chi^2 = 5.161, \text{df} = 3, P > 0.1$).

Mean ($\pm$ 1 SE) *B. tryoni* recaptures in cuelure and sticky traps after each release are shown in Figure 3-2. Average male:female ratio captured in cuelure traps was 375:1, but 1:1 for all sticky trap colours. Analysis of variance including cuelure trap recaptures indicated a significant difference between trap treatments, with sticky trap captures being much lower than cuelure trap captures ($F = 10.697, \text{df} = 3,6, P < 0.01$). There was no significant difference between male and female recaptures despite the large number of males captured in cuelure traps. Recaptures were not significantly different between releases. There was a very highly significant interaction between trap recaptures and sex ($F = 9.938, \text{df} = 3,6, P < 0.01$) (Figure 3-2).

Where cuelure trap recaptures were excluded from the analysis, daylight fluorescent yellow and green sticky traps were not significantly better than unpainted sticky traps for capturing *B. tryoni* ($F = 2.018, \text{df} = 2,4, P > 0.05$). There was no significant difference between male and female recaptures. A very significant difference in recaptures was found between releases ($F = 36.010, \text{df} = 2,2.793, P < 0.01$), with more flies being recaptured in October 2001 than March and April 2002. A non-significant interaction between trap type and sex ($F = 1.662, \text{df} = 2,4, P >0.05$) indicates that no colour was found to be more attractive to female *B. tryoni*. 
Figure 3-2. Mean (± 1 SE) *B. tryoni* male and female recaptures in lure and sticky traps. (a) Recaptures following September 2001 release. (b) Recaptures following March 2002 release. (c) Recaptures following April 2002 release. Male (□); Female (■).
3.5. Discussion

For the purpose of monitoring dispersal of B. tryoni cuelure traps are the standard against which other trap types must be compared to determine their effectiveness. Coloured, unbaited sticky traps were found to be relatively ineffective for monitoring dispersal of sterile B. tryoni. Even within 200m of the release point, sticky traps captured, on average, only 12% as many flies as were captured by cuelure traps within the first week of trapping.

Use of sticky traps painted with colours assumed to be attractive to insects should increase captures to levels higher than the probability of chance landings. Sticky trap colours in previous studies and pest-monitoring practice have been chosen to mimic that of host fruits or foliage. Unbaited and baited sticky red spheres have been used to monitor the apple maggot fly, Rhagoletis pomonella (Tephritidae) (Prokopy 1968; Duan & Prokopy 1994). Unbaited, bright yellow-green tennis ball fruit models are attractive to B. tryoni, although fruit models baited with amyl acetate and cuelure proved to be more attractive to females and males, respectively (Dalby-Ball & Meats 2002). Hill and Hooper (1984) found that daylight fluorescent Saturn Yellow on flat rectangular sticky traps (15 x 20 cm) was significantly better at attracting B. tryoni than other colours in the absence of olfactory cues. This was attributed to a peak reflected wavelength (540 nm) similar to that of green leaves (550 nm). Hill and Hooper (1984) also found that male captures outnumbered female captures, although it was suggested that this might have reflected population composition rather than a differential rate of attraction. In the study reported here the use of painted daylight fluorescent yellow and green sticky traps did not increase recaptures of B. tryoni relative to unpainted sticky trap controls (Figure 3-2). Neither sex was found to be more responsive to either colour in preference to unpainted sticky traps (Figure 3-2). Although the number of recaptures on sticky traps in October 2001 was higher than in both March and April 2002, seasonality of sticky trap performance is beyond the scope of this paper owing to a lack of temporal replication.
In an experiment investigating the effect of fruit abundance within a tree canopy on the behaviour of wild and cultured *B. tryoni*, Dalby-Ball and Meats (2000a) found that neither sex of a laboratory strain on *B. tryoni* responded differentially to trees with differing amounts of fruit. This behaviour was in contrast to the tendency of both male and female wild *B. tryoni* to accumulate in fruiting host trees (Dalby-Ball & Meats 2000a). The results presented in this study showed that there was no association between recaptures of sterile *B. tryoni* in sticky traps and host status of the tree in which the trap was placed. This supports the concerns raised by Dalby-Ball and Meats (2000a) that released sterile flies used in sterile insect technique may not tend to accumulate in fruiting trees as wild flies are expected to do.

Despite their shortcomings in monitoring dispersal of *B. tryoni*, sticky traps may provide an indication of emergence foci. Captures of moderate numbers of unmarked flies at locations distinct from the release point were made on two occasions during the period of this study (Figure 3-1). These captures in October 2001 and March 2002 presumably reflect emergence sites of flies endemic to the orchard. The pupal stage of *B. tryoni* is completed in soil after fruit infested with larvae drops to the ground. After eclosion, and wing expansion and hardening, *B. tryoni* adults fly from the ground to nearby vegetation. At this early stage of dispersal local density of newly emerged flies would be relatively high, with an associated high probability of fly captures on sticky traps by chance.

Knowledge of the location of emergence foci may be of benefit to *B. tryoni* control. Regular (weekly) monitoring of sticky traps would provide evidence of a fly incursion at least one week earlier than cuelure traps, owing to the time required for adult males to reach sexual maturity and begin responding to cuelure. The time delay between fly emergence and cuelure response corresponds with the period of highest dispersal of *B. tryoni* (Bateman & Sonleitner 1967; Fletcher 1973). Therefore, earlier detection using sticky traps has the potential to reduce the area requiring control measures, and enable intensification of control efforts in a localised area.
CHAPTER 4. Odoriferous Sticky Spheres for Monitoring Dispersal: Male and Female Dispersal is Similar.

4.1. Summary

The effectiveness of baited sticky sphere traps for monitoring dispersal of male and female Queensland fruit fly, Bactrocera tryoni (Froggatt) (Diptera: Tephritidae), from a point-release was examined. Lynfield traps baited with cuelure recaptured considerably more released B. tryoni than either odoriferous yellow or black sticky spheres baited with food-lure (protein autolysate). Mean recapture rate per trap on yellow and black sticky spheres was only 1.7% and 1.5%, respectively, of the recapture rate of Lynfield traps within one week after release. Both kinds of sticky sphere recaptured female B. tryoni. Dispersal patterns of post-teneral male and female B. tryoni were compared using recapture data obtained from the sticky spheres. Similarity between Linear regression analysis of the relation of recapture rate to the logarithm of distance indicates that there was no difference between dispersal patterns of male and female B. tryoni in the week following release. This is the first study to explicitly document that dispersal patterns of post-teneral male and female B. tryoni are similar.
4.2. Introduction

Knowledge of the dispersal patterns of released sterile insects is important for effective implementation of sterile insect technique (SIT). In theory, dispersal of individuals through a homogeneous environment from a single point in space will result in a two-dimensional Gaussian distribution that can be predicted by simple diffusion (e.g. see Turchin 1998). Accordingly, sterile insect density should be highest immediately around a release point, and decrease approximately exponentially with distance. At some distance away from the release point, the density of sterile insects will drop below a level at which sterile male-wild female encounters are sufficient to control population growth, thus if release points are spaced too far apart the area-dilution effect will result in voids within the target area where sterile male density is below the threshold required (Meats 1996).

Queensland fruit fly, Bactrocera tryoni (Froggatt) (Diptera: Tephritidae) is an important horticultural pest that is endemic to eastern Australia, and has been inadvertently introduced to several Pacific islands. Females of this species oviposit in ripening fruit of numerous commercial and native host species (Hancock et al. 2000), with subsequent fruit damage by developing larvae. Suppression of B. tryoni populations using SIT has a long history in Australia, beginning in late 1961 when the first SIT trials were conducted in Manilla, New South Wales (Andrewartha et al. 1967). Successful eradication of B. tryoni was achieved in Western Australia in a program incorporating SIT (Fisher 1992), and SIT is used in suppression programs to control outbreaks in important horticultural areas in south-eastern Australia (James 1992; Horwood & Keenan 1994; Dominiak et al. 1998; Dominiak et al. 2000b; Dominiak et al. 2003).

Earlier papers indicate that B. tryoni possesses high dispersal capabilities. The maximum recorded dispersal distance from a sterile fly release at Wangaratta, Victoria, was 94 km, although the average recapture distance was within 1.5 km of the release point (MacFarlane et al. 1987). The two successful releases at
Wangaratta involved a large number of male flies (470,000) so this result is not unexpected. If density at any point is related to release rate, density declines with distance and recapture rate at any point is related to density (Fletcher 1974a; Meats 1998b). It has been reported that recapture rate of sterile \textit{B. tryoni} per trap declines as the reciprocal of the square of distance from the release point, conforming to an ‘inverse square rule’ (Fletcher 1974b; Meats 1998a). Meats (1996) estimated that if release rates were sufficient to achieve a sterile:wild ratio of 100:1 at the release point, the ratio would drop to only 25:1 at a distance 0.5 km and 4:1 at 1 km. More recent studies confirm that most sterile \textit{B. tryoni} do not disperse far from the release point (Dominiak & Webster 1998; Meats 1998b; Dominiak \textit{et al.} 2003).

The attractant most commonly used in traps deployed to monitor populations of \textit{B. tryoni} is cuelure, 4-(3-oxobutyl) phenyl acetate. Cuelure is attractive to sexually mature male \textit{B. tryoni}, and immature females to a lesser extent (Drew 1987). Drew (1987) suggested that this response is due to the chemical similarity of cuelure with a female feeding attractant and male sex attractant, 2-butanone. However, cuelure is not an ideal attractant for monitoring populations of \textit{B. tryoni}. Inseminated sexually mature females, that do not require mates, are repelled by the high cuelure concentration used in monitoring traps (Hill 1986; Drew 1987; Dalby-Ball & Meats 2002) and males form the majority of trap captures (Drew 1974; Meats \textit{et al.} 2002a).

Fletcher (1974b), using flies reared from infested fruit, found that within 24 hours a single cuelure trap could recapture 1% of mature male \textit{B. tryoni} that were released in an area of approximately 200 m radius of the trap. In the same study, he found that a grid of 16 traps, 100 m apart in a 4 x 4 array, would recapture approximately 8% within 24 hours. Total recapture rates of 0.04% (Dominiak & Webster 1998), 0.07% (Dominiak \textit{et al.} 1998), and 0 – 2.05% (Dominiak \textit{et al.} 2000b) have been reported for sterile \textit{B. tryoni} released in sterile insect programs on a 400 m grid. Differences between recapture rates of wild and sterile male \textit{B. tryoni} may be due to competitiveness and survivability of mass-
reared, sterile flies (Dominiak et al. 1998; Dominiak et al. 2000b), although there has been no direct comparison of wild and sterile recaptures in cuelure traps following simultaneous release.

Mature male B. tryoni possess a capacity for limited mechanoreceptive anemotaxis, with movement only occasionally in response to both wind and the odour of cuelure (Meats & Hartland 1999). This imprecise odour tracking system could explain low recapture rates of B. tryoni. Meats and Hartland (1999) suggested that the strength of cuelure vapour emitted by a cuelure trap may only attract flies within the immediate vicinity (within a metre or so) of the trap.

Given that cuelure is ineffective in the field for attracting immature male and adult female B. tryoni it would be useful to identify a better method to monitor dispersal of these animals. This is particularly important as undirected dispersive movement is predominantly associated with the post-teneral (immature adult) phase (Bateman & Sonleitner 1967; Fletcher 1973). Traps based on both attractive colours and food odours have been investigated for monitoring tephritid fruit fly populations. It has been found that flat sticky traps painted with daylight fluorescent colours Saturn Yellow, Lime, Blaze Orange and Emerald attract tephritid fruit flies (Hill & Hooper 1984). The most attractive colours caught significantly more males than females (Hill & Hooper 1984). Chapter 3 reported that there was no difference between male and female recaptures on flat, circular plastic sticky traps painted with daylight fluorescent colours Chartreuse and Green, and unpainted sticky traps. Yeast-based food-lure traps capture low numbers of male and female flies but overall are much less successful than cuelure traps for detecting fly populations (Meats et al. 2002a). Presence of a red fruit model as a visual cue in addition to the odour of yeast autolysate provided a stimulus for orientation and landing of B. tryoni (Dalby-Ball & Meats 2000b), and the interaction of odour and visual cues have been found to increase efficiency of traps for other tephritid fruit flies (Duan & Prokopy 1992; Aluja & Prokopy 1993; Liburd et al. 1998).
The aim of this study was to examine the effectiveness of a food-lure (protein autolysate) baited sticky trap for monitoring dispersal of *B. tryoni* from a point-release. Total recapture rates and recapture rates of males and females on yellow and black baited sticky spheres were compared with cuelure (Lynfield) trap recaptures. Dispersal patterns of post-teneral male and female *B. tryoni* were compared using recapture data obtained from sticky spheres.

### 4.3. Materials and Methods

#### 4.3.1. Site description, source and treatment of flies

The study was conducted in an orchard at the Hawkesbury campus of the University of Western Sydney, near Richmond, New South Wales, and contained various species and varieties of citrus, stonefruits, and pears, as well as ornamental trees. Management practices in the orchard were restricted to grass-mowing, pruning and minor manipulation for teaching purposes.

A laboratory-reared strain with a homozygous recessive colour mutation (*white marks*) was used in this study. *White marks* differ from wild-type flies in that dorsal thoracic markings are white rather than yellow, and this feature can be used to distinguish them from wild *B. tryoni* captured in monitoring traps. *White marks* were obtained by inbreeding of family groups; pure breeding lines for these characters were retrieved after outcrossing to flies of stocks of recently wild origin (Meats et al. 2002b). Irradiated (sterile) *B. tryoni* were also used and were obtained as pupae from the mass rearing facility at Camden, New South Wales, managed by NSW Agriculture on behalf of the Tri-State Fruit Fly Committee. Pupae were irradiated 3 days before adult eclosion with 71.3 – 73.8 Gy of gamma radiation from a cobalt-60 source, in a normal atmosphere.

*White marks* and sterile pupae were allowed to emerge in large laboratory sleeve cages (45 x 33 x 34 cm). Within each sleeve cage, pupae were placed in
600 mL plastic trays with an equal quantity (by volume) of saw dust. Cages contained a sealed, plastic water container with a cotton dental roll wick, and an open plastic container filled with granulated sucrose to provide emerged flies with water and food *ad libitum*. Each sleeve cage was stocked with approximately 3000 emerged flies.

Within three days of eclosion, post-teneral (immature) adult flies were transported to Richmond in an air-conditioned vehicle and released from a single point within a grid of traps spaced at approximately 20 m (Figure 4-1). To ensure that human movements did not assist fly dispersal, cages were carried from the car to the release point, and flies were removed by hand from all equipment and persons before leaving the release point. *White marks* were released on 2 occasions: 2 April 2003 (~21000 post-teneral adults), and 8 April 2003 (~12000 post-teneral adults). *White marks* in the second release were marked with fluorescent pigment (Fiesta ®, FEX Series, Astral Pink 1) using a self-marking technique (Steiner 1965). This was done so that these flies could be distinguished from the *white marks* of the earlier release. Fluorescent pigment-marked (Astral Pink 1) sterile flies were released on a single occasion (16 April 2003; ~12000 post-teneral adults).

4.3.2. Trap types and deployment

Sticky spheres were either yellow tennis balls coated with a transparent, odourless polybutane gel to which flies would adhere on contact (Rentokil ®, Tac-Gel Formula 3) (Plate 4-1a), or tennis balls that were painted black before being coated with Tac-Gel (Plate 4-1b). Sticky spheres were suspended from branches in the tree canopy by wire. When suspended, the holes drilled in the upper hemisphere of the ball enabled bait solution to be loaded directly into the hollow centre of the ball. All sticky traps of both colours were loaded with 5 mL of protein autolysate solution from a 250 mL plastic wash bottle immediately before flies were released. The bait solution was 4% protein autolysate, by volume, in water, that was exposed to air for a few days until pH rose from ~ 5.5 to neutral
(pH 7.0), and then refrigerated until use. Yellow and black sticky spheres supplemented an existing grid (see Chapter 3) of disposable plastic pot-type lure traps baited with cuelure (‘Lynfield’ trap) (Cowley et al. 1990). Each Lynfield trap (n = 27) was accompanied by a sticky trap of each colour in an adjacent tree (yellow: n = 27; black: n = 27), sampling a distance of approximately 3 – 88 m from the release point (Figure 4-1). Sticky sphere and Lynfield traps were inspected after one week, with B. tryoni captures transferred to labelled plastic vials for transport back to the laboratory. Trap captures were examined under blue light (475 nm) with a dissecting microscope to identify marked individuals.

4.3.3. Data analysis

Recaptures from each trap were expressed as a proportion of total adults introduced during each release. Comparison of trap effectiveness was analysed using mixed-model analysis of variance (ANOVA). Fixed factors were trap type (Lynfield trap, yellow sticky sphere, black sticky sphere), and sex (male, female). Release (2 April, 8 April, 16 April) was treated as a random factor. The same analysis was repeated after Lynfield trap data was removed. Homogeneity of variance between treatments was tested using Levene’s test of equality. Preliminary analysis of data indicated that the distribution of recaptures was highly skewed and data were subsequently logarithmically transformed (log10(x + 1)) (Underwood 1997). Where Lynfield trap data was included in the analysis, variances were found to be heterogeneous despite transformation. In large experiments, ANOVA is robust despite departures from the assumption of homogeneity of variances (Underwood 1997) and analysis was continued using logarithmically-transformed data.

Linear regression was applied to the relationship between the logarithm of sticky sphere recaptures and the logarithm of distance from the release point. Male and female recaptures were considered separately. For male and female recapture models, regression equations, $R^2$ coefficient, and overall ANOVA were generated.
Plate 4-1. Sticky ball traps suspended within the canopy of a tree in an orchard, Richmond, New South Wales, Australia. (a) Yellow sticky ball trap; (b) Black sticky ball trap.
Figure 4-1. Location of Lynfield traps baited with cuelure and yellow and black sticky spheres baited with protein autolysate solution relative to the release point. Yellow and black sticky spheres were placed in trees next to those where Lynfield traps had been installed. Lynfield trap: black cross. Sticky sphere: solid black diamond.
4.4. Results

4.4.1. Trap effectiveness

Lynfield traps baited with cuelure recaptured considerably more released *B. tryoni* than either yellow or black sticky spheres. Mean (± 1 SE) total recapture rate by Lynfield traps during one week following release was 10.03 ± 3.53%. Yellow and black sticky spheres recaptured an average total of 0.47 ± 0.09% and 0.23 ± 0.05% in one week, respectively.

Mean (± 1 SE) recapture rate per trap in Lynfield, and yellow and black sticky spheres is presented in Figure 4-2. Mean male:female ratio recaptured in Lynfield traps was 1980:1. In yellow and black sticky spheres, mean male:female ratio was 1.2:1 and 1.5:1, respectively. Analysis of variance including Lynfield trap recaptures indicated that there was a significant difference between recaptures of *B. tryoni* per trap, with Lynfield trap recaptures being much higher than either sticky sphere colour (*F* = 7.546, df = 2, 4, *P* = 0.044). There was no significant difference between male and female recaptures (*F* = 8.321, df = 1, 2, *P* = 0.102). There was a significant interaction between trap and sex (*F* = 7.963, df = 2, 4, *P* = 0.040). Recaptures per trap were not significantly different between releases, but there was a very highly significant interaction between trap, sex and release (*F* = 9.799, df = 4, 468, *P* < 0.001) which reflected a higher proportion of (sterile) males being caught in Lynfield traps following the release on 16 April 2003 (Figure 4-2).
Figure 4-2. Mean (± 1 SE) male and female *B. tryoni* recaptures in cuelure, yellow sticky sphere and black sticky sphere traps one week after release. (a) Recaptures following April 2, 2003 release. (b) Recaptures following April 8, 2003 release. (c) Recaptures following April 16, 2003 release. Male (□); Female (■).
Following removal of Lynfield trap data from analysis, there was no significant
difference found between recaptures on yellow and black sticky spheres \((F = 0.365, \text{df} = 1,2, P = 0.607)\). There was no significant difference between male and
female recaptures on yellow and black sticky spheres \((F = 2.745, \text{df} = 1,2, P = 0.239)\), and no interaction between trap and sex \((F = 0.248, \text{df} = 1,2, P = 0.668)\).
Recaptures per trap were not significantly different between releases.

### 4.4.2. Dispersal pattern of males and females

The relationship between logarithmically-transformed male and female recaptures
on sticky sphere traps with the logarithm of distance from the release point is
presented in Figure 4-3. For both males and females there was a significant
relationship between the log-transformed proportion of \(B. \text{tryoni}\) recaptured on
sticky spheres and the logarithm of distance from the release point (male: \(F = 93.748, \text{df} = 1,160, P < 0.001\); female: \(F = 104.396, \text{df} = 1,160, P < 0.001\)). Linear
regression accounted for 36.9% of variance for male recaptures and 39.5% for
female recaptures. The lines that best predicted male and female recapture rates
against the logarithm of distance from the release point were very similar.
Figure 4-3. Log-transformed recaptures of male and female *B. tryoni* on sticky spheres plotted against the logarithm of distance (m) from the release point. Solid lines represent linear regression models fitted to male and female recaptures. Dotted lines represent upper and lower 95% CI. Male: hollow grey diamond, grey line ($y = 0.000253 - 0.000146x$; $R^2 = 0.369$). Female: black cross, black line ($y = 0.00026 - 0.000154x$; $R^2 = 0.395$).
4.5. Discussion

4.5.1. Low recapture rates on odoriferous sticky spheres

Yellow and black sticky spheres baited with protein autolysate solution were relatively inefficient for monitoring dispersal of *B. tryoni* compared to Lynfield traps baited with cuelure. Mean recapture rate per trap on yellow and black sticky spheres was only 1.7% and 1.5%, respectively, of recaptures in Lynfield traps within one week after release. Effectiveness of any alternative trap for monitoring field populations and dispersal of *B. tryoni* must be compared to traps baited with cuelure. Current practice for monitoring *B. tryoni* populations involves use of Lynfield traps baited with cuelure in a 400 m grid (Meats 1996; Dominiak *et al.* 2000b). Lynfield traps baited with cuelure are inexpensive, easy to operate and inspect (Cowley *et al.* 1990), and require little maintenance as traps do not need to be recharged with cuelure and insecticide for six months (Meats *et al.* 2002a).

Traps utilising protein (yeast) hydrolysate and autolysate as a lure have been found to be difficult to inspect and require more frequent recharging as they dry out (Fletcher 1974a; Meats *et al.* 2002a). In addition, this study indicates that trap recaptures in sticky spheres baited with yeast autolysate decline rapidly with distance from the release point, with the majority of flies being trapped within 60 m of the release point (Figure 4-3). Spacing between sticky sphere traps would need to be much lower than that of Lynfield traps if used to supplement existing monitoring grids owing to lower efficiency of sticky spheres for monitoring post-teneral *B. tryoni*.

4.5.2. Sticky sphere colour and sex of recaptures

Sticky sphere colour did not significantly influence the number of female recaptures, although the sex ratio of recaptured flies on both yellow and black sticky spheres were slightly male-biased. Several studies on tephritid fruit flies
have shown that visual cues provided by fruit are important for host-finding by females, and coloured traps painted to mimic host fruit can be used successfully to monitor and control pest populations. Female apple maggot flies, *Rhagoletis pomonella* (Walsh), are strongly attracted to the visual stimulus of red spheres and ripe Red Delicious apples (Duan & Prokopy 1992; Aluja & Prokopy 1993; Duan & Prokopy 1994). In the walnut fly, *R. juglandis* (Cresson), both males and females landed most often on models painted green to appear ripe and uninfested (Henneman & Papaj 1999). In contrast, earlier studies have shown that *Bactrocera* species are attracted to traps coloured yellow and green, which has been considered to reflect a supernormal plant foliage stimulus that is used to locate suitable habitats (Hill & Hooper 1984). Captures of Oriental fruit flies, *Bactrocera dorsalis* (Hendel), were higher on yellow and white sticky spheres than spheres painted green or red (Cornelius et al. 1999; Vargas et al. 2000).

Similarly, bright yellow-green tennis ball fruit models similar to those used in the present study were attractive to male and female *B. tryoni* (Dalby-Ball & Meats 2002). However, it has also been shown that *B. tryoni* and the wild tobacco fruit fly, *B. cacuminata* (Hering), are attracted to blue and orange fruit models, respectively, reflecting preference for the colour of possible native host fruit (Drew et al. 2003).

Despite the difference between total recaptures of Lynfield traps baited with cuelure and yellow and black sticky spheres baited with yeast autolysate solution, both sticky sphere colours recaptured female *B. tryoni*. That male and female *B. tryoni* were recaptured on black and yellow sticky spheres, but no difference was found between recaptures between sticky sphere colours, indicates that the odour of yeast autolysate solution is more important than colour for attraction of post-teneral flies. This has practical implications for monitoring *B. tryoni* with odoriferous sticky spheres; black spheres are easier to maintain because yellow spheres rapidly become covered and discoloured with non-target insects species. The results of the present study support an earlier study on *B. tryoni* that found that odour was more important for orientation of female *B. tryoni* than fruit model colour (Dalby-Ball & Meats 2000b). Odour also plays a
dominant role in orientation of males and females in blueberry maggot fly, *R. mendax* (Liburd *et al.* 1998), and the odour of orange pulp has been shown to be attractive to male and female Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Katsoyannos *et al.* 1997). In *R. pomonella* females, fruit odour only appeared to aid host-finding where fruit or fruit model density on a tree was low (Aluja & Prokopy 1993).

4.5.3. Male and female dispersal is similar

Similarity between linear regression models fitted to male and female sticky sphere recaptures indicates that there is no difference between dispersal patterns of male and female *B. tryoni* in the week following release. This is the first study to explicitly document that dispersal patterns of post-teneral male and female *B. tryoni* are similar. Using a mark-recapture technique, Fletcher (1973) found similar estimates of male and female *B. tryoni* remaining in an orchard at Wilton, New South Wales, after release as young adults, but it was not possible to determine whether these values reflected emigration from the orchard or mortality.

Confirmation that female dispersal during the post-teneral period is similar to that of males in *B. tryoni* indicates that immature adults of both sexes exhibit a propensity for undirected dispersive flight irrespective of the suitability of the immediate area for breeding (Fletcher 1973). Consequently, models predicting post-teneral dispersal patterns of male *B. tryoni* based on recaptures in traps baited with cuelure may be used reliably to predict dispersal of both sexes as immature adults. Movements of mature adult *B. tryoni* associated with locating mates, food and oviposition sites remain relatively unknown and require further study.
4.5.4. Dispersal of gamma-irradiated *B. tryoni*

A significant interaction was detected between trap type, sex and release when recaptures from Lynfield traps baited with cuelure were included in the analysis. A higher proportion of males were recaptured in Lynfield traps following release on 16 April 2003 (Figure 4-2) which involved release of gamma-irradiated, sterile *B. tryoni*. There are several possible explanations for this result, although further study is required to test their validity. Firstly, sterile males may be more highly attracted to traps baited with cuelure, although a causal mechanism for this response is not known. Another possibility is that sterile male *B. tryoni* are attracted to cuelure earlier than other strains of *B. tryoni* that only become attracted to cuelure when sexually mature. In *C. capitata*, irradiated males responded to trimedlure at a younger age than wild flies, which may have been due to inadvertent selection for decreased development time in laboratory-reared flies (Barry et al. 2003a). If sterile male *B. tryoni* respond to cuelure at an earlier age, lower recaptures of *white marks* represent a longer period of post-teneral dispersal and it may be expected that *white marks* disperse further than sterile flies as young adults. Alternatively, sterile *B. tryoni* may have limited flight capabilities compared to conspecifics that have not been irradiated, and remained in the area of the release site. Mass-reared melon fly, *B. cucurbitae* (Coquillett), were found to have lower flight durations than wild flies (Nakamori & Simizu 1983), and mass-reared and sterile *B. cucurbitae* could be identified by differences in acoustic properties of tethered flight sounds (Kanmiya et al. 1987b).
CHAPTER 5. Dispersal of males: effect of season, laboratory-domestication and sterilisation.

5.1. Summary

This study documented dispersal of post-teneral male Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), released from a point within an orchard. Marked male *B. tryoni* were recaptured in the trap furthest from the release point, representing a distance greater than 1000 m, although 97.42 ± 1.32% of males were recaptured less than 500 m from the release point. Recaptures of wild and sterile males per Lynfield trap baited with cuelure were highest within one week after release, whereas recaptures of white marks males per trap increased in the second week. This result may offer evidence to support the hypothesis that sterile male *B. tryoni* respond to cuelure at an earlier age, indicating earlier sexual maturity. Comparison of wild, laboratory-reared and gamma-irradiated male *B. tryoni* indicated that mean dispersal distance and redistribution pattern was not significantly affected by fly origin. Analysis of temporally replicated releases between 2001 – 2003 unexpectedly revealed that dispersal of male *B. tryoni* in an orchard near Richmond, New South Wales, was higher in Spring than in Autumn. Low mean dispersal distances in Autumn may be explained by the presence of fruiting hosts in the orchard, or the availability of resources required by over-wintering flies. Maximum daily temperature was significantly higher in Spring than in Autumn, but there was no significant correlation between temperature and mean dispersal distance. Recapture rate per trap was significantly negatively correlated with increasing daily maximum and average temperature.
5.2. Introduction

Dispersal is a fundamental ecological process that couples dynamics within and between populations. Defined as an increase in the mean distance between individuals, dispersal may include movements within the territory of a population, as well as movements away from it (Southwood 1962). Consequently, dispersal enables gene flow between populations, and (re)colonisation of new habitat (Stinner 1983). Dispersal of pest insect species has received much attention owing to the critical importance of understanding pest movement for effective control (Stinner 1983). Application of sterile insect technique (SIT) requires knowledge of dispersal from a release point so that effective release rates can be determined (Meats 1996). Similarly, dispersal can influence the release rate required for insect biological control agents, as release rates below a critical threshold may lead to an Allee effect (Hopper & Roush 1993; Grevstad 1999).

Although some form of dispersal is possible in all species, evidence indicates that the incidence of dispersal in insects is highest in species whose habitats are temporary and relatively short-lived in a particular location (Southwood 1962, 1981). The ability to encounter and utilise changing or temporary habitats is the prime selective force for evolution and maintenance of a phase of dispersal in a species (Southwood 1962). Insect dispersal primarily occurs during the adult stage of the life cycle prior to reproductive maturity, with energy invested into flight muscle and movement away from the larval habitat before egg maturation. Deferment of reproductive capacity for migration is a physiological necessity to reach new habitat in which a reduced reproductive rate is compensated by higher survival of offspring (Southwood 1981), although reproductive potential of the adult is dependent on locating sexually mature conspecific adults in the new habitat (Bateman 1977; Hopper & Roush 1993).
5.2.1. Dispersal of *B. tryoni*

Earlier papers indicate that *B. tryoni* possesses high dispersal capabilities. Wild male *B. tryoni* that had been reared out of infested fruit and marked by hand before release in an orchard near Wilton, New South Wales, were caught up to 24 km from the release point (Fletcher 1974a). Peak trap captures of *B. tryoni* in rainforest with limited fruit host species at Cooloola, Queensland, were recorded 2 – 3 weeks after peak fruiting time of major hosts located 60 km away from the study site (Drew *et al.* 1984). The abundance of fruit in surrounding areas is likely to influence the extent to which *B. tryoni* disperse (Fletcher 1974a).

Movement of adult *B. tryoni* is characterised by two distinct phases. Firstly, post-teneral, sexually immature adults disperse away from the site of pupal emergence regardless of the suitability of the immediate habitat for survival and reproduction (Bateman & Sonleitner 1967; Fletcher 1973). When adults become sexually mature, post-teneral dispersal is followed by trivial, local, non-dispersive movement in search of food, mates, and oviposition sites (Fletcher 1973). Sexually mature adult *B. tryoni* may also exhibit dispersive movement in response to adversity (Fletcher 1973), including lack of adequate oviposition sites (Bateman & Sonleitner 1967; Zalucki *et al.* 1984). Similarly, at the southern end of its range and at high altitude, commencement of warm weather in spring influences over-wintering adults to disperse (Fletcher 1979; Drew & Hooper 1983).

Availability of fruiting host trees that are limited in space and time may explain high capacity for dispersal in *B. tryoni*. At Wilton, when fruit was available for oviposition, large numbers of *B. tryoni* moved into the area and remained until fruit became scarce (Fletcher 1973). Trapping of *B. tryoni* at Mount Glorious, near Brisbane, Queensland, an area containing open eucalypt and rainforests interspersed with residential areas and small farms, showed that peak trap captures were associated with fruiting times of major hosts; loquats, mulberries, peaches, mangoes and guavas (Drew & Hooper 1983). At an altitude
of 700 m, Drew and Hooper (1983) proposed that \textit{B. tryoni} caught in traps during early spring represented flies migrating into the area, coinciding with fruiting of loquats and mulberries.

\subsection*{5.2.2. Sterile insect technique and dispersal}

Sterile insect technique (SIT) aims to reduce the growth rate of a target population to below unity by saturating wild females with released mass-reared, sterilised males. Suppression of \textit{B. tryoni} populations using SIT has a long history in Australia, beginning in late 1961 when the first SIT trials were conducted in Manilla, New South Wales (Andrewartha \textit{et al.} 1967). Successful eradication of \textit{B. tryoni} was achieved in Western Australia in a program incorporating SIT (Fisher 1992), and SIT is used in suppression programs to control outbreaks in important horticultural areas in south-eastern Australia (James 1992; Horwood & Keenan 1994; Dominiak \textit{et al.} 1998; Dominiak \textit{et al.} 2000b; Dominiak \textit{et al.} 2003).

The release rates necessary for successful suppression of insect populations using SIT are determined partly by the competitiveness of sterile males relative to their wild counterparts, and partly by their ability to survive and distribute themselves within the environment (Fried 1971; Itô & Koyama 1982; Meats \textit{et al.} 1988). Wild and laboratory-reared \textit{B. tryoni} exhibit no difference in patterns of tethered flight (Chapman 1982), although propensity for flight is highly variable (Chapman 1983). The maximum recorded dispersal distance from a sterile fly release at Wangaratta, Victoria, was 94 km, although the average recapture distance was within 1.5 km of the release point (MacFarlane \textit{et al.} 1987). The two successful releases at Wangaratta involved a large number of male flies (470,000) so this result is not unexpected considering that trap recaptures are density-dependant (Fletcher 1974a; Meats 1998b). More recent studies indicate that most sterile \textit{B. tryoni} do not disperse far from the release point (Dominiak & Webster 1998; Meats 1998b; Dominiak \textit{et al.} 2003). Recapture rates of sterile male \textit{B. tryoni} in traps less than 100 m from a release point in an orchard near
Richmond, New South Wales, were higher than that of a non-sterile laboratory-reared strain (Chapter 4). This could indicate earlier maturation of sterile *B. tryoni*, and subsequent reduction in dispersal distance due to a shorter post-teneral dispersal phase. Alternatively, the flies may have dispersed at a lower rate or sterile flies may exhibit higher survivability than non-sterile *B. tryoni*. It is only possible to differentiate between these possibilities by conducting simultaneous releases. For effective SIT it is important that sterile males are distributed in a similar way to that of their wild counterparts when they become sexually mature, ensuring that they are positioned to be competitive with wild males for wild females (Meats 1998c). No direct comparisons of post-teneral dispersal have been made between wild, laboratory-reared and sterile *B. tryoni*.

The objective of this study was to document dispersal of post-teneral male *B. tryoni* released from a point within an orchard. Temporally replicated releases were conducted in Spring and Autumn to investigate the effect of season on dispersal of male *B. tryoni*. Dispersal of wild male *B. tryoni* was compared to dispersal of laboratory-reared and gamma-irradiated male *B. tryoni*. In addition, recaptures of wild, white marks and sterile *B. tryoni* to Lynfield traps baited with cuelure were compared to determine if laboratory-domestication or gamma-irradiation influence responsiveness of males, as an indicator of male sexual maturity. Analysis of the influence of wind was not considered necessary in view of the results of Fletcher (1974a) and MacFarlane (1987). Furthermore, analysis of the influence of wind was not possible with the available trapping grid, which was long and thin (Figure 5.1).
5.3. Materials and Methods

5.3.1. Study site and trap deployment

The study was conducted at the Hawkesbury Campus of the University of Western Sydney, near Richmond, New South Wales. The property has a mixed orchard containing various species and varieties of citrus, stonefruits, and pears, as well as ornamental trees. Fruit trees were arranged in distinct blocks for management and research purposes (Figure 5-1). Management practices in the orchard were restricted to grass-mowing and pruning. The ‘Apiary’ block contained navel oranges, peaches, nectarines, plums and ornamental species (Plate 5-1a). Adjacent and north-east of the Apiary block was the ‘Horticulture’ block, containing plums, cherries, apricots, pears, kiwi fruit and ornamental trees (Plate 5-1b). ‘Orchard Square’ block consisted of valencia oranges, tangelos and lemons (Plate 5-2a). The ‘GACB Citrus’ block contained valencia oranges (Plate 5-2b), and the adjacent ‘Nashi Pear’ block contained nashi pears (Plate 5-3a). A distance of approximately 200 m, consisting of open fields with little tree cover, separated the Horticulture and Orchard Square blocks from the GACB Citrus and Nashi Pear blocks. A matrix of grazing land, a eucalypt plantation and ornamental trees extended beyond the edge of the orchard, including ornamental trees planted along College Drive (Plate 5-3b).
Figure 5-1. Location of Lynfield traps baited with cuelure relative to the release point.
Plate 5-1. Orchard at the Hawkesbury Campus of the University of Western Sydney, near Richmond, New South Wales. (a) Apiary block; (b) Horticulture block.
Plate 5-2. Orchard at the Hawkesbury Campus of the University of Western Sydney, near Richmond, New South Wales. (a) Orchard Square block; (b) GACB Citrus block.
Plate 5-3. Orchard at the Hawkesbury Campus of the University of Western Sydney, near Richmond, New South Wales. (a) Nashi Pear block; (b) College Drive.
Temperature records for the period of the study (1 April 2001 – 30 November 2003) were obtained from the Bureau of Meteorology for a weather station located approximately 3 km from the orchard (Site 67105, Richmond RAAF, Lat. -33.6004, Long. 150.7761). Data requested were daily maximum temperature (°C), daily minimum temperature (°C), and average daily air temperature using all available observations (°C).

A grid of 88 cuelure traps was used to monitor dispersal of *B. tryoni*. The trap was a disposable pot-type lure trap made of clear plastic (Lynfield trap) (Cowley et al. 1990). Approximately 2 mL of a cuelure and malathion mix (8:1), was placed on three dental rolls held in place at the centre of the trap by a clip. The trap was suspended within the canopy of host and non-host trees by a piece of wire tied onto a metal loop incorporated with the lid. The base of the traps had four 2 mm water drainage holes. There were four 25 mm entry holes 90° apart in the clear plastic trap body. Traps were spaced at approximately 20 m intervals within orchard blocks, sampling a distance of 3 – 1087 m from the release point (Figure 5-1).

5.3.2. Source and marking of flies

Three strains of *B. tryoni*, wild, white marks and sterile, were used during the study. Wild *B. tryoni* were reared out of infested fruit collected from various locations in the Sydney Basin (loquat, peach, nectarine, apricot, nashi pear, mango). First generation offspring from these flies (G1) were also used. First generation offspring show no difference from their wild-caught sires in rates of development or fecundity (Meats et al. 2004). Fruit infested with *B. tryoni* larvae was placed in metal wire trays stacked inside standard laboratory sleeve cages (base 45 cm x 33 cm and height 34 cm). The metal wire trays containing infested fruit were stacked on top of metal dissection trays filled with perlite and folded newspaper to absorb juices from the rotting fruit. Perlite-filled dissection trays were wrapped in fine mesh fabric to provide a barrier that prevented larvae from pupating in the perlite. A layer of sawdust was scattered on the base of the
laboratory cage for larvae to settle and enter the pupal phase. Pupae were sifted out of the sawdust every 2-3 days.

*White marks* were a laboratory-reared strain of *B. tryoni* exhibiting a natural colour mutation in adults. Rather than yellow markings typical of wild-type *B. tryoni*, *white marks* possess white markings (Plate 5-4). The *white marks* colour mutation is caused by a homozygous recessive allele on chromosome 2 of *B. tryoni* (Zhao *et al.* 2003). This feature can be used to distinguish *white marks* from wild *B. tryoni* captured in monitoring traps. *White marks* were obtained by inbreeding of family groups; pure breeding lines for these characters were retrieved after outcrossing to flies of stocks of recently wild origin (Meats *et al.* 2002b).

Sterile *B. tryoni* were obtained as pupae from the mass rearing facility at Camden, New South Wales, managed by NSW Agriculture on behalf of the Tri-State Fruit Fly Committee. Sterile pupae were produced by irradiation 3 days before adult eclosion with 70.3 – 73.8 Gy of gamma radiation from a cobalt-60 source, in a normal atmosphere, by the Radiation and Dosimetry Service of the Australian Nuclear Science and Technology Organisation.

Wild, *white marks* and sterile *B. tryoni* were mass-marked with fluorescent pigment using a self-marking technique (Norris 1957; Steiner 1965). On several occasions it was not necessary to mark *white marks* as their white markings could be used to distinguish them from wild-coloured *B. tryoni* (Table 5-1, Table 5-2). Pupae were gently sifted into an equal volume of sawdust and fluorescent pigment. Fluorescent pigment was applied at a rate of 0.2 g per 1000 pupae. During emergence, a small quantity of fluorescent pigment adhered to the ptilinum enabling identification of released flies when examined under blue light (475 nm). Ptilinal fluorescent pigment marking does not affect survival of adult *B. tryoni* (Chapter 2).
Plate 5-4. Dorsal thoracic colour forms of *B. tryoni*. The *white marks* (left) colour mutation is caused by a homozygous recessive allele on chromosome 2. This feature can be used to distinguish *white marks* from wild type *B. tryoni* (right).
5.3.3. Pupal releases

*Bactrocera tryoni* pupae were allowed to emerge in the field within Styrofoam boxes at a single release point in the centre of the Apiary block. Pupal releases were conducted only in Spring, at the beginning of the fruit fly season. Wild *B. tryoni* were not released as pupae, as the availability of wild flies was limited and pupal releases conducted using *white marks* and sterile *B. tryoni* indicated that total recapture rates of males released as pupae were low (Table 5-1). Two types of Styrofoam emergence boxes were used during the study to house emerging pupae: a large box placed on the ground, and several small boxes suspended from branches of orchard trees (Table 5-1). The ground release box was a broccoli box (base 50 cm x 30 cm and height 35 cm) with two holes (8 cm diameter) cut into two sides for emerging adults to escape (Plate 5-5a). The box was covered with a flat panel of Styrofoam that extended beyond the sides of the box to prevent rain from entering the box, and was held in place by two bricks. A strip a transparent, odourless polybutane gel to which insects would adhere on contact was applied around the base of the box to prevent ant predation on pupae and newly-emerged adults. Six plastic trays each containing approximately 10000 pupae in an equal volume of sawdust mixed with fluorescent pigment powder were placed in the ground release box.
Table 5-1. Releases of *B. tryoni* as pupae in an orchard near Richmond, New South Wales.

<table>
<thead>
<tr>
<th>Release</th>
<th>Pigment mark colour</th>
<th>Release method</th>
<th>Pupae released</th>
<th>Males released</th>
<th>Males recaptured (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spring</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>October 2001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterile</td>
<td>Lunar Yellow</td>
<td>Ground</td>
<td>60000</td>
<td>17156</td>
<td>0.71</td>
</tr>
<tr>
<td>October 2003</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>White marks</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterile</td>
<td>Chartreuse</td>
<td>Suspended</td>
<td>32000</td>
<td>6680</td>
<td>0.06</td>
</tr>
<tr>
<td>November 2003</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>White marks</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterile</td>
<td>Chartreuse</td>
<td>Suspended</td>
<td>55000</td>
<td>27376</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

69
Suspended release boxes were small Styrofoam ice boxes with a rope carrying handle. Each suspended release box had two holes (diameter 7 cm) cut into two sides so that emerging flies could escape (Plate 5-5b). Suspended boxes were hung within the canopy of host trees using the rope carrying handle and wire to secure the box at the release point in the centre of the Apiary orchard block. A layer of pupae mixed with an equal volume of sawdust mixed with fluorescent pigment powder was added directly to each release box. Up to 20000 pupae were placed in each suspended release box.

Emergence rates (Table 5-1) were estimated by taking 2 - 5 25 mL samples from the pupal layer one week after emergence of flies. Counts were made of pupae that did not emerge, partly emerged adults, empty pupal cases (Dominiak et al. 2000b), and emerged adults that died before escaping from the pupal release box. Successfully emerged adults were deemed to be the proportion of empty cases corrected by the number of adult flies that died before leaving the release box. The number of males that emerged and were released was calculated assuming an even sex ratio.
Plate 5-5. Styrofoam boxes used to house emerging pupae in the field. (a) Ground release box; (b) Hanging release boxes.
5.3.4. Adult releases

Post-teneral adult *B. tryoni* were released at the beginning of the fruit fly season in Spring, and late Summer/Autumn at the end of the season (Table 5-2). Wild *B. tryoni* could not be released as post-teneral adults in Spring as a source of an adequate number of wild flies for release was not available at that time of year. Two methods were employed to release *B. tryoni* as adults. Firstly, approximately 6000 pupae in plastic trays of sawdust mixed with fluorescent pigment powder were allowed to emerge in 50 L black storage bins (Nally Crates, Viscount Plastics (NSW) Ltd.) covered with mesh fabric that was secured over the top of the bin by black nylon elastic (Plate 5-6). Folded cardboard was placed inside the storage bin to provide additional area for emerging flies to perch. Raw sugar was liberally scattered onto the mesh fabric each day until release to provide emerging flies with a source of food. Water was provided 4-5 times per day until release using a plastic spray bottle to apply a fine mist of water onto and through the mesh.

Standard laboratory sleeve cages were also used to release post-teneral *B. tryoni*. Approximately 3000 pupae in plastic trays of sawdust mixed with fluorescent pigment powder were allowed to emerge. Cages were kept near a window that provided natural light-dark conditions. Cages contained a sealed plastic water container with a cotton dental roll wick, and an open plastic container filled with granulated sucrose to provide emerged flies with water and food *ad libitum*. 
Table 5-2. Releases of *B. tryoni* as immature (post-teneral) adults in an orchard near Richmond, New South Wales. Wild (G₁) flies were first generation offspring of wild flies reared out of infested fruit.

<table>
<thead>
<tr>
<th>Release</th>
<th>Pigment mark colour</th>
<th>Release method</th>
<th>Males released</th>
<th>Males recaptured (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spring</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>October 2001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterile</td>
<td>Astral Pink</td>
<td>Storage bin</td>
<td>30000</td>
<td>12.52</td>
</tr>
<tr>
<td>November 2002</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>White marks</em></td>
<td>none</td>
<td>Sleeve cage</td>
<td>4000</td>
<td>1.65</td>
</tr>
<tr>
<td>Sterile</td>
<td>Astral Pink</td>
<td>Storage bin</td>
<td>21708</td>
<td>0.07</td>
</tr>
<tr>
<td>October 2003</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>White marks</em></td>
<td>Arc Yellow</td>
<td>Sleeve cage</td>
<td>6000</td>
<td>0.95</td>
</tr>
<tr>
<td>Sterile</td>
<td>Deep Green</td>
<td>Sleeve cage</td>
<td>6000</td>
<td>2.07</td>
</tr>
<tr>
<td><strong>Autumn</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>March 2002</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild</td>
<td>Orange</td>
<td>Sleeve cage</td>
<td>3000</td>
<td>5.67</td>
</tr>
<tr>
<td>Wild (G₁)</td>
<td>Lunar Yellow</td>
<td>Sleeve cage</td>
<td>4500</td>
<td>2.42</td>
</tr>
<tr>
<td>Sterile</td>
<td>Astral Pink</td>
<td>Storage bin</td>
<td>24000</td>
<td>5.11</td>
</tr>
<tr>
<td>April 2002</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>White marks</em></td>
<td>none</td>
<td>Storage bin</td>
<td>1329</td>
<td>2.33</td>
</tr>
<tr>
<td>Sterile</td>
<td>Astral Pink</td>
<td>Storage bin</td>
<td>14548</td>
<td>8.72</td>
</tr>
<tr>
<td>February 2003</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild</td>
<td>Chartreuse</td>
<td>Sleeve cage</td>
<td>2000</td>
<td>2.85</td>
</tr>
<tr>
<td>Wild (G₁)</td>
<td>Orange</td>
<td>Sleeve cage</td>
<td>1500</td>
<td>1.67</td>
</tr>
<tr>
<td>Sterile</td>
<td>Astral Pink</td>
<td>Storage bin</td>
<td>19856</td>
<td>4.04</td>
</tr>
<tr>
<td>April 2003a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>White marks</em></td>
<td>none</td>
<td>Sleeve cage</td>
<td>10500</td>
<td>44.04</td>
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<td>April 2003b</td>
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<td></td>
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</tr>
<tr>
<td><em>White marks</em></td>
<td>Aurora Pink</td>
<td>Sleeve cage</td>
<td>6000</td>
<td>20.12</td>
</tr>
<tr>
<td>April 2003c</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sterile</td>
<td>Astral Pink</td>
<td>Sleeve cage</td>
<td>6000</td>
<td>35.70</td>
</tr>
</tbody>
</table>
Plate 5-6. Black plastic storage bin used to release post-teneral adult *B. tryoni*.
Within three days of eclosion, post-teneral adult flies were transported to Richmond in an air-conditioned vehicle and released from a single point within the trapping grid at the centre of the Apiary block. To ensure that human movements did not assist fly dispersal, cages were carried from the car to the release point, and flies were removed by hand from all equipment and persons before leaving the release point. The number of males released as post-teneral adults was estimated visually or by following the same method as for the pupal release.

5.3.5. Sampling and data analysis

Efficiency of the trapping grid was determined by inspecting Lynfield trap recaptures 24 hours after release of post-teneral adults within a 5 x 5 trapping array immediately surrounding the release point (the Apiary block; Figure 5-1). Trapping grid efficiency was examined using white marks released twice in April 2003 (April 2003a and 2003b), and in October 2003. After release of flies as pupae and post-teneral adults, Lynfield traps were inspected at weekly intervals, with contents emptied into labelled plastic vials for transport back to the laboratory.

Recaptures from Lynfield traps were expressed as a proportion of total adults introduced during each release. SPSS for Windows Release10.0.1 was used for all analyses (except comparison of regression slopes). Trap recaptures from pupal and adult releases were analysed separately using fixed-factor analysis of variance (ANOVA). For pupal releases, factors were strain (white marks, sterile) and week after release (one, two). For adult releases, factors were strain (wild, white marks, sterile), week after release (one, two) and season (Spring, Autumn). Homogeneity of variance between treatments was tested using Levene’s test of equality. Preliminary analysis of data indicated that the distribution of recaptures was highly skewed and data were subsequently logarithmically transformed (\(\log_{10}(x + 1)\)) (Underwood 1997). Variances were found to be heterogeneous despite transformation. In large experiments, ANOVA is robust despite departures
from the assumption of homogeneity of variances (Underwood 1997) and analysis
was continued using logarithmically-transformed data. Post-hoc multiple
comparisons between strains of *B. tryoni* were made using Dunnett’s C test.
Dunnett’s C is based on the Studentized range and is appropriate when variances
are unequal.

Linear regression was applied to the relationship between the logarithm of
the proportion of flies recaptured in each Lynfield trap and the logarithm of
distance from the release point. Wild, *white marks* and sterile male recaptures, one
and two weeks after release, in Spring and Autumn were considered separately.
For each model, regression equations, $R^2$ coefficient, and overall ANOVA were
generated. For the first week following release of *B. tryoni* released as post-teneral
adults, slopes of regression lines were tested for equality (Sokal & Rohlf 1995).
Slopes of regression lines for recaptures in the second week following release
were not compared as regression lines poorly described variation in recaptures
with the logarithm of distance (Figure 5-5).

Using recapture data from adult releases, mean recapture distance from
each release was calculated. Mean recapture distance of flies released as pupae
was not analysed as there was an insufficient number of replicates of *white marks*
releases. Average recapture distance of wild, white marks and sterile *B. tryoni* was
compared using fixed-factor ANOVA. Factors were strain (wild, *white marks*,
sterile), week after release (one, two) and season (Spring, Autumn). When mean
distance of recaptures was zero in the second week after release (October 2003:
sterile and *white marks*) the value was excluded from analysis. Homogeneity of
variance between treatments was tested using Levene’s test of equality. Variances
between groups were heterogeneous and data were subjected to square-root
transformation ($\sqrt{x + 1}$). Post-hoc multiple comparisons between strains of *B.
tryoni* were made using Dunnett’s C test.

Temperatures in Spring ($n = 82$) and Autumn ($n = 84$) were compared
using the non-parametric Mann-Whitney test (test statistic $U$). The relationship
between temperature and total recaptures, mean recaptures per trap, and mean recapture distance was analysed with Spearman’s rank correlation (test statistic $r_s$). Total recaptures, mean recaptures per trap, and mean recapture distance for wild, white marks and sterile males were pooled and related to mean maximum, minimum and average daily temperatures (°C) from the week after release and for two weeks after release. Recaptures of flies released as pupae were not analysed as there were an insufficient number of replicates.

5.4. Results

5.4.1. Response to cuelure traps

Male recaptures from each pupal release are presented in Table 5-1. Following pupal releases of *B. tryoni*, Lynfield traps baited with cuelure recaptured a mean (± 1 SE) of 0.26 ± 0.22% and 0.03 ± 0.03% of white marks and sterile males, respectively. Table 5-2 presents the proportion of males recaptured from each post-teneral adult release. Mean total recaptures of wild males released as post-teneral adults was 2.99 ± 0.70 %. Recaptures of white marks and sterile male *B. tryoni* released as post-teneral adults were 20.43 ± 7.95% and 9.75 ± 4.30%, respectively. Lynfield trap recaptures within 24 hours after release of post-teneral adult white marks was 1.42 ± 1.00% in Lynfield traps spaced 20 m apart in a 5 x 5 array.

Recaptures with time after release for white marks and sterile males released as pupae in Spring are given in Figure 5-2. Recaptures of sterile males released as pupae were significantly higher than that of white marks males recaptured in Lynfield traps ($F_{1,876} = 6.172, P = 0.013$). Trap recaptures in the second week after release were significantly lower than in the first week ($F_{1,876} = 4.905, P = 0.027$). The interaction between strain and week was not significant ($F_{1,876} = 3.740, P = 0.053$).
White marks and sterile male recaptures in Lynfield traps were higher than recaptures of wild B. tryoni following release as post-teneral adults ($F = 54.164$, df = 2,2982, $P < 0.001$; Figure 5-3). Recaptures per trap in Spring (Figure 5-3a) were significantly lower than recaptures in Autumn (Figure 5-3b) ($F = 93.539$, df = 1,2894, $P < 0.001$). Male recaptures did not significantly differ with time after release ($F = 37.97$, df = 1,2892, $P = 0.051$). There was a significant interaction between strain and season ($F = 29.158$, df = 1,2892, $P < 0.001$), and strain and week after release ($F = 13.083$, df = 2,2892, $P < 0.001$).

5.4.2. Dispersal from pupal releases

The proportion of males released as pupae that were recaptured in Lynfield traps over two weeks, in relation to distance from the release point, is shown in Figure 5-4. In both white marks and sterile male B. tryoni released as pupae, one week after release recaptures were highest around the release point and decreased with distance away from the release point (Figure 5-4a, Figure 5-4b). There was a significant but very weak relationship between the logarithm of distance from the release point and the logarithm of recaptures in the first week following release of both white marks and sterile male B. tryoni. Recaptures of both white marks and sterile male B. tryoni in the second week after release were low (Figure 5-4c, Figure 5-4d). The highest recorded recaptures in the second week after release were more than 100 m from the release point. White marks and sterile male recaptures were not linearly related with distance.
Figure 5-2. Recaptures with time after release for white marks and sterile male *B. tryoni* released as pupae in Spring (mean ± 1 SE). No wild flies were released as pupae. White bars: first week after release; grey bars: second week after release.
Figure 5-3. Recaptures per trap of wild, white marks and sterile male *B. tryoni* released as post-teneral adults in Autumn and Spring (mean ± 1 SE). (a) Autumn. (b) Spring. No wild flies were released as post-teneral adults in Autumn. White bars: first week after release; grey bars: second week after release.
Figure 5-4. Proportion of white marks and sterile male *B. tryoni* released as pupae that were recaptured in Lynfield traps over two weeks, in relation to distance from the release point. (a) White marks and (b) sterile male recaptures in the first week after release. (c) White marks and (d) sterile male recaptures in the second week after release. All pupal releases were conducted in Spring.
5.4.3. Dispersal from adult releases

Within one week of release, all recaptures of males released as adults were made within a distance of 500 m from the release point. In the second week after release, marked male *B. tryoni* were recaptured in the trap furthest from the release point, representing a distance greater than 1000 m, although 97.42 ± 1.32% of males were recaptured less than 500 m from the release point. The proportion of males released as post-teneral adults that were recaptured in Lynfield traps over two weeks, in relation to distance from the release point, is shown in Figure 5-5. Similar to pupal releases, recaptures of male *B. tryoni* released as post-teneral adults one week after release were highest around the release point and decreased with distance away from the release point. This was evident from both Spring and Autumn releases of wild (Figure 5-5a), *white marks* (Figure 5-5b) and sterile releases (Figure 5-5c). Recaptures in the second week after release were not centred around the release point. In Spring releases, there was no relationship between *white marks* (Figure 5-5e) and sterile (Figure 5-5f) male *B. tryoni* recaptures and the logarithm of distance in the second week. Wild (Figure 5-5d) and sterile (Figure 5-5f) male recaptures were not linearly related with distance in the second week after release in Autumn. There was a weak, but significant regression between distance and male *white marks* recaptures in the second week after release during Autumn (Figure 5-5c).

Regression slopes of *white marks* recaptures one week after release in Spring and Autumn did not differ significantly (*F* = 2.263, df = 1,436, *P* > 0.05; Figure 5-5b). There was also no difference between slopes of sterile male recaptures one week after release in Spring and Autumn (*F* = 0.490, df = 1,612, *P* > 0.05; Figure 5-5c). Slopes of *white marks* and sterile male recaptures in Spring (*F* = 1.159, df = 1,260, *P* > 0.05; Figure 5-5b – Figure 5-5c), and slopes of wild, *white marks* and sterile male recaptures in Autumn were not significantly different (*F* = 0.000, df = 2,698, *P* > 0.05; Figure 5-5a – Figure 5-5c).
Mean recapture distance was not significantly different between wild, white marks and sterile *B. tryoni* released as post-teneral adults (*F* = 1.162, df = 2,12, *P* = 0.332; Figure 5-6). There was a significant difference between mean recapture distance in Spring and Autumn (*F* = 4.727, df = 1,21, *P* = 0.041). Mean recapture distance from the release point one week after release (Figure 5-6a) was significantly less than mean recapture distance two weeks after release (Figure 5-6b) (*F* = 17.288, df = 1,21, *P* < 0.001). The interaction between season and week was highly significant, with the difference between mean recapture distance in the second week after release being much higher in Spring than in Autumn (*F* = 7.107, df = 1,21, *P* = 0.014).

**Effect of temperature**

Daily maximum temperatures during releases of *B. tryoni* in Spring were significantly higher than those in Autumn (*U* = 2802.000, *P* = 0.038). There was no difference in daily minimum (*U* = 3334.500, *P* = 0.723) or daily average temperatures (*U* = 3148.500, *P* = 0.281) between Spring and Autumn.

In the first week after release total male recaptures were significantly negatively correlated with daily maximum and average temperature (Table 5-3). Male recaptures per trap were also significantly negatively correlated with daily maximum and average temperature in the week following release. Total male recaptures and recaptures per trap were not correlated with temperature over both weeks of the study. Daily maximum, minimum and average temperatures were not correlated with mean recapture distance of male *B. tryoni* during a one- or two-week period after release.
Figure 5-5. Proportion of wild, white marks and sterile male *B. tryoni* released as post-teneral adults that were recaptured in Lynfield traps over two weeks, in relation to distance from the release point. (a) Wild, (b) white marks, and (c) sterile male recaptures in the first week after release. (d) Wild, (e) white marks, and (f) sterile male recaptures in the second week after release. Grey markers: Spring; black markers: Autumn.
Figure 5-6. Mean recapture distance of wild, *white marks* and sterile male *B. tryoni* released as post-teneral adults (mean ± 1 SE). No wild flies were released as post-teneral adults in Autumn. (a) Distance in the first week after release. (b) Distance in the second week after release. White bars: Spring; grey bars: Autumn.
Table 5-3. Spearman’s rank correlation ($r_s$) of total male recaptures (%), males recaptured per trap (%), and mean recapture distance with mean maximum, minimum and average daily temperatures (°C). Means for recapture data and temperatures were calculated for one week and two weeks after release. Temperature data were obtained from a meteorological station operated by the Bureau of Meteorology that was approximately 3 km from the orchard where releases were conducted (Site 67105, Richmond RAAF, Lat. -33.6004, Long. 150.7761).

<table>
<thead>
<tr>
<th>Time elapsed after release</th>
<th>$N$</th>
<th>Maximum temperature (°C)</th>
<th>Minimum temperature (°C)</th>
<th>Average temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$r_s$</td>
<td>$P$</td>
<td>$r_s$</td>
</tr>
<tr>
<td><strong>One week</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total male recaptures (%)</td>
<td>17</td>
<td>-0.729</td>
<td><strong>0.001</strong></td>
<td>-0.445</td>
</tr>
<tr>
<td>Males recaptured per trap (%)</td>
<td>17</td>
<td>-0.656</td>
<td><strong>0.004</strong></td>
<td>-0.323</td>
</tr>
<tr>
<td>Mean recapture distance (m)</td>
<td>17</td>
<td>0.291</td>
<td>0.399</td>
<td>0.197</td>
</tr>
<tr>
<td><strong>Two weeks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total males recaptured (%)</td>
<td>16</td>
<td>-0.211</td>
<td>0.433</td>
<td>-0.053</td>
</tr>
<tr>
<td>Males recaptured per trap (%)</td>
<td>16</td>
<td>0.056</td>
<td>0.835</td>
<td>0.238</td>
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<tr>
<td>Mean recapture distance (m)</td>
<td>16</td>
<td>0.045</td>
<td>0.870</td>
<td>0.283</td>
</tr>
</tbody>
</table>
5.5. Discussion

5.5.1. Recapture rates of strains

Recapture rates of male *B. tryoni* in Lynfield traps baited with cuelure were high relative to most other mass mark-release-recapture studies on this species. Total recapture rates of 0.04% (Dominiak & Webster 1998), 0.07% (Dominiak *et al.* 1998), and 0 – 2.05% (Dominiak *et al.* 2000b) have been reported for sterile *B. tryoni* released in sterile insect programs on a 400 m grid. In the present study, one day following post-teneral adult release of *white marks* males, an average of approximately 1% were recaptured in the trapping grid used in this study. By two weeks, the mean total recapture rate of *white marks* males released as post-teneral adults was almost 19%. For wild and sterile male *B. tryoni* released as post-teneral adults, mean total recapture rates two weeks after release were almost 3% and 10%, respectively. The relatively high recapture rate in this study was probably due to the close spacing of traps within the grid, of approximately 20 m. However, recapture rates in the present study were lower than reported by Fletcher (1974b). Using flies reared from infested fruit, Fletcher (1974b) found that within 24 hours a single cuelure trap could recapture 1% of mature male *B. tryoni* that were released in an area of approximately 200 m radius of the trap. In the same study, he found that a grid of 16 traps, 100 m apart in a 4 x 4 array, would recapture approximately 8% within 24 hours, although this study used fully mature flies.

A significant difference was detected between recapture rate per Lynfield trap of wild male *B. tryoni* released as post-teneral adults, relative to *white marks* and sterile males (Figure 5-3). In this study, this observation may be explained by the relative number of flies released of each strain, as recapture rate at any point is related to fly density (Fletcher 1974a; Meats 1998b). However, this does not explain higher recaptures of sterile males in Lynfield traps where release rates of *white marks* and sterile males were similar (Chapter 4). In Chapter 4, one suggested explanation for higher recapture of sterile male *B. tryoni* was that sterile
male *B. tryoni* were attracted to cuelure earlier than *white marks*, that only become attracted to cuelure when sexually mature. In the current study, there was a significant interaction between strain and week after release (Figure 5-3). Recaptures of wild and sterile males per trap were highest within one week after release. In contrast, recaptures of *white marks* males per trap increased in the second week, owing to high recapture rates of *white marks* in Autumn (Figure 5-3b). This result may offer evidence to support the hypothesis that sterile male *B. tryoni* respond to cuelure at an earlier age. Eggs from the *white marks* culture are normally harvested at 6 – 8 weeks after adult eclosion (A. Meats, pers. comm.), whereas eggs are harvested at an average age of two weeks from the culture maintained to produce sterile flies (Bateman 1967). The rearing conditions used to obtain sterile *B. tryoni* for SIT may artificially select for earlier sexual maturity. Domestication during the first four generations in the laboratory significantly reduces age of first mating in *B. tryoni* (Meats *et al.* 2004). In Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann), irradiated males responded to trimedlure at a younger age than wild flies, which may have been due to inadvertent selection for decreased development time in laboratory-reared flies (Barry *et al.* 2003a).

### 5.5.2. Dispersal of strains is similar

This study found that there was no difference between the dispersal capacity of wild, laboratory-domesticated *white marks*, and sterile male *B. tryoni*. Within one week of release, the linear relationship between the logarithm of distance from the release point and male recaptures in Lynfield traps baited with cue lure was similar between strains. This indicated that no significant difference existed between the pattern of wild, *white marks* and sterile *B. tryoni* dispersal. Similarly, mean recapture distance of wild, *white marks* and sterile male *B. tryoni* released as adults was not significantly different. Values for mean recapture distance apply only to flies on the trapping grid at Richmond, but are a useful index of dispersal ability with which to compare different releases. Due to low trap efficiency, dispersal distance is much greater than the size of the trapping grid used in the
current study. Despite this, recaptures are relative to density (Fletcher 1974a; Meats 1998b), so trap recaptures provide an indication of male distribution.

Wild, *white marks* and sterile males all exhibited a significant, and similar increase in recapture distance from the release point associated with time after release. This observation is consistent with observations of dispersal in other tephritid fruit flies. Dispersal of adult male olive fruit flies, *Bactrocera oleae* (Gmelin), increased linearly with time after release in the absence of host fruit, flying a mean distance of 400 m from the release point in one week (Fletcher & Kapatos 1981). In *C. capitata*, most of the dispersal of released populations occurred within the first three days after release, increasing from 113.8 m on the first day after release, to a mean distance of 156.5 m by the third day after release (Plant & Cunningham 1991). In the current study, wild, *white marks* and sterile male *B. tryoni* were centred around the release point one week after release, but drifted away from the release point during the second week after release (Figure 5-4, Figure 5-5). Recaptures of *C. capitata* remained symmetrically distributed around the release point, drifting only slightly in the direction of the prevailing wind (Plant & Cunningham 1991). As the trapping grid at Richmond was not symmetrically arranged around the release point, it is not possible to determine if wind direction influenced redistribution patterns of male *B. tryoni*.

Lack of any difference in dispersal between wild, *white marks* and sterile male *B. tryoni* is unexpected. Studies on *B. tryoni* and other tephritid fruit flies in the field and laboratory show that laboratory domestication and sterilisation affect dispersal in the field, flight capacity and acoustic properties of flight. Wild melon flies, *B. cucurbitae* (Coquillett), travelled a greater distance than those from a mass-reared strain (Nakamori & Soemori 1981). It has also been shown that gamma-irradiation reduces the dispersal capacity of male adult *B. cucurbitae* (Hamada 1980). Wild *B. oleae* were recaptured considerably further away from a release site than laboratory-reared flies (Fletcher & Economopoulos 1976; Economopoulos *et al.* 1978). Flight ability of wild *B. oleae* collected near Athens,
Greece, was significantly higher than that of laboratory reared flies (Konstantopoulou & Economopoulos 1997).

Spontaneous flight activity of wild male and female *B. tryoni* in laboratory cages was considerably lower than that of laboratory-reared flies (Chapman 1983). Using a flight mill, Chapman (1982) found that when fruit was available, laboratory-reared *B. tryoni* predominantly performed short flights lasting less than five seconds. It was suggested that a difference between wild and laboratory-reared flight behaviour in *B. tryoni* reflected a greater level of excitability in the laboratory-reared stock due to the method of rearing in the laboratory (Chapman 1982, 1983). In *B. cucurbitae*, evidence from a flight mill study indicates that wild flies fly longer than mass-reared flies (Nakamori & Simizu 1983). The average duration of continuous flights reached a peak earlier in mass-reared than in wild *B. cucurbitae*, and then declined with age. In wild *B. cucurbitae* the peak average duration was reached later, but the high level was maintained for longer than in the mass-reared flies. In the laboratory, flight ability of a temperature-sensitive lethal strain of *C. capitata* was reduced by irradiation (Barry et al. 2003a).

Development of acoustic properties of tethered flight sounds with age from wild *B. cucurbitae* was significantly different from that of a mass-reared and sterile strain, but there was no difference between mass-reared and sterile strains (Kanmiya et al. 1987a). Acoustic parameters of flight sounds, including fundamental frequency, peak power density and harmonic distortion, reflect physiological processes and are a qualitative parameter of behaviour that can be influenced by fly maturity and flight ability (Kanmiya et al. 1987a). In the same study, Kanmiya et al. (1987a) found significant differences between *B. cucurbitae* strains among properties of flight sounds as flies aged, including fundamental frequency and total harmonic distortion, although there were fewer differences between mass-reared and sterile flies. Multivariate analysis of flight sounds from tethered flight sounds of *B. cucurbitae* also indicated that mass-reared and sterile flies exhibited characters that were distinct from that of wild flies (Kanmiya et al. 1987b).
5.5.3. Dispersal distance and season

Although overall recapture distance from the release point increased with time after release, recapture distance was significantly affected by season of release. Temperatures during Autumn may explain lower average dispersal distances of male *B. tryoni* during this period. Maximum temperatures during Autumn releases of wild, white marks and sterile males were lower than during releases in Spring. However, no correlation was detected between mean recapture distance and mean daily maximum, minimum or average temperatures (Table 5-3). This indicates that factors other than temperature were important in determining redistribution patterns of male *B. tryoni* following release in Spring and Autumn. On the contrary, recapture rate per trap was significantly negatively correlated with increasing daily maximum and average temperature. This may indicate that high temperature leads to high mortality rates for released *B. tryoni* in the field. Another possibility is that high field temperatures may lead to suppression of trivial movement in response to high temperatures, thereby reducing the chance that flies move into the vicinity of male lure traps and respond to an odour gradient (Meats & Hartland 1999).

Previous studies have shown that *B. tryoni* exhibit dispersal away from an area in response to adversity, including lack of oviposition sites with the onset of Winter in cooler climates (Bateman & Sonleitner 1967; Fletcher 1973). As such, it was expected that dispersal following release of wild, *white marks* and sterile male *B. tryoni* would be greater in Autumn than in Spring, when host fruits, especially stonefruits, were plentiful. This was not observed in the current study; mean recapture distance for wild, *white marks* and sterile *B. tryoni* was higher in Spring than in Autumn (Figure 5-6). The orchard at Richmond contained many host and non-host trees, with fruiting hosts available all year, including winter. In Autumn, fruiting navel orange trees were present in the Apiary block in close proximity to the release point, and may have reduced dispersal of Autumn releases. Fletcher (1979) suggested that emigration of female *B. tryoni* from an
over-wintering site in Camden, New South Wales, was delayed by the presence of 
fruiting loquat trees, an early season host of this species. In addition, resources 
required by over-wintering flies may have been plentiful in the orchard, including 
shepherd and leaf surface bacteria that are the major source of nutrients for 
developing flies (Drew et al. 1983).

5.5.4. Dispersal of B. tryoni is highly variable

Although mean recapture distance of B. tryoni was below 500 m in both Spring 
and Autumn, by the second week after release, recaptures were made greater than 
1000 m from the release point. Other studies on dispersal of B. tryoni have also 
shown that the majority of flies released are recaptured close to the release point, 
but a small proportion fly considerable distances (Fletcher 1974a; MacFarlane et 
al. 1987; Dominiak & Webster 1998; Dominiak et al. 2003).

Chapman (1982) suggested that great variability in flight propensity may 
be characteristic of B. tryoni. High variability in flight variables was found among 
flies of the same sex, age and origin during laboratory experiments on B. tryoni 
(Chapman 1982, 1983). Moreover, the propensity of groups of B. tryoni to flight 
was similar between observation periods, indicating that activity levels of 
individuals were retained over time and may have a genetic component (Chapman 
1983). Variability between duration of flight has also been observed in B. 
cucurbitae (Nakamori & Soemori 1981). Dispersal of C. capitata has been 
modelled based on a diffusion equation that incorporated a parameter for ‘settling’ 
(Plant & Cunningham 1991). The model of Plant and Cunningham (1991) 
assumed that a released population of C. capitata consists of a subpopulation that 
is actively dispersing at a given time and one that is not.

Maintenance of high variability in dispersal in tephritid fruit flies may be 
associated with the various costs and benefits of dispersal. Long-distance dispersal 
may increase the chance of encountering spatially and temporally distributed 
resources, such as oviposition and feeding sites (Bateman & Sonleitner 1967;
Fletcher 1973; Bateman 1977; Fletcher & Kapatos 1981; Drew & Hooper 1983; Zalucki et al. 1984). Conversely, dispersal may decrease the chance of encounters between males and females in small populations (Bateman 1977; Hopper & Roush 1993), leading to low or negative population growth; a phenomenon known as an Allee effect. Recent examination of trap captures in quarantine areas normally free of *B. tryoni* between 1974 and 2000 indicates that 71% of low level incursions failed to establish an outbreak (Meats et al. 2003).