8. Ground sterile fly release

Ground releases are commonly used where aerial releases are neither cost-effective nor efficient (discontinuous distribution and relatively small areas), or where additional releases are required to provide a higher density of flies for a particular reason, i.e. hotspots as indicated by monitoring traps, or where a high risk area is known to exist and needs to be treated with more flies than can normally be supplied aerially.

Ground releases can be divided into two general methods, adult and pupal. Adult release is the most widely used method and the pupal release method could be applied only under certain specific conditions.

8.1 ADULT GROUND RELEASE

This method is generally based on pupae being delivered into the release centre and the pupae being held in containers (e.g. paper bags, plastic bins, cardboard boxes, etc), allowed to emerge, held for a period of time to allow a full emergence and development to maturity, and then released by a ground mechanism. This method minimizes predation compared to the pupal release, however, conditions in the holding containers need to be well managed to ensure released adults have good survival and competitiveness. Adults are usually released 2 to 5 days (varies with species) after emergence and are approaching sexual maturity (to greater degrees with different species). This procedure hopefully facilitates minimum adult losses prior to sexual activity. The main variations in this technique are the release containers and the adult holding densities.

Generally, adults in containers should be transported from the release centres to the release sites in cool conditions (<20°C) to minimise stress within the container. The frequency of release may be affected by circumstances such as supplies of pupae, staggered emergence, and unfavourable weather conditions.

8.1.1 Containers used for ground release

The most widely used containers for ground release are plastic cylindrical bins, PARC boxes and paper bags.

8.1.1.1 Cylindrical bins

Ideally only 15,000 pupae (Sproule *et al.* 1992, Horwood and Keenan 1994, Perepelicia *et al.* 1994) should be placed in release bins (45 litre plastic) with a maximum of 25,000 (this is for Queensland fly with an average weight of 10 mg — thus needs to be adjusted for other species). Crumpled paper should be placed in the bottom of the bin to provide additional resting

FIGURE 8.1
Bins in the back of a trailer ready for release. The trailer is usually covered to afford shelter to the adults during transport. Longer distance transport should be by airconditioned vehicle. Road transport should be kept to a minimum as this form of transport causes stress to the adult over extended travel. The small air vent can be seen and may be a limitation to this method.

FIGURE 8.2
PARC boxes with and without ventilation in the sides. The internal divider for additional standing space can also be seen. Pink pupae can be seen in the bottom of boxes and emergence has not started. Boxes are stacked five high and room ventilation must ensure that waste gases do not accumulate in the boxes.

space to allow expanding of wings and to absorb excreta. The inside of the bin should be sand blasted to allow adults to grasp the walls. Round bins are not as space efficient as square or rectangular containers and require larger vehicles for transport to release the equivalent number of flies (**Figure 8.1**). Ventilation within bins (through screen-covered openings) is more important than with PARC because the bins are deeper (James 1992, Horwood and Keenan 1994). Respiration gases such as carbon dioxide and ammonia from excretia may pool in the bottom of any container, particularly bins, and adversely effect adults emerging in the bottom of the container (see Sections below).

8.1.1.2 PARC boxes

A variant of the adult release method is the release using PARC boxes. These boxes have a 50 litre volume and have a larger floor area than the bins. Pupae are not as deep on the floor and are less likely to overheat. Additional crumpled paper or other dividers can be added to increase resting space for adults. Both these methods may have ventilation problems and volatile waste products (carbon dioxide, ammonia, humidity) may build up in containers and decrease survivability (Horwood and Keenan 1994, James 1992). Holes may be cut in the PARC boxes and covered with gauze or fly screen to assist volatiles to escape the containers. Generally these containers may result in larger numbers of flies (>15,000) deposited in a smaller number of locations, compared with techniques which use smaller release containers such as paper bags.

Ventilation is very important for these methods to draw waste products out of containers. In the holg room, containers are frequently stacked in pyramids to maximise space. These pyramids interfere with air flow and managers needs to ensure that air flow and waste removal is optimised (**Figure 8.2**).

With both these methods, managers must assess overcrowding stress. Additionally, an excess depth of pupae (particularly in containers with a small base) may contribute to pupal overheating and be detrimental to emergence (Dominiak *et al.* 1998).

Where methods re-use containers or parts of them (plastic bins, PARC boxes, tubs, drinkers, etc), managers need to be aware that cleaning is an important component to minimise the chance of fungal or other pathogens adversely impacting the programme. Care needs to be taken in the choice of cleaning agents as some residues may be detrimental to adults. Sometimes minor changes in cleaning agents (due to supply problems) may significantly adversely impact on adults.

8.1.1.3 Paper bags

Pupae are placed in paper bags (e.g. Kraft No. 20) and adults emerge in and are distributed in the same bags. One linear meter of paper is placed inside the bag to provide adult flies with a resting surface of approximately 2,400 cm^2. This method

places smaller numbers of pupae in bags and is suited for more releases to be made from more points. This should result in a better distribution of flies over the landscape, compared with plastic bins, PARC boxes or large cages. Commonly bags are about 20 cm length 10 cm width × 35 to 45 cm height and contain about 4,000 to 8,000 pupae with an expected 80 to 85% emergence.

FIGURE 8.3
Two large mesh cages on a trailer. The sides are held with Velcro (i.e. material which has two sides, sticky hook side and a fur side – it can be pulled apart and pushed together to make a seal) and can be easily pulled open to release adult flies. Cages are transported in utility vehicles or trailers because of their size. This method usually releases large numbers of flies in a small number of confined release points.

8.1.1.4 Other types of release containers

There are other containers that could be used for ground release such as the mesh cages and nylon mesh bags (**Figures 8.3, 8.4** and **8.5**). These containers have been tested experimentally in Australia, however, have not been used in large scale operational programmes (Dominiak *et al.* 1998, 2000a, 200b, 2003, Meats *et al.* 2003). They were developed to overcome ventilation problems of solid walled containers such as plastic bins and PARC boxes. These may carry higher adult numbers than bins/boxes as there is no accumulation of waste volatiles. The mesh sides provide easy surface for adult flies to stand. Cages with dimensions of 1.8 m length × 0.7 m width × 1.2 m height may be seeded with 200,000 pupae, with an expected 86% emergence (Dominiak *et al.* 1998). Larger cages often suffer the same distribution problems as bins in view of the limited number of release points.

Distribution can be improved by the use of smaller cages (50 cm length × 50 cm width × 50 cm height) which contain 16,000 pupae (Meats *et al.* 2003). Field managers need to determine which cage size is suitable for their circumstances. Pupal depth should not exceed 9 mm as the accumulated heat results in decreasing emergence and increases deformed adults (Dominiak *et al.* 1998). Some species also have a lower emergence resulting in different adult populations in cages. This factor may determine the number of pupae placed in cages.

Another similar method is the nylon mesh bag. These bags (~90 cm length × 90 cm width) may contain as many as 80,000 pupae and result in 80% emergence (Dominiak *et al.* 2000a). Bags have Velcro joins in the side panels to facilitate adult release and subsequent washing. These bags are hung on wire racks for emergence. The nylon mesh allows air to circulate through the bag and waste products do not accumulate.

8.1.2 Description of adult ground releases procedures

Once the adult fruit flies have emerged in bags, they are loaded in the releasing vehicles, which must have a shelter to protect the bags from direct sun, rain, wind, etc. Precautions must be considered to avoid excess of movement of the bags during transport. Also,

FIGURE 8.4
Small mesh cages are easily opened using velcro lids. These do not have the ventilation problems associated with bins or boxes. Smaller release containers such as these cages allow smaller releases at many more release points than the large cages.

FIGURE 8.5
Nylon bags hung on a rack. Pink pupae can be seen in the bottom and adults can also be seen on the bag sides. Water is provided by the wettex or cloth at the top. The bag has Velcro joins in the side panels for easy opening and cleaning.

FIGURE 8.6
This paper bag has been torn open to show the flies inside. Normally the adult flies leave the bag through opening. These empty bags are removed from the tree during the next bag distribution cycle.

it is not recommended to pile up or compress the bags to avoid unnecessary damage to adults caused by the excessive handling. These release vehicles should be conditioned with at least two levels of racks where paper bags are placed to avoid piling and compressing.

Prior to release, it is critical to know the location of the hosts, in order to efficiently release flies in the field. For this purpose, a host census or data base, as well as the location of detection sites must be determined in advance.

To help the flies to escape from the paper bags, the bags are torn from top to bottom. Handling needs to be with care to avoid damaging the flies.

Traditionally, paper bags and other release containers (e.g. PARC box, plastic bins, etc) are taken by air-conditioned vehicle to pre-designated release points. These locations should preferably be more than 100 m from any monitoring site. The vehicle is stopped and the container is taken from the vehicle to the site and the adults released under or into the tree canopy. These activities usually take several minutes to complete. This process requires a series of stops and may be considered time inefficient. This may be a minor concern where labour costs are low. The number of release points per hectare needs to be determined, depending on the desired coverage, and the estimated flight distance of insects. Standard or pre-determined release points have been commonly used in the past, however, there is an increasing trend to roving releases where small numbers are released from a moving vehicle from many points. Fixed point (James 1992, Dominiak *et al.* 1998) and roving releases result in slightly different distributions in the field, and use varying levels of resources – managers need to assess which method is appropriate for their circumstances. Fixed point releases may be located by GPS coordinates and researchers and managers can better understand flight distances, dispersion and distribution factors (See Section 11).

Paper bags may be placed in host trees usually on a weekly basis and old bags are removed in the following distribution cycles (**Figure 8.6**). Some countries have concerns about environmental pollution issues and this may need permission from local authorities. These large numbers of small releases allows better distribution of sterile flies, however, are labour intensive and may be less acceptable in countries where labour costs are high.

FIGURE 8.7
Paper bags are stacked on trays in the back of a small truck. Bags may be inverted to save space. Bags need to be torn open to allow flies to escape the bag at release sites.

In the PARC box or plastic bin releases where larger numbers of pupae are involved, unemerged pupae should be returned to the vehicle for re-use and possibly a subsequent second release however this does not occur with bag releases. Unemerged pupae should not be poured on the ground as dye may become lost from the pupal case before emergence and hence compromise the integrity of identification services. Unemerged pupae should be returned to base for destruction.

An alternative is to release flies from a slow moving vehicle in a roving release (**Figure 8.7**) (Salvato *et al.* 2003). This is more time efficient however requires some other considerations. This method minimises the stop/start nature of the fixed point release method and is commonly used in paper bag release, however, other small containers may be used.

Adult flies may be distributed mechanically from a machine, similar to aerial release, however, this adds significantly to the cost of the programme.

Bags or other small containers may be stacked on the back of a tray-back vehicle and the release person tears the bags or opens the containers and introduces adults into the air stream. Releases are made at regular times or distances but the vehicle does not stop. This option may have occupational health and safety aspects which are strongly regulated in some countries. There also needs to be a systematic approach to ensure spent containers are kept separate from unused containers. Fruit flies tend not to fly in winds >4/hr and therefore releases from an open cage while the vehicle is moving is unlikely to be successful (Dominiak *et al.* 2002a).

An additional option is to chill the adult flies (3 to 6°C depending on the species) prior to release. This ensures that only adults are placed in the release containers. This avoids the need to return the puparia to the release centre. Generally these containers are held at below flight threshold temperatures (~17°C) up to the point of release. After release from the aircraft, adult flies quickly warm up and fly to trees.

Both these approaches have some general limitations. High temperatures (>30°C) should be avoided as many fly species prefer not to expend energy and not fly at these temperatures. It is generally not recommended to release during rain. Releases when ambient temperatures are below flight threshold are also discouraged as released flies have a low probability of reaching the protection of trees.

8.1.3 Situations under which to conduct ground releases

There are various possible situations to conduct adult ground releases (some of these can also apply to other containers used for ground release):

- **Routine ground releases in predetermined spots:** According with the particular conditions of the area (host distribution, urban v/s rural, accessibility given by roads, topography, distances, required permission to enter properties, etc), the distribution of the material is pre-determined, identifying every spot where a bag is to be placed. A specific list containing the places is prepared and must be taken in the vehicle during the process of releasing. In order to meet the desired density, the number of bags for every spot must be specified. This releasing method makes it difficult to cover the area homogenously, and because of that, it is not recommended for general use in extended areas. To conduct this releasing method, the bags are distributed as homogenously as possible. Two general methods are commonly used, namely from vehicles in movement and stopping at every releasing spot:

  – Releases carried out stopping the vehicle: At every pre-established releasing spot, the vehicle stops and the bags are placed within the canopy of host trees having both, foliage and fruit. Avoid placing bags within a radius of 100 meters from a trap. As an example, a small vehicle can carry 150 to 300 bags, to cover an area of 400 to 500 has, releasing a density of ca. 2,500 to 3,500 adults per ha (8000 sterile pupae per bag × 85% emergence).
  – Releases from moving vehicles: For releases carried out from moving vehicles, the bags are torn and released at regular intervals of 50 to 100 meters. The vehicle usually moves at a speed of 40 km/h. As an example, a large vehicle with capacity of 1,200 bags to cover an area of 3,000 ha per day.

- **Complementary preventive ground releases in high risk areas:** Some areas require more flies as a preventive measure, because of the risk associated based on historical data. The number of additional bags should be such that the regular fly density in the area is increased.

- **Complementary ground releases in hot spots or detection areas:** Increased fly releases are sometimes required in a hotspot or following a detection that meets the emergency response trigger, which is 2 or more adult flies, a gravid female or an immature stage detected for the case of Medfly. For implementation of eradication actions the area where the fly find occurred can be defined as:
  – 200 meters radius around the detection point (12.5 ha). 10 bags are placed within that radius. Based on experience, ca. 40,000 flying males are expected in the area of 12.5 ha (8,000 sterile pupae per bag × 85% emergence × 60% fliers) (ca. 5,500 sterile flies per hectare).
  – 1 km^2 (100 ha) around the detection point, where 100 bags are placed. Based on experience, ca. 400,000 sterile flying males are expected in the area of 100 ha (ca. 4,000 sterile flies per hectare).

- **Complementary ground releases in places difficult to access:** Complementary releases may be required to cover places not easily reached by airplanes (deep valleys, mountainous zones, foggy or hazy zones or other climatic adversities) or zones with aircraft exclusions (airports, military zones). According to the pest situation in the area, the release procedures can be matched either with regular release or high risk zone release.

- **Back-up ground releases:** Ground releases may be required as a back-up to aerial release when flights are cancelled due to adverse climatic conditions. Regular ground release is used to cover the area.

8.2 PUPAL GROUND RELEASE

Pupal release has been conducted as a routine operation with success only in the case of Australia. Other experiences using this release method have generally not been satisfactory mainly because of substantial sterile fly losses during emergence and wing stretching due to predation by birds, ants and other predators. Thus a critical pre-condition for use of this release method must be low predation rates.

8.2.1 General concepts

Pupal ground release is based on pupae being distributed directly into the field, and the emergence and maturation occurring with minimum human interference. In general, these methods are likely to gain best results if predation (by birds, ants, lizards and other creatures) is minimal. It is also important to produce pupal body weight as higher pupal weights are usually associated with higher survival and competitiveness attributes (Dominiak *et al.* 2002). The main advantage of this method is its low release cost and the virtual absence of any infrastructure requirement. However there are many areas where pupal release would be unsuited and managers need to assess their circumstances. It appears best suited to small release programmes where predation is not a major concern.

One advantage is that there are also indications that adults become acclimatised to the local weather as the pupae are exposed to variable temperatures for the two days between release and adult emergence (Meats 1973, 1984). Indications are that this is particularly valuable when releases are done in autumn and spring, when adults held at constant temperatures are unlikely to fly at lower temperatures (lower than 17°C) (Dominiak *et al.* 2000a). Apart from the adaption to local climate, pupal releases do not suffer any overcrowding stress and adults leave the site when they are ready. Therefore emergence may be extended and is not limited to particular time constraints required by most adult releases. Adults emerge and disperse daily into the environment compared with the sudden large delivery of flies in one day using adult ground releases.

This regular flow of adults leaving the site results in a steady delivery of adults into the environment without any requirement for human operators to revisit the area. Both overcrowding and irregular delivery of flies into the field are potential short comings of adult ground release programmes.

8.2.2 Covered pupal releases

Unsheltered pupal release, involving the distribution of unprotected pupae onto the ground is not successful, even with low predation rates, due to climatic influences, particularly heat. Even if these pupae emerge into adults, there is a high chance that the dye on the pupal case may be removed by rain or dew formation. This method has little chance of being successful and is not generally supported.

Ground release of covered pupae therefore attempts to replicate nature where adults emerge from pupae placed underground. In these methods, pupae are poured directly onto the ground and covered in material called a “bed”, and beds may be up to 1 m across and contain 800,000 pupae with 80% emergence possible (Dominiak and

FIGURE 8.8
A bed on the ground using sawdust. Researchers are evaluating emergence and adult survival. Coverings which do not crust maximise adult emergence from the bed. Dry abrasive coverings may damage the fly.

Webster 1998). The material holds the pupal case firmly and minimises energy loss during emergence, compared with circumstances where pupal cases can move during emergence in adult releases. Several materials have been evaluated.

8.2.2.1 Sawdust

Dry sawdust has been tried, but hard woods appear to contain toxic compounds which decrease emergence. Dye maybe added to the sawdust to supplement the normal dyeing process associated with dye on the pupal case **Figure 8.8** (MacFarlane and Betlinski 1987).

8.2.2.2 Sand

Several types of sand have also been evaluated. In general, sand which forms a crust after drying out does not affect emergence of the adults from the pupal case, but adults have difficulty breaking through the crust. Double washed river sand is recommended (Dominiak *et al* 2000b).

8.2.2.3 Vermiculite

Emergence through dry materials often results in superficial damage to the insects cuticle and predisposes the insect to moisture loss and early death. Therefore it is considered that moist vermiculite is better than dry vermiculite, using a mixture of 4 litres of water per 4 litres of vermiculite (Dominiak *et al.* 2003b). A layer of approximately 5 to 10 cm of vermiculite seems to be ideal, however, this needs to be evaluated for different fruit fly species and for different grades of vermiculite. Moist vermiculite appears to be the ideal covering, providing a medium to hold the pupal cases during emergence and to prevent the loss of body weight (Dominiak *et al.* 2002). Moist vermiculite also does not remove dye from pupal cases, however, free water does and this should be avoided.

FIGURE 8.9
Tray release being prepared. Pupae are first poured onto the base and are being covered by vermiculite. The two house bricks will hold the top tray above the base (see background) and allow the flies to escape from between the two trays.

8.3 PUPAL RELEASE METHODS

There are several methods to house pupae and bedding materials. The basic "bed" technique is to pour the material (vermiculite, sand, sawdust or other) on the ground to a depth of 25 mm, pour evenly the pupae over the bed, and cover the pupae with up to 10 mm of the material. This method has several disadvantages. If placed in full sun, pupae may overheat and die. In some areas, meat eating ants may predate on emerging adults. Ants appear unlikely to harvest covered pupae but some species of ants may take exposed pupal cases. Birds (such as crows or seagulls) may learn that scratching through the bed may offer and easy meal – this will vary in different areas and different bird species (Dominiak *et al.* 2000a). Rain may minimise the dye marking of emerged adults and therefore this method may be more suited to dry regions. The advantages are that up to 800,000 pupae may be deployed at one site with virtually no resources (Dominiak and Webster 1998).

Beds may be protected from ant predation by placing the material and pupae in a tray, bin, box or other container. These containers frequently have lids or covers to shelter the pupae from the sun and to minimise bird predation on the unemerged pupae and adults (**Figure 8.9**).

The ideal container appears to be a white styrene foam box (commonly used to supply vegetables to markets — 30 cm × 58 cm × 29 cm). These are low cost and commonly available. They provide insulation against extremes of temperature. These containers need to allow holes or portals (~3 cm × 10 cm) for the adults to leave the container. These containers can comfortably hold 240,000 pupae, although 80,000 was more commonly used, covered with 6 litre of moist vermiculite (Dominiak *et al.* 2003b). Ideally these portals should have some covering to prevent rain from entering the container and drowning the pupae or adults. Pupal frass should be returned each week when the container is recharged with pupae. During cooler periods, the emergence can be encouraged by placing containers at least 1 m off the ground, this prevents the effects of the cold ground on the pupae. The styrene foam also affords some protection from extremes of temperature.

FIGURE 8.10
Foam box for release being used in late winter.

FIGURE 8.11
Bucket release

Flies emerge from containers and obtain nutrition from the two drinkers. Food in the drinkers may be water and sugar, or also include protein, depending on the research results for different species. Bricks create weight to prevent wind turning the container over. In wetter climates, there should be some mechanism to prevent rain entering the boxes (**Figure 8.10**).

The bucket release allows pupae to emerge and for the adults to leave via the holes. Food and water are suspended from the lid in small containers (**Figure 8.11**). Buckets can be hung in trees however branch pruning is necessary to avoid ants predating on pupae or adults. Buckets require a lid to keep out rain and minimise bird predation.

For ground pupal release a low cost water based food source may be made available by a pet drinker (Dominiak *et al.* 2003b). These containers often have a three litre capacity and would provide food and water for a week.

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