

Short communication

Bacillus thuringiensis as a marker for insect dispersal studies

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Abstract Laboratory studies confirmed that commercial formulations of *Bacillus thuringiensis* (Bt) could be used to mark potato tuber moths, *Phthorimaea operculella*. Moths were successfully marked by direct application or through indirect acquisition by contact with marked surfaces such as a leaf or a Petri dish. Marked moths were identified by the distinctive crystalline morphology of different subspecies of Bt, allowing the separation of individual moths marked with either Btk (*kurstaki*) or Bti (*israelensis*). A simple microbiological method for processing the trapped moths before definitive, microscopic identification of the subspecies is described. Field marking was verified by capturing light brown apple moths, *Epiphyas postvittana*, from areas that had been subjected to large-scale spraying of Btk for control of an exotic pest species. Btk was also detected in moths from isolated traps at c. 1 km from the treated area, but not in moths from traps at greater distances. These results indicate that Bt preparations could be used to mark naturally occurring populations as well as laboratory-reared individuals to study their dispersal.

Keywords marker; insect dispersal; *Bacillus thuringiensis*; moths

INTRODUCTION

A range of methods has been used for marking insects for studies of dispersal. Mark-release-recapture studies use physical marking or abrasion, trace elements, dyes or dusts, or protein markers to label insects in the laboratory before release. These methods and other marking techniques have been reviewed by Hagler & Jackson (2001). The efficacy of these markers varies between insects, and the techniques also vary greatly in expense and technology requirements. In previous studies, we have found fluorescent dusts to be more efficient than felt tips for marking potato tuber moth, *Phthorimaea operculella* (Zeller), and diamondback moth, *Plutella xylostella* (L.) (Cameron et al. 2002) although the latter approach provided a greater number of different marking options.

Marking in the field using a mark-capture technique allows mass marking of naturally occurring insects, thereby avoiding any effects of rearing and handling on dispersal. This approach requires higher volumes of markers such as specifically developed resin dyes that can be identified by fluorescence (Schellhorn et al. 2004), or inexpensive protein markers that can be identified using ELISA techniques (Hagler 2004). Bacteria from the genus *Serratia* have been considered as markers for insect dispersal studies (Jackson et al. 2004). However, this bacterium occurs commonly in potato tuber moth (Madhusudhan unpubl. data) and would not be a unique marker for this species. We have investigated an alternative method based on the application of commercially available formulations of Bt and the use of microscopy to differentiate subspecies of Bt, based on their crystal morphology. These techniques are used as a novel method to mark moths in the laboratory and field for preliminary studies of dispersal.

METHODS

Laboratory

Solutions of commercially available formulations of *Bacillus thuringiensis kurstaki* (Btk; Foray 48B™, Abbott Laboratories, North Chicago, IL, United States) and *Bacillus thuringiensis israelensis* (Bti; Vectobac™, Abbott Laboratories, North Chicago, IL, United States) in distilled water were used to mark adult potato tuber moths. Adults were anaesthetised with CO₂ to allow spraying *in situ* and solutions of these formulations at 1.0% concentration were sprayed using a perfume sprayer either directly onto the moths in a Petri dish, or on the surface of a clean Petri dish, or on bean plants. Moths that were sprayed directly were then held in a cage containing a sugar source (dental wick dipped in 10% sugar solution). Unmarked moths were released into Petri dishes that had previously been sprayed directly with the solution, or were released into a cage with sprayed bean plants. Control moths or surfaces or bean plants were sprayed with sterile distilled water. Twenty-eight to 30 moths were used for each treatment. The moths were held at 23°C for up to 16 h after which they were collected and frozen individually in 1.7 ml microcentrifuge tubes. Individual moths were macerated with 100 µl of 0.1%, filter-sterilised Tween 20. To this was added 100 µl of sterile distilled water and the tubes were incubated in a water bath at 65°C for 10 min. The tubes were agitated vigorously and allowed to stand for 2 h after which 60–70 µl of the supernatant was plated on to selective media containing Lennox Broth (10 g/litre; Difco Laboratories, United States), agar (15 g/litre), and penicillin (6 mg/litre). The plates were incubated for 4 days at 25°C. This method provided selective isolation of Bt from marked moths by the combined use of heat as well as incorporation of penicillin in the media.

Preliminary screening based on colony morphology was carried out for Bt-like colonies after 4 days and confirmatory evidence of presence of the Bt marker was sought using stained bacterial smears prepared as described by Chilcott & Wigley (1988). A speck of the colony was removed from the plate using a sterile toothpick, smeared onto a water droplet placed on a glass slide, allowed to air-dry and baked at 100°C for 1 h. The slide, while still hot, was dipped in 1.5% naphthalene black (BDH Laboratories) in 37% acetic acid for 2 min, rinsed in water, and stained in 2% Giemsa stain for 2 min. The slides were then rinsed briefly in running water and allowed to dry. Smears were then viewed under a light

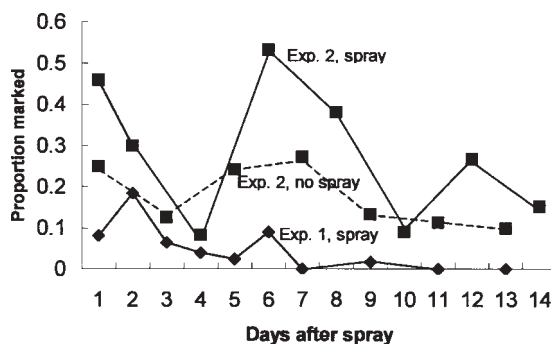


Fig. 1 Proportion of Btk-marked light brown apple moths (*Epiphyas postvittana*) from pheromone traps in the sprayed area (spray) or 1 km from the sprayed area (no spray) at various periods after aerial spraying.

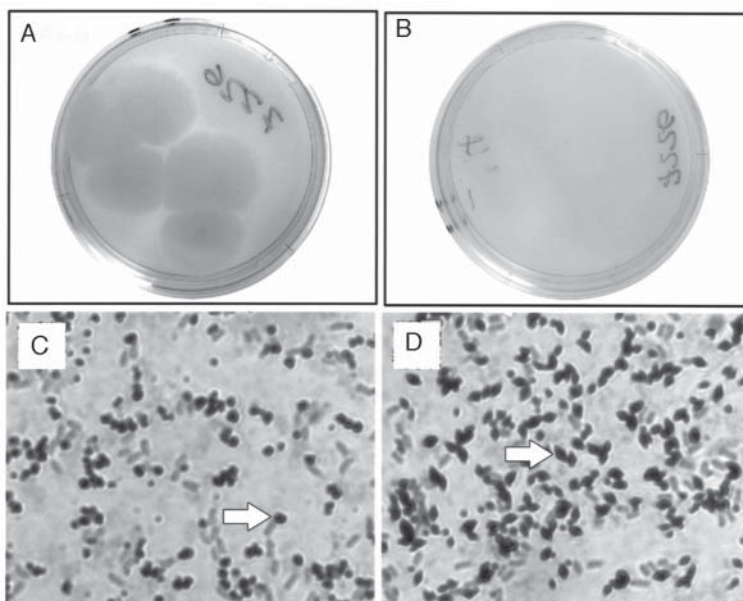
microscope using oil immersion for presence of crystal inclusions characteristic to the Bt subspecies (Höfte & Whiteley 1989).

Field

The opportunity for a field experiment was provided by the occurrence of localised aerial spraying with Btk (Foray 48B) as part of a control programme for an exotic lepidopteran pest in the Auckland region. Another lepidopteran pest, the ubiquitous light brown apple moth, *Epiphyas postvittana* (Walker), was used as an indicator species to assess marking resulting from this spraying. In a preliminary control experiment to determine if Bt occurred on moths from unsprayed parts of the greater Auckland area, pheromone traps (Desire®, HortResearch, Mt Albert, Auckland, New Zealand) were placed at nine sites and light brown apple moths were collected over a 4-day period in November 2003. Eight of the traps were at least 7 km from the aerial spraying area, and one trap was c. 1 km from this area.

The extent of marking of light brown apple moths within the sprayed area was examined at a site located in a 100 ha cemetery that was being sprayed at 2-weekly intervals with Btk (Foray 48B) from the air. Preliminary trapping revealed that moths occurred in the site at the rate of c. 7/trap per day. Two experiments were performed. Six pheromone traps, c. 200 m apart on an irregular transect spanning 800 m, were placed in the cemetery grounds on 25 February 2004 one day after spraying. The traps were attached to the lower branches of small trees in open areas at heights of c. 1 m from the ground. The sticky bases were changed daily for 7 days and

Fig. 2 **A**, Colonies of *Bacillus thuringiensis* var. *israelensis* isolated from a marked adult potato tuber moth (*Phthorimaea operculella*). **B**, Blank plate from unmarked adult potato tuber moth. **C**, Stained crystals (typically, angular/irregular) of *B. thuringiensis* var. *israelensis*. **D**, Stained crystals (typically, bipyramidal) of *B. thuringiensis* var. *kurstaki*.



then at 2-day intervals until day 13. The experiment was repeated starting on 10 March 2004, with the addition of one trap c. 1 km from the sprayed site. Captured light brown apple moths from all field experiments were individually removed with sterile implements, transferred to individual microcentrifuge tubes and frozen until being processed as described above.

RESULTS

Laboratory

All three methods of delivery, direct application or indirect acquisition by contact with a Petri dish or leaf surface, proved to be highly efficient in marking the adult moths, and both Btk and Bti were detected in all individuals. Control moths produced no characteristic colonies and no Bt was detected in the 89 moths that were processed. We were able to process c. 300 moths over a 6-h period.

Field

A total of 273 moths were caught over a 4-day period in traps placed outside the sprayed area in nine different sites in the greater Auckland area. No moths from the traps situated at greater than 7 km from the sprayed area tested positive for Bt, but Btk was present in five moths collected 1 day after spraying in the trap 1 km from the sprayed area.

In the first experiment in the sprayed area, a total of 273 light brown apple moths were captured and tested for the presence of Bt. Marking with Btk was detected for 9 days, reaching a peak at day 2 and then declining, but did not exceed 20% (Fig. 1, Experiment 1). Rainfall exceeded 20 mm on days 1, 2, and 4. In the second experiment, 292 moths were captured and marking persisted for the full 14 days of the experiment. Although higher marking rates were achieved in the second experiment, declines were associated with rainfall exceeding 20 mm per day on days 2 and 8 (Fig. 1; Experiment 2, spray). One kilometre from the sprayed area, 175 moths were caught, and marked moths were identified up to day 13 after spraying (Fig. 1; Experiment, 2 no spray). In addition to recovery of Btk from moths, we also found Btk on a cerambycid beetle, thrips, a bumble bee, and ants, all caught incidentally on the pheromone traps.

DISCUSSION

Results presented here from preliminary field and laboratory experiments indicate that both Btk and Bti could be used in mark-release-recapture and mark-capture experiments aimed at investigating insect dispersal. The 100% marking achieved in all the laboratory tests indicated that the combination of direct and indirect marking that would occur in the

field could lead to high marking rates. The field test used here was an extreme test of the field application of a marker. Given that aerial applications of Bt are not likely to penetrate under dense canopy cover, it is likely that much of the marking achieved in the field was indirect self-marking. The combined results indicate that although marking generally decreased over time, some increases may occur as moths move around the environment. The results also suggest that the duration of self-marking in the field is dependant on weather conditions, particularly rain. Our preliminary measures of persistence of the Bt marker in the field indicate that it would allow useful estimates of dispersal. The method of processing marked individuals is not labour-intensive and does not require specialised equipment. The microbiological methods including the staining method are routine and can be followed quite easily in a basic laboratory. We were able to process c. 300 moths over a 6-h period. The method of preliminary screening based on colony morphology is straightforward as is the method for staining. Stained Btk and Bti crystals are easy to identify using light microscopy (Fig. 2).

Various researchers (Hagler & Jackson 2001) working in the area of insect movement and dispersal have reiterated that markers, especially those applied in the field, should fulfill certain basic criteria including environmental safety, cost-effectiveness, durability, lack of effects on the target insect, ease of application, as well as being clearly identifiable. We believe that Bt-based formulations suit these criteria, particularly because they provide a source of inexpensive markers for which efficient field application techniques already exist. Although we have shown that Bt is not routinely recovered from trapped moths in unsprayed areas, it is possible that Btk and Bta (*aizawai*) formulations may be present as the result of their applications in some agricultural areas. We suspect that the recovery of five marked moths at 1 km indicated dispersal from the sprayed area, as use of Btk in the surrounding urban area is rare. However, we have shown that any possible contamination is overcome by the use of Bti formulations that are readily distinguishable from

other Bt formulations. Preliminary tests to confirm the absence of a Bt marker would be advisable before all dispersal experiments. To advance the use of this marking system for studies of dispersal in the field, we are currently investigating the effects of marking techniques and doses on the intensity and duration of marking.

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