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Maintaining DNA quality in stored-grain beetles caught in Lindgren funnel traps

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ABSTRACT

Lindgren funnel traps baited with aggregation pheromones are effective tools for monitoring flight activity in the red flour beetle (*Tribolium castaneum*) and lesser grain borer (*Rhyzopertha dominica*). Beetles caught in these traps are a potentially valuable resource for genetic studies, provided their DNA remains intact. In a series of laboratory and field experiments we evaluated a range of liquid preservatives and dry preservation to determine which approach would provide the highest yield of quality DNA for use in molecular analyses after short-term preservation. Preservatives containing propylene glycol produced an initial decline in PCR yield from extracted DNA in both beetle species after 3 days exposure, but subsequent declines in yield were comparatively slow. Water and phosphate-buffered saline provided good short-term preservation, but the rate of decline accelerated as exposure time increased. Dry preservation (achieved using a section of dichlorvos pest strip as a killing agent) provided the best level of DNA preservation for both species for up to 14 days provided humidity remained low. Hygroscopic water uptake significantly reduced the effectiveness of propylene glycol as a DNA preservative. Whilst propylene glycol is known to be an effective long-term DNA preservative, our results indicate that for typical pheromone trap deployment periods of up to 7 days, *T. castaneum* and *R. dominica* are best preserved dry if this is operationally feasible.

1. Introduction

Molecular techniques are becoming increasingly important in many areas of entomology, including studies on pesticide resistance and insect ecology (Brown and Brogdon, 1987; Reiss et al., 1995; Mandrioli et al., 2006). Appropriate preservation techniques for maintaining DNA integrity are critical for subsequent molecular analyses, and a large number of studies have been undertaken to identify the best preservatives and storage conditions, taking into account the characteristics of particular groups of organisms and the objectives of the proposed molecular studies. Poor storage conditions can result in DNA shearing, where endo- and exonucleases break the DNA into smaller fragments that may not be suitable for further analyses, and also DNA interstrand cross-linking, which blocks the progression of DNA polymerase on the template and thus reduces PCR efficiency (Seutin et al., 1991; Dean and Ballard, 2001; Mandrioli et al., 2006). Low temperature storage helps to maintain DNA quality by slowing or stopping enzymatic activity, but the necessary facilities are not always available, particularly during field work. Seutin et al. (1991) showed that a DMSO-salt solution could be used to preserve avian tissues without refrigeration, since nucleases are dependent on divalent cations and can be largely inactivated by solutions containing high EDTA concentrations, whilst lysis buffers such as that developed by Muralidharan and Wemmer (1994) contain non-ionic detergents that both lyse cells and inactivate nucleases. Lysis and isolation buffers often require sample homogenisation to be effective (Reiss et al., 1995), limiting their usefulness in passive traps or where parallel morphological studies are planned.

In insects and other arthropods good quality DNA can often be extracted from dried specimens (Post et al., 1993; Hammond et al., 1996; Dillon et al., 1996; Austin and Dillon, 1997), but this is not always the case (Mandrioli et al., 2006; Mtambo et al., 2006; Bisanti et al., 2009). Dry storage is unacceptable for some invertebrates (such as arachnids and molluscs) if they are to be used in morphological studies, and liquid preservatives such as propylene glycol and ethanol are more appropriate. In general, higher ethanol concentrations at lower temperatures are more effective, both in the laboratory and in short-term field trials (Post et al., 1993; Austin

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and Dillon, 1997; Quicke et al., 1999; Gurdebeke and Maelfait, 2002; Oliveira et al., 2002; Vink et al., 2005; Mandrioli et al., 2006; Bisanti et al., 2009), although some studies suggest that either temperature or ethanol concentration (but not both) may have no significant effects under particular sets of experimental conditions. Some liquids, such as ethyl acetate, 2-propanol, formal saline, chloroform, methanol and Carnoy's fixative are ineffective as DNA preservatives (Post et al., 1993; Reiss et al., 1995; Dillon et al., 1996; Fukatsu, 1999; Mandrioli et al., 2006; Rivero et al., 2007), whilst others such as formaldehyde solution have produced variable results (Dillon et al., 1996; Gurdebeke and Maelfait, 2002; Stoeckle et al., 2010) but on balance are considered as unsuitable for maintaining quality DNA suitable for PCR amplification. Acetone was found effective for preserving aphid (Fukatsu, 1999), moth (Mandrioli et al., 2006) and beetle (Bisanti et al., 2009) DNA, whilst hexane has been reported as effective for the short-term preservation of DNA in mosquitoes (Narang and Seawright, 1990).

When insect specimens are collected alive and can be rapidly transferred to the laboratory, maintaining their DNA quality is relatively unproblematic. More complex issues have to be faced when researchers want to collect specimens using passive traps which are only serviced at prolonged or irregular intervals (Rubink et al., 2003). In these situations liquid sample preservatives generally have a dual role as killing agents, and when dry preservation is sought, alternative killing agents such as dry-release insecticides are required. Logistic considerations can limit the choice of liquid preservatives, since evaporation can be an important issue (Dillon et al., 1996) and consequently ethanol or other volatile solvents generally cannot be used. In hot dry conditions even water may evaporate too quickly. Propylene glycol can help solve the evaporation problem, and is generally used in preference to ethylene glycol because of its lower toxicity and reduced environmental risk (Rubink et al., 2003; Vink et al., 2005). Whilst propylene glycol is considered a good DNA preservative, particularly at low temperatures, it is also hygroscopic, absorbing large quantities of water from the atmosphere which may reduce its effectiveness over time. In trap containers with relatively small apertures a dry killing agent (such as a section of dichlorvos 'pest strip') without a liquid preservative may be a viable option, provided the captured insects do not get damaged or dislodged from the traps once dry.

Lindgren funnel traps (Lindgren, 1983) used in conjunction with aggregation pheromones are an effective method for monitoring *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.) flight activity in agricultural landscapes, and we are currently using this technique to study the ecology of these beetles in southern Australia. Material collected during our trapping program will be potentially valuable for molecular studies on the distribution of pesticide resistance, and we conducted this study to determine which approach to short-term DNA preservation provides the best results for these beetle species. The approach we took was to expose beetles to a range of different preservation scenarios under laboratory and field conditions, extract the DNA from individual beetles, and then attempt to amplify a section of genomic DNA using PCR. Provided the primer concentrations are not limiting, the PCR yield reflects the initial number of amplifiable copies of the template in the sample aliquot, which in turn is a function of both DNA yield and integrity.

2. Materials and methods

2.1. Beetle cultures

Tribolium castaneum was cultured in the laboratory on a diet of rolled oats and wholemeal wheat flour, whilst *R. dominica* was cultured on whole wheat. Both cultures were established from local wild populations and had been maintained in the laboratory for several generations before use. Cultures were maintained at 30 ± 1 °C with a 15L:9D lighting regime (cool fluorescent lights).

2.2. Experiment 1. Maintenance of DNA yield and quality under controlled conditions of temperature and humidity

We assessed seven different treatments over a period of 14 days, the maximum period over which pheromone traps are likely to remain in the field without servicing. All treatments were prepared in single glass crystallising dishes 75 mm in diameter and 40 mm deep. Propylene glycol (PG, 99.5+%) and Triton® X-100 (a non-ionic surfactant) were obtained from Sigma–Aldrich Inc, St. Louis, MO. Deionised water was used in the preparation of all relevant treatments. Phosphate-buffered saline (PBS) was prepared with laboratory-grade reagents using the protocol provided by Sambrook and Russell (2001). Abbreviations for the five liquid treatments and their components (liquids as % v/v) were: PG, 100 PG; PG + water, 50:49.8:0.2 PG:water:Triton® X-100; PG + PBS, 50:49.8:0.2 PG:PBS:Triton® X-100; Water, 99.8:0.2 water:Triton® X-100; PBS, 99.8:0.2 PBS:Triton® X-100.

The five crystallising dishes assigned to the liquid treatments were weighed and then 60 mL of the designated liquid was added. The dishes were weighed again and 18 adult *R. dominica* were added to each dish and, when necessary, were pushed beneath the solution surface, since in the field the rocking movement of suspended funnel traps leads to captured beetles rapidly breaking through the surface tension and sinking into liquid preservatives. The dishes were weighed a third time, and then placed in a glass desiccator above a saturated aqueous solution of NaCl in order to maintain the relative humidity (r.h.) at 75% (Winston and Bates, 1960; Rockland, 1960).

The remaining treatments involved dry preservation at two different humidities. Killmaster Zero® pest strips containing 186 g/kg dichlorvos were obtained from Barmac Industries Pty Ltd, Blackstone, QLD, and sections approximately 1 cm^2 in area were cut and placed in the dry treatment dishes immediately after groups of 18 beetles had been added. Dichlorvos has been shown to be an effective killing agent in Lindgren funnel traps, and does not interfere with the attraction of *R. dominica* to its aggregation pheromones (Edde et al., 2005). Glass Petri dish lids were placed on the two dishes for 30 min, by which time all beetle movement had ceased. The Petri dish lids were then removed and the dishes were placed separately in two additional glass desiccators, one containing saturated NaCl (75% r.h.) and the other containing saturated $MgCl_2$ to provide 32% r.h. (Winston and Bates, 1960; Rockland, 1960). The two treatments are abbreviated as 'Dry 75% RH' and 'Dry 32% RH'. The Dry 75% RH treatment was kept in a separate desiccator to the liquid treatments maintained at this humidity to ensure dichlorvos volatilisation did not contaminate any of the liquid preservatives.

The three sealed desiccators were placed in a controlled temperature room at 30 ± 1 °C with a 15L:9D lighting regime (cool fluorescent lights). Beetles were sampled from each treatment at 3, 7 and 14 days post-establishment. On each occasion, the dishes containing liquid preservatives were weighed before and after six beetles were removed to allow the calculation of water uptake or loss from the dishes. Beetles were placed in individual 1.5 mL microcentrifuge tubes each containing 500 μL absolute (200 proof) ethanol (Sigma–Aldrich, molecular grade). A further six live beetles were removed from the laboratory culture on each sampling day to act as controls, and were also placed in absolute ethanol. After 30 min with occasional agitation, the ethanol in all tubes was replaced and the tubes were stored at −80 ± 3 °C until DNA extraction and analysis.

This experiment was conducted twice, with *R. dominica* and *T. castaneum* adults being evaluated in separate experiments. Dish weights were only monitored in the *R. dominica* experiment.