Elimination of microsporidian infections from quarantined heather beetle populations

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1. Introduction

This report is a summary of work conducted for Dr H. Keys, DOC Turangi, under contract to the Science and Research Division, Department of Conservation, Wellington (reference number: FLO 017). The purpose of the research was to provide advice and diagnostic assistance to Landcare Research in order to detect and eliminate a microsporidian (Protozoa) disease from quarantined laboratory populations of the heather beetle, *Lochmaea suturalis*. Heather beetle populations are being imported from the U.K. by Landcare for evaluation as a biocontrol agent of heather. In previous work (Significance of a microsporidian disease of the Heather Beetle, *Lochmaea suturalis*, Report to DOC, July 1994), the author reported the detection of a microsporidian disease in quarantined heather beetle populations imported from southern England and advised on possible approaches to obtaining disease-free stock. The current contract aimed to utilise these suggestions in order to obtain healthy populations prior to possible release and to answer specific questions regarding mechanisms of microsporidian transmission. The contract asked for advice and services relating to four specific questions:

(1) How is the microsporidian disease transmitted?

(2) What tissues does the microsporidan infect?

(3) What specific methods should be used in intensive line breeding to eliminate the disease?

(4) Is cross-transmission of microsporidia already present in New Zealand likely to pose a serious threat to the beetle if it is released here?

2. Microsporidia

Microsporida are spore-forming protozoa that are obligate parasites of a wide range of insects and other invertebrates. They are amongst the most widely occurring pathogens of insects, with many species described from beetle and lepidopteran larvae. They often occur at high frequencies in field populations where they can cause significant mortality and reduce the fertility and fecundity of infected survivors. Microsporidian infections occur in some of our most important insect pests, including leafrollers, grass grub, porina caterpillar and Argentine stem weevil.

Microsporidia infect a wide range of body tissues including the gut, leading to transmission between individuals via faecal contamination. Some microsporida infect developing ova and are transmitted between generations within the egg. Microsporidian infections are an especially troublesome problem in insect rearing due to their ready means of transmission both within and between generations. Infections of laboratory populations often go un-

recognised due to their insidious nature, manifest in reduced growth rates, slowly accumulating larval mortality, and impaired reproductive performance.

Disease transmission and tissues infected

There are two major mechanisms of microsporidian infections in insects: via faeces, or internally via the egg. In order to determine likelihood of each transmission mechanism in the heather beetle, stained smears of faecal material (frass), eggs, and reproductive tissues from infected individuals were examined under the light microscope at magnifications ranging from 340x to 1440x. Abundant spores were detected in the frass and gut tissues of infected beetles. Microsporidian lifestages were not detected in eggs or in the undeveloped ova of infected females. Microsporidan spores or vegetative stages were also detected in haemacytes, salivary glands, epithelial tissue and fat-body cells.

These results strongly indicate that consumption of spores shed in frass is the primary mechanism by which microsporidian infections are transmitted between heather beetles. The failure to detect egg infections, or infections of ova within ovarioles, does not completely rule out transovarial transmission as a source of new infections but does suggest that it is unlikely to be a key mechanism of disease transmission.

4. Elimination of disease from quarantined heather beetle populations

The establishment of a disease-free population of quarantined heather beetles was attempted using a combination of methods. These were developed in consultation with Dr Pauline Syrett, Project Leader, and Mr Lindsay Smith, technician responsible for rearing the beetles within the insect quarantine facility at Lincoln. Approaches adopted included:

(i) the collection of beetles from a range of geographical locations in the hope of discovering a population free of microsporidian infections;

(ii) confining beetles individually in tubes until their disease status was determined from microscopic examination of their frass;

(iii) setting up isolated male-female pairs of apparently disease-free beetles and rearing their progeny as isolated lines until the disease-free status of adults could be confirmed by post-mortem examination;

(iv) sacrificial examination of a proportion of the progeny of each reproducing pair;

(v) adoption of rigorous hygiene procedures to prevent the possibility of disease transmission between breeding colonies.

Beetles were collected at three locations: Yately (205 beetles) and Chobham Common (113 beetles) southern England, and at Oakworth, Yorkshire (59 beetles). Individually confined beetles were shipped to New Zealand where their frass was examined shortly after arrival for the presence of microsporidian spores. Spores were found at a high incidence in the frass of beetles: from both Yately and Chobham Common, as well as in dead beetles from these sites, but only in the frass of one beetle from Oakworth. All beetles originating from Yately and Chobham Common were destroyed. Twenty pairs of heather beetles from Oakworth were established in separate breeding cages. Four of these pairs laid fertile eggs which hatched to produce rapidly-growing and apparently healthy larvae. These were reared through to yield approximately 300 adults. In postmortem examinations of Oakworth beetles an infection was discovered in a male from a non-laying pair. Infections were not detected in any of the other beetles from Oakworth, in sacrificed eggs and larvae from the breeding pairs, or in larvae dying during the rearing process.

We conclude that is highly likely that the F1 generation of quarantined Oakworth heather beetles contains is free of microsporidian infection.

5. Likelihood of cross-infection from microsporidia infecting New Zealand insects

Microsporidia from a range of genera infect some of our most important insect pests, such as grass grub, porina caterpillar, Argentine stem weevil and leafrollers, as well as important beneficial insects such as bees. If released into the field, heather beetles will share their geographical range with such insects together with a multitude of less common species. Some of these lesser known species may also harbour microsporidia. However, experience demonstrates that most microsporidia are specific to their host and that species with a high degree of cross-infectivity are rare in the field. We conclude that the most serious disease risk for the heather beetle stems not from species of New Zealand microsporidia but from its indigenous pathogens and that rigorous efforts should be maintained to ensure the disease-free status of beetle populations established in quarantine and of beetles released into the field.

6 . Late result

On the 24th of June we examined frass smears taken from heather beetles collected in Scotland and recently quarantined in New Zealand. Spores were not detected in the frass of beetles collected from Loch Linnhe or Rannock Moor but were observed in the frass of beetles from Glencoe Village.

These latest results show that the microsporidian disease is widely distributed within British heather beetle populations. However, the failure to detect infections in two of the Scottish sites and in beetles from Oakworth, Yorkshire, demonstrates that it is possible to locate populations in which the disease is either absent or at a very low incidence. Sites such as these will be suitable for providing beetles for quarantined parent stock from which disease-tested progeny could be drawn for release into the field.