The Nosema Sampling & Knowledge Transfer Project – An Update – Alan Riach

The above project originated from a *Nosema* analysis research programme conducted by Dr Chris Connolly at Ninewells Research Centre in Dundee. The funding body for that that research (The Biotechnical & Biosciences Research Council (BBSRC)) were keen to have the techniques used in the research program pushed out into the beekeeping community – a process sometimes referred to as "citizen science". It was agreed that a small team of SBA members would be trained up in the processes, as that would provide the association with some valuable in-house capability in the diagnosis of *Nosema* and possibly also viral diseases.

The Scottish Science & Advice for Scottish Agriculture's (SASA) management very generously agreed to provide laboratory & equipment facilities at Roddinglaw, Edinburgh in which to carry out the training and subsequently to allow the trained up members to carry out testing. This has involved a huge effort from Fiona Highet and her staff & from Chris Connolly & his staff, who have provided excellent support.

This is an update to let SBA members know what progress has been made in this leading edge project.

BBSRC approved the knowledge transfer programme, provisional on running the programme on a 6 month timescale starting immediately. The late autumn starting date was not ideal for the beekeeping world as the bees were being fed and bedded down for winter.

The first part of the program required about 250 twin samples, each of 30 bees, to be collected from all over Scotland in order to have sample stock for the training program and the subsequent testing. Of the twin samples, one was sent to SASA for fast freezing to minus 80 C for later use in testing for *Nosema apis* and *Nosema ceranae* using microscopy measurement and Polymerase Chain Reaction (PCR) techniques. PCR involves molecular methods of finding DNA remnants (of e.g. disease pathogens) which are undetectable by other methods & rapidly duplicating these remnants to a level where they can be easily detected.

The fast freezing of the live bee samples to these low temperatures preserves the integrity of the DNA/RNA material in the disease pathogens.

The second sample was used by the beekeeper or local association microscopist to pre-test for *Nosema*, positive sample liquid being sent to SASA in an Eppendorf sample phial. This will enable SASA to quickly zone into suspect deep frozen samples.

John Durkacz & the author developed a method of gathering & shipping the live bee samples which caused minimal disturbance to the colonies. This involved using small transparent plastic 5cm x 5cm x 3cm "snack boxes", ventilated by creating holes with a hot skewer. John devised a method of gathering the samples at a reduced entrance of the hive which meant that the hives did not need to be opened. See diagrams.

Live bees shipped in these containers arrived at SASA in perfect condition.

I found that the knock & collect method worked brilliantly although I should perhaps not have trialled it on the "strong" hive. In a flurry of pressure & excitement, John managed to accidently mis-label a postage sample to himself – the bees arrived back home in Dunfermline next day buzzing happily – proves your system works John!

Response from the Local Associations (LA's) has been excellent; over 250 samples have been received by SASA and deep frozen. Thanks are due to all those who sent samples; your response to the urgent request was impressive. We shall endeavour to get feedback to all those who sent samples as testing gets underway in January.

It would be ideal if the same colonies could be re-sampled in the spring, say in April, as we could then develop a very valuable pre-winter & post- winter database for the two *Nosemas*. I would therefore ask that those who sent in samples please consider re-sampling the same colonies in late April/May and again send the live samples to SASA, as before.

Fig.1

Small food containers (5cm x 5cm x 3cm), purchased from Poundland in packets of 6 or 8. These are cheap and sturdy. Using a hot needle or small drill bit make a double row of holes down either side of the lid. (The centre part is left clear as a band of sellotape can be used to secure the lid after the bees are collected). Label two containers for each hive and place quarter of a teaspoonful of fondant in the one to be shipped to SASA.

Fig. 2

The entrance closed down to about 3 or 4 cm. The container is placed over this reduced entrance, the side of the hive is tapped and the bees appear and "self-fill" into the containers.

Fig.3

If a lot of bees emerge the box can be filled by scraping it across the front of the hive. The fact that the box is transparent encourages the bees to enter and move towards the light. The lid can then be slid into place & a wrap of sellotape applied to secure, round the middle of the box. (The lids do snap on but not very securely.

Fig.4

The boxes were labelled with Beekeepers name, Apiary identity, Hive number & date. One was kept for home freezing and examining for *Nosema* later. The one with the added fondant was clearly labelled, placed in a jiffy bag with holes punched for ventilation & posted to SASA.

Fig.5

The hives soon settled after this treatment and the bees in the box for posting were soon feeding on the fondant previously inserted.

It was considered that about eight SBA volunteers could be trained with the laboratory facilities available and that these 8 would provide a worthwhile “testing team”. Nine candidates were selected from volunteers.

An intensive 3 day training programme was held at SASA’s lab in Edinburgh & Ninewells Medical Laboratories in Dundee, at the beginning of December 2013. The programme used samples that

were sent in by members and some interesting results came out of the assays carried out during the training programme. A small number of the samples that had been confirmed as *Nosema* positive by field microscopy were shown to be predominantly *Nosema ceranae* by the PCR testing. If we can re-test in the spring it would enable us to determine whether the two *Nosema* species are seasonal.

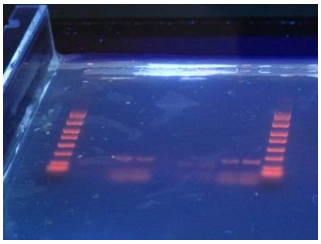
The following SBA volunteers enthusiastically gave of their time at the training days. Ruth Anderson (Largs & Ayr), Beth Bader (Edinburgh), Mark Barnett (Borders), Tony Harris (Moray), Kevin Russell (Dunblane & Stirling), Christine Matthews (South of Scotland) & Bron Wright (SBA VP). John and I completed the team. Preparing PCR samples and running the tests is exacting work requiring sustained concentration and none of the participants had any difficulty sleeping after the 3 days of activities. Our tutors were clear (& patient) and had prepared excellent test protocols.

A very big thank you to the many beekeepers who responded to our urgent call for samples at what was a rather late seasonal timing.

Ruth Anderson loading the Kingfisher DNA extraction machine

Careful concentration under the watchful eye of Vince in the SASA lab.

The PCR Thermal Cycler does its work taking the sample through various temperature stages as it duplicates the genetic information over and over to produce a large & measurable sample.



Results: Two sets of results showing positives for *Nosema ceranae*. The fluorescent dyed material has migrated along the gel bed (from the top of the photo to the bottom, driven by an applied electric potential of 80volts).The columns from left to right are:

1st column is a standard "ladder guide" a mixture of different molecular weights –the smaller molecules make their way through the gel faster than the heavier ones. These ladders provide a guide, against which the size of the test genetic molecules can be compared.

2nd & 3rd columns – blank - a zero result for *Nosema apis*

4th & 5th columns – a positive result for *Nosema ceranae* & its control (ignore blurs at the very bottom of the picture)

The columns at the right are the results for the second sample viz *N.apis* negative, *N.ceranae* positive and guidance ladder.

Lab testing by the two PCR teams started in January and the testing so far, has continued to identify higher levels of *Nosema ceranae* than *Nosema apis.* PCR testing for the presence of Deformed Wing Virus (DWV), a virus associated with varroa has also shown significant levels.

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