

COST 850

Workshop Programme: Nematodes for mushroom fly pest control

Purpose and market

The workshop aim is to provide training in the use of entomopathogenic nematodes for control fly pests in commercial mushroom production. It is designed for European mushroom advisors, extension workers and practitioners.

Logistics

The workshop will be held at Warwick HRI, Wellesbourne, UK, a specialist mushroom research institute with facilities for work in mushroom houses and specialist laboratories. Warwick HRI is located near Stratford-upon-Avon, Warwickshire, UK about 45 minutes from Birmingham international airport and 1 ½ hours from Heathrow airport.

Date

The workshop will start early on Wednesday 27 October and be completed in one full day. Participants should arrive Tuesday 26 October and depart Thursday 28 October unless they want to stay on for the Mushroom Conference held at the same site on 28 October.

Workshop Programme

- 08:00 Buses depart from Stratford Moat House Hotel for HRI
- 08:30 Welcome (David Chandler)
- 08:35 Introduction to Workshop Aims (Ralf Ehlers)
- 08:45 Brief introduction to nematodes, with open questions (Ralf Ehlers)
- 09:00 Overview of current practice for use of nematodes, (best practice and integration with other products (chemical and biological) (Roma Gwynn & Stephen Jess).
- 09:30 Questions and discussion on current practices (Ralf Ehlers)
- 10:00 Coffee and Demonstration of commercially-produced nematode products.
- 10:30 Group 1: Nematode practical – in laboratory handling, observation and counting of viable versus non-viable nematodes.
- Group 2: Application practical - tour of HRI mushroom facilities, application and sampling of nematodes.
- Group 3: Sciariid/Phorid practical - presentation on identification of both sciarids and phorids and practical taxonomy.
- 12:00 Lunch.
- 13:00 Group 1: Sciariid/Phorid practical - presentation on identification of both sciarids and phorids and practical taxonomy.
- Group 2: Nematode practical – in laboratory handling, observation and counting of viable versus non-viable nematodes.
- Group 3: Application practical - tour of HRI mushroom facilities, application and sampling of nematodes.
- 14:30 Group 1: Application practical - tour of HRI mushroom facilities, application and sampling of nematodes.
- Group 2: Sciariid/Phorid practical - presentation on identification of both sciarids and phorids and practical taxonomy.
- Group 3: Nematode practical – in laboratory handling, observation and counting of viable versus non-viable nematodes.
- 16:00 Tea Break
- 16:30 Question and answer session and discussion
- 18:00 Return to Accommodation

Dr. Roma Gwynn

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Workshop : Nematodes for mushroom fly pest control

Application Practical

Aim

Explore techniques for calibrating application equipment for nematodes, application methods and sampling to assess nematodes in crop.

Methods

Dose rate calculation

Tank - 200 l recirculation tank, 6 l remains in tank
Nematodes - 1 x 50 million nematodes per pack applied in 30 l water
- rate = applied at 3×10^6 nematodes per m^2 in 1.8 l water (= 1.67 million nematodes per l)
Tray - 0.6 m^2 , treating 10 trays

Total area to be treated = $0.6 \text{ m}^2 \times 10 = 6 \text{ m}^2$

Number of nematodes needed per tray = $0.6 \text{ m}^2 \times (3 \times 10^6 \text{ nems}/\text{m}^2) = 1,800,000$

Water per tray = $0.6 \text{ m}^2 \times 1.8 \text{ l} = 1.08 \text{ l}$

Nematodes required for 10 trays = $10 \times 1,800,000 = 18,000,000$

Water required for 10 trays = $10 \times 1.08 = 10.8 \text{ l}$
plus need 6 l extra for volume left in tank,
Total = 16.8 l

Nematode solution required for 10 trays = 18 million
plus the extra 6 l = $6 \text{ l} \times 1.67 \text{ million} = 10.02 \text{ million}$
Total = 28.02 million

Dilution

Pack contains 50 million nematodes therefore need to dilute it for this small use.

1. Put entire contents of 50 million nematode pack into 1 l water = 50,000 nematodes/ml
2. Amount needed for 10 trays plus extra 6 l = $28.02 \text{ million} / 50,000 = 560.4 \text{ ml}$
3. Therefore, agitate the solution and then put 560 ml nematode concentrate into tank plus $(16.8 - 0.560 \text{ l}) = 16.24 \text{ l}$ water.
4. Agitate in tank before application.

Sprayer calibration and Application

To apply nematode solution to trays we need to know how many seconds are required to get 1 l, this will mean the dose applied is equivalent to 3×10^6 nematodes/ m^2 .

Calculate the rate by setting up the sprayer then measuring the time required to deliver 1 litre liquid into a measuring beaker.

This is then the time that is needed to cover each tray.

Sampling casing for sciarids

At times during the crop cycle it can be useful to know the relative number of sciarids in the crop. This can be pre-application of nematodes and post-application. This method does not give immediate results. However, over time by collecting this data you can get a good measure of how your control is working.

For this method to be effective you need to standardise where samples are made, for example select areas in the middle of a stack of trays/shelves or bags in the middle of a house.

1. You will need to take at least 5 sets of samples.
2. For each selected bag/tray/shelf, take 10 small samples of casing, each sample about one tablespoon.
3. To standardise this and to make it quick take the sample using an adapted 50 ml plastic syringe. Cut the end off the syringe, push the syringe into the casing, raise the plunger, twist and take the sample.
4. Using the plunger push the core into the sample box, repeat this at ten randomly chosen spots across the selected area. Repeat this 5 times to give 5 boxes.
5. The sample box should be a small container with a tight fitting lid, but large enough so the casing samples do not touch the lid.
6. Put a small piece (3 cm x 3 cm) of sticky trap stuck to the inside of the lid.
7. Leave in the house or a similar area and monitor the trap for sciarid emergence.
8. Identification of the emerging flies will be discussed during the sciarid identification practical.

This sampling method can be used to collect nematodes, they can be extracted from the casing to monitor nematode numbers, although this method can only give an estimate relative to the original dose of nematodes added. The method used to extract nematodes will be discussed during the laboratory practical.

Protocol quality assessment of entomopathogenic nematodes (V. 1)

1. Unit to be assessed
Nematodes in commercial unit (50 million unit)
2. COUNTING
 - 2.1. Open package and pour content in 5 dm³ tap water of 10 to 20°C in bucket of 15 to 25 cm diameter
 - 2.2. Stir suspension vigorously for 1 minute and keep agitated by bubbling air in from a tube leading to the bottom of the bucket
 - 2.3. Take 3 x 100 µl samples in 3 clean test tubes (use pipette with ≤ 1% error).
 - 2.4. Add 4,9 ml tap water to the test tubes (use glass-pipette with < 2% error)
 - 2.5. Mix tube by shaking and immediately after shaking take 5 x 100 µl from each tube and place them on clean Petri-dishes.
 - 2.6. After having placed 15 droplets count living nematodes in droplets using a dissecting microscope with ≥ 40-fold magnification.
 - 2.7. To distinguish living from dead nematodes use the following characters

Living	Dead
<ul style="list-style-type: none"> • Movement • Resting individuals: Never completely straight. Coiled or at least bent at head or tail <p>⇒ the nematodes usually start moving slowly after rehydrating in water.</p>	<ul style="list-style-type: none"> • Straight shape with shrivelled uneven surface • Nematodes with air bubbles inside their body

2.8. Results of the counting are entered in this table

	Droplet 1	Droplet 2	Droplet 3	Droplet 4	Droplet 5	Sum
Tube 1						
Tube 2						
Tube 3						
TOTAL:						

2.9. The estimated number per 50 million unit is calculated as

TOTAL x 166667 * Nematodes (± 12% as a conf-limit_{α=95%})

This number is needed to calculate the adjustment factor (AF) for adjusting the dosages for assessing nematode efficacy (see 3.4):

$$AF = TOTAL / 300$$

* Obtained from:

50 [dilution in test tube] · 5000 [ml total suspension] · 10 [100µl pro ml] / 15 [number of droplets counted]

3. Efficacy testing using the lesser mealworm (*Tenebrio molitor*)
 - 3.1. Dry Quartz-sand (grain-size 0.1 to 0.4 mm) is adjusted to 10% water content by adding non-chlorinated tap-water. If all dosages are to be tested each nematode batch requires 900 g sand (900 g sand + 100 g water).
 - 3.2. The moist sand is mixed and 200 g filled in plastic boxes (10 x 10 cm, height \geq 2 cm). Testing one nematode batch requires 2 (for quick assessment) or 6 (thorough assessment) boxes.
 - 3.3. 1 ml of the nematode suspensions are added to the middle of the boxes. The nematodes are applied in the following dilutions:

With 40 mealworms per box	dose 0	dose 1	dose 2	dose 3	dose 4	dose 5
<i>Steinernema feltiae</i>	0	80	200	400	800	1600
<i>H. bacteriophora</i>	0	200	400	800	1200	2400
<i>H. megidis</i>	0	200	400	800	1200	2400
<i>Steinernema carpocapsae</i>	0	40	80	200	400	800

Doses to be used in **quick assessment** are printed **bold**. All doses are tested in the thorough test scheme

- 3.4. The doses are each added in 1 ml volume. For that purpose, suspensions are diluted accordingly. Each 1000 μ l from the initial suspension (see 2.1) is diluted with water according to the following table. The numbers given must be multiplied with the adjustment factor *AF* (see 2.9)

Volume of tap water to be added to 1000 μ l of initial suspension to get different doses						
With 40 mealworms per box	dose 0	dose 1	dose 2	dose 3	dose 4	dose 5
<i>Steinernema feltiae</i>	-	125.00	50.00	25.00	12.50	6.25
<i>H. bacteriophora</i>	-	50.00	25.00	12.50	8.33	4.17
<i>H. megidis</i>	-	50.00	25.00	12.50	8.33	4.17
<i>Steinernema carpocapsae</i>	-	250.00	125.00	50.00	25.00	12.50

Doses to be used in **quick assessment** are printed **bold**. All doses are tested in the thorough test scheme

- 3.5. After adding 1000 μ l of each dose to boxes 40 fresh and living mealworms (100 to 300 mg) are added to each box. Boxes are closed with the lid having 20 needle-pin holes to allow for sufficient aeration. Plates are incubated at 22 to 25°C.
- 3.6. Mortality of mealworms is assessed after 7 days incubation and values are entered in this table:

Nematode:						
Dose						
Number dead larvae						

Material:

Adjustable Pipette (<i>Fisherbrand</i>): 50-200 μ l	Art. Nr. 64890511	101,75 €
Adjustable Pipette (<i>Fisherbrand</i>): 100 – 1000 μ l	Art. Nr. 64890510	101,75 €
Tips: 1000 pieces	Art. Nr. 64039207	14,32 €
Glass-pipette (1ml)	Art. Nr. 62752070	2,20 €
Glass-pipette (5ml)	Art. Nr. 62752075	2,58 €
Pipetting ball	Art. Nr. 35494450	6,39 €

All from Fisher Scientific (In Germany Fon: 01805-258221; Fax 01805-258223)

QC Protocol in Spanish

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Protokolo sobre el control de calidad de Nematodos (V. 1)

4. Unidades

Unidad de 50 millones de nematodos en formulación comercial

5. Conteo

- 5.1. Vierta y diluya el contenido del paquete en 5 litros de agua (10-20°C). Para este propósito necesitara un envase de 15 a 25 cm de diámetro.
- 5.2. Remueva la suspensión vigorosamente por un periodo de 1 minuto y manténgala agitada utilizando una bomba de aire. El tubo de aire debera localizarse en el fondo del envase.
- 5.3. Tome 3 muestras de 100 μ l en tubos de ensayo limpios (utilize una pipeta con error $\leq 1\%$)
- 5.4. Añada 4.9 ml de agua a los tubos de ensayo (utilize una pipeta de cristal con error $> 2\%$)
- 5.5. Agite el contenido del tubo de ensayo tomando inmediatamente 5 muestras de 100 μ l de cada tubo. Coloque las muestras en placas de petri limpias.
- 5.6. Luego de haber colocado las 15 gotas en la placa de petri , procedera a contar el número de nematodos vivos en cada gota, utilizando un microscópio de disección con una magnificación ≥ 40 .
- 5.7. Para distinguir entre nematodos vivos y muertos utilice los siguientes criterios:

Vivos	Muertos
<ul style="list-style-type: none">• Movimiento• Reposo: Poseen curvatura, o por lo menos un pequeño doblez en el area anterior o posterior del cuerpo.	<ul style="list-style-type: none">• Forma elongada sin curvaturas• La superficie del cuerpo se presenta arrugada• Presencia de burbujas de aire dentro del cuerpo

5.8. Los resultados de los conteos serán anotados en esta tabla:

	gota 1	gota 2	gota 3	gota 4	gota 5	Suma
tubo 1						
tubo 2						
tubo 3						
TOTAL:						

5.9. El número promedio de nematodos para una unidad de 50 millones se obtendrá utilizando la siguiente ecuación:

TOTAL x 166667* nematodos ($\pm 12\%$ como límite de confianza $\Delta=95\%$)

Partiendo del resultado total de la suma de los conteos se calculará el Factor de corrección (AF) el cual se utilizará para ajustar las dosis necesarias para las pruebas de eficacia de los nematodos (vea 3.4):

$$\text{Factor de corrección (AF)} = \text{TOTAL} / 300$$

6. Prueba de eficacia utilizando el gusano de la harina (Tenebrio molitor)

6.1. Utilizaremos arena de cuarzo seca con un grano de 0.1-0.4 mm. El contenido de agua de la arena se ajustará al 10% añadiendo agua de clorinada. En el caso de que se hagan pruebas para cada dosis se requerirán 900 g de arena + 100 g de agua.

6.2. Luego de humedecer la arena deberá ser mezclada. Llene envases plásticos con tapa de un tamaño aproximado 10 x 10 cm x 2cm con 200 g de arena húmeda. Para cada prueba se requerirán 2 envases plásticos (prueba corta) o 6 envases (prueba científica).

6.3. Un mililitro (1 ml) de las suspensiones de nematodos será añadido en el medio del envase. Los nematodos serán aplicados utilizando las siguientes diluciones:

40 tenebrios/caja	Dosis 0	Dosis 1	Dosis 2	Dosis 3	Dosis 4	Dosis 5
<i>Steinernema feltiae</i>	0	80	200	400	800	1600
<i>H. bacteriophora</i>	0	200	400	800	1200	2400
<i>H. megidis</i>	0	200	400	800	1200	2400
<i>Steinernema carpocapsae</i>	0	40	80	200	400	800

Impreso en negrilla son presentadas las dosis a utilizarse en la *prueba corta*. La *prueba científica* se llevará a cabo con todas las dosis.

6.4. Las diferentes dosis serán añadidas en 1 ml de volumen. Para este propósito las dosis serán preparadas partiendo de la suspensión original. 1000 μ l de la suspensión original serán diluidos con agua de acuerdo a los valores de la tabla a continuación. Los valores de la tabla deberán ser multiplicados por el factor de producción (AF) (vea 2.9)

* Esta constante se obtiene de la siguiente fórmula:

50 [dilución tubo ensayo] · 5000 [ml suspensión original] · 10 [100 μ l gotas por ml] / 15 μ l [número de gotas contadas]

Cantidad de agua en ml que debera ser añadida a cada 1000µl dela suspensión original						
40 tenebrios/caja	Dosis 0	Dosis 1	Dosis 2	Dosis 3	Dosis 4	Dosis 5
<i>Steinernema feltiae</i>	-	125.00	50.00	25.00	12.50	6.25
<i>H. bacteriophora</i>	-	50.00	25.00	12.50	8.33	4.17
<i>H. megidis</i>	-	50.00	25.00	12.50	8.33	4.17
<i>Steinernema carpocapsae</i>	-	250.00	125.00	50.00	25.00	12.50

Impreso en negrilla son presentadas las dosis a utilizarse en la *prueba corta*. La *prueba científica* se llevara a cabo con todas las dosis.

- 6.5. Una vez han sido añadidos los 1000µl en los envases plásticos, se añadirán 40 tenebrios en cada caja (100-300 mg). Se tapan las cajas. En la tapa se haran 20 orificios con una aguja para proveer aeración. Las cajas seran incubadas a 22-25°C durante un periodo de siete días. Después del periodo de incubación se contarán el número de larvas muertas. Los resultados seran anotados en la siguiente tabla:

Nematodos:						
Dosis						
Larvas muertas						

Información para la compra de materiales:

Einstellbare Pipetten (<i>Fisherbrand</i>): 50-200 µl	Art. Nr. 64890511	101,75	€
Einstellbare Pipette (<i>Fisherbrand</i>): 100 – 1000 µl	Art. Nr. 64890510	101,75	€
Spitzen dazu: 1000 Stück	Art. Nr. 64039207	14,32	€
Glaspipette (1ml)	Art. Nr. 62752070	2,20	€
Glaspipette (5ml)	Art. Nr. 62752075	2,58	€
Pipettierbälle	Art. Nr. 35494450	6,39	€