Twinning BA/12/IB/AG 01 "Further strengthening of capacities of phytosanitary sector in the fields of plant protection products, plant health and seeds and seedlings, including phytosanitary laboratories and phytosanitary inspections"

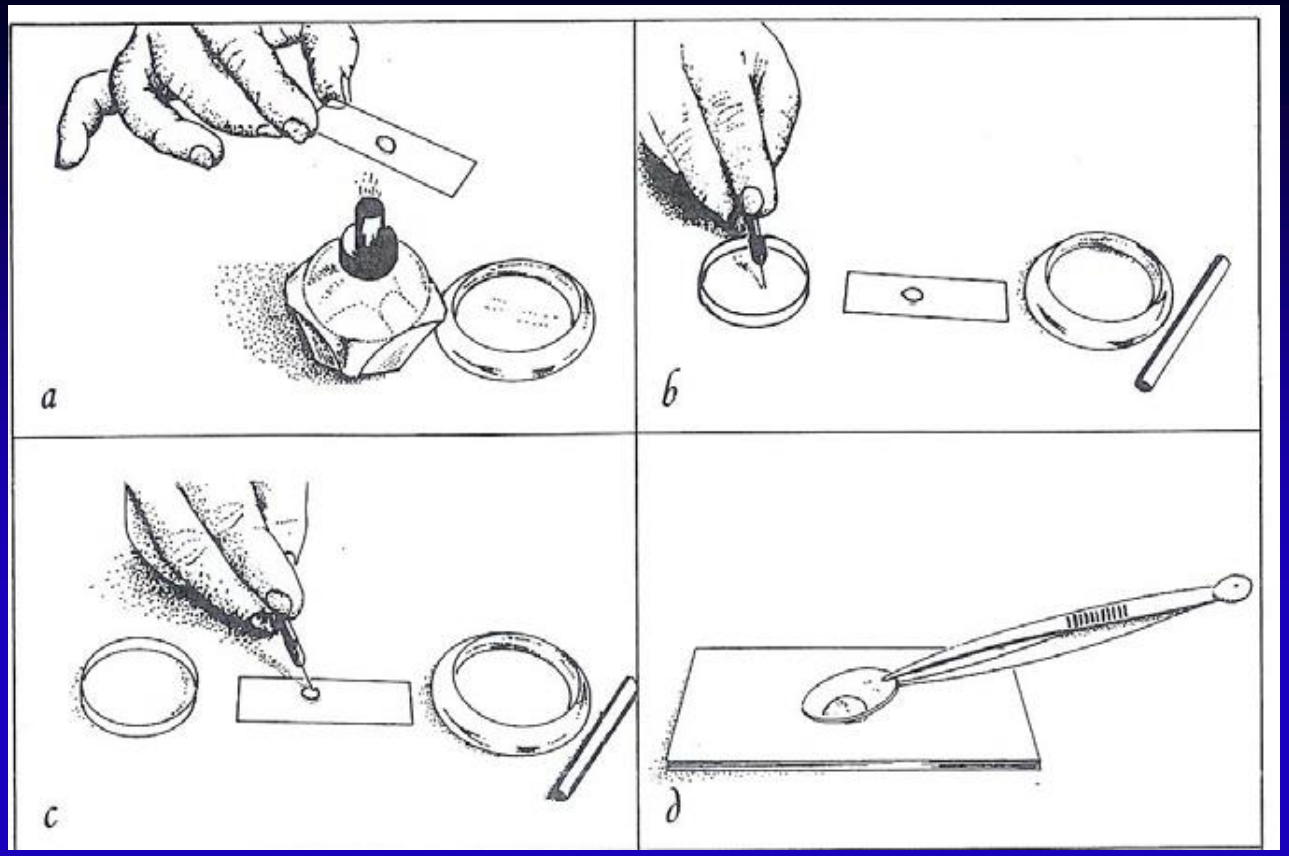
Training course on agricultural nematology

Mostar, March 7-11, 2016

PREPARATION OF FREE LIVING AND ROOT-KNOT NEMATODES GLASS SLIDE MOUNTS

PREPARATION OF TEMPORARY GLASS SLIDE MOUNT OF FREE LIVING NEMATODES

- Temporary mounts (slides) of free living nematodes in water are useful for a few hours or days if kept refrigerated.
- If the specimens are fixed in a 2% formaldehyde solution or other fixative (TAF, etc.), temporary mounts may be prepared in fixative used.
- Temporary mounts are commonly used in plant diagnostic laboratories.



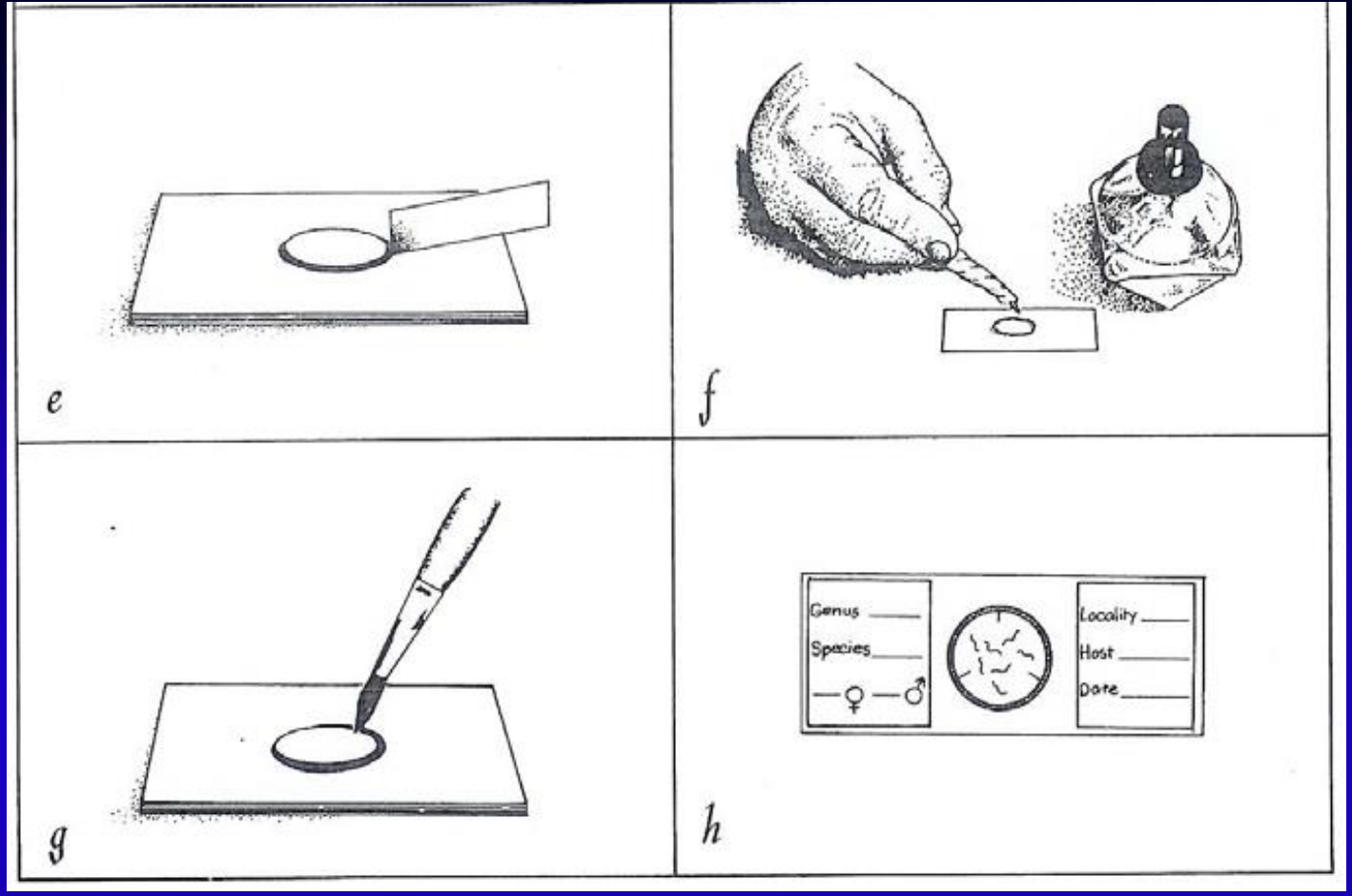
Procedure

A) Transfer 10-15 extracted nematodes with a fine dissecting needle, toothbrush bristle, or bamboo pick to the drop of water on the slide. Kill or relax nematodes in a drop of water by passing the bottom of a glass slide several times over the flame of an alcohol burner (this is not necessary if the drop contains fixative or mounting fluid).

B) Pick up several short sections of glass wool with the moistened tip of a transfer needle. They should be slightly thicker than the diameter of the nematodes.

C) Place the glass wool spacers on three sides of the drop of water.

D) Pass a coverslip through the flame of the alcohol burner to eliminate lint and moisture, and then slowly place it over the drop of water.



E) Remove excess water with the tip of a paper towel or facial tissue while viewing the nematodes under a dissecting microscope, taking care to avoid drawing the nematodes out from under the coverslip.

(F, G) Seal the edge of the coverslip to the glass slide with wax from the tip of a heated (not burning) birthday candle (F), using the wick as an applicator, or with clear nail lacquer or some other sealant (G), applied with a small brush.

(H) Label the slide.

PREPARATION OF TEMPORATY MOUNTS OF PERINEAL PATTERN OF ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.)

a) Dissect females from roots, etc. placed in water or 0.9% NaCL solution, with a needle, etc. and place them in the middle of drop of water on a microscopic slide and slowly, gently place a coverslip over this drop.

b) Place the slide under compound microscope trying to find out the position of the perineal pattern. The found perineal pattern should be projected directly to the top.

c) Gently press the coverslip. The female will be crushed (flattened). Seal the slide (nail lacquer, etc.).

d) Good prepared slide shout allows for observation of perineal pattern and sometimes also female stylet.

e) Alternatively transfer female to a drop of water in the middle of weigh dish made of plastic (4 – 10 cm in diameter) or any other piece of soft plastic sheet of similar diameter.

f) Cut off female posterior end with a fine scalpel, injection needle etc.

g) Remove content of cut-off part with nematological handling needle to leave cuticle, only and place it in the middle of drop of water on a microscopic slide and slowly, gently, place a coverslip over this drop.

h) Place the slide under compound microscope trying to find out the position of the perineal pattern. The found perineal pattern should be projected directly to the top.

i) Gently press the coverslip. Seal the slide (nail lacquer, etc.).

PREPARATION OF PERMANENT GLASS SLIDE MOUNT OF FREE LIVING NEMATODES

Rapid lactoglycerol method

Prepare:

- 4% formalin solution (dissolve about 100 ml of 40% formaldehyde with water to obtain 1 l of the formalin solution)
- lactoglycerol:

  lactic acid 100 ml;
  glycerol 200 ml
  distilled water 100 ml

Procedure

A) Transfer 10-15 extracted nematodes with a fine dissecting needle, toothbrush bristle, or bamboo pick to the very small drop of water on the watch glass.
B) Kill or relax nematodes by pouring on drop of water of about 1 ml of boiling 4% formalin solution (heated over the flame of alcohol burner).
C) Fixing of the nematodes should be continued for 1-2 days, but sometimes nematodes are good for mounting after 1-2 days of fixing.
D) Transfer nematodes to drop of cold lactoglycerol on the microscopic slide, where they become distorted.
E) Pass the bottom of a glass slide several times over the flame of an alcohol burner (until „first smoke”). Check under stereoscopic microscope if they are still distorted. If yes, repeat the procedure.
F) Place drop of glycerol in the middle of second microscopic slide.
G) Pick up several short sections of glass wool with the moistened tip of a transfer needle. They should be slightly thicker than the diameter of the nematodes.
H) Place the glass wool spacers on three sides of the drop of glycerol.
I) Transfer nematodes from lactoglycerol to glycerol drop.

Procedure

J) Pass a coverslip through the flame of the alcohol burner to eliminate lint and moisture, and then slowly place it over the drop of glycerol.
K) Seal the edge of the coverslip to the glass slide with wax from the tip of a heated (not burning) birthday candle, using the wick as an applicator, or with clear fingernail polish or some other sealant, applied with a small brush.
L) Label the slide.
M) Microscope slides could be prepared with paraffin wax rings (rectangular or circular) of similar proportions to the margins of the coverslips to be used to cover the finished preparations.
N) The ring of wax should be complete, and there must be sufficient wax in the ring to effectively surround the sample and to seal the coverslip.
O) Melt paraffin wax (melting point 57-60 °C) in a crystallising basin or glass Petri dish and leave the applicator in the wax to warm up and prepare a ring on the microscopic slide.
P) Transfer the nematodes into a minute drop of pure glycerol on a slide glass with the paraffin.

Procedure

Q) Place the cover glass at the slide glass above the drop of glycerol with nematodes; it is supported by two paraffin pieces situated at both sides of the drop laterally.
R) Heat the slide glass is gently at 80-85 °C till the paraffin melts and seals the glycerol drop with nematodes. Because of the slope of the cover glass, the liquid paraffin moves first to the lowest edge of the cover glass and then spreads gradually around the paraffin drop.

Another fixatives may be used instead of 4% formalin. One of them is TAF containing:

formaldehyde 40%	**7 ml;**
trimethylamine	**2 ml**
distilled water	**91 ml.**

The nematodes may be placed in cold TAF or fixed in hot TAF as using similar procedure as in case of fixing with 4% formalin.

Seinhorst glycerol method

a) Transfer nematodes fixed in formalin (or other fixative) to a small cavity block (e.g. glass staining block) containing the following solution (S1):

- ethanol 96% 20 ml;
- glycerol 1 ml
- distilled water 79 ml.

b) Place the cavity block with nematodes to desiccator containing 96% ethanol for at least 12 h (preferably in an oven at 35-40 °C, to remove almost all the water).

c) Transfer nematodes to a cavity block filled with the following solution (S2)

- ethanol 96% 95 ml;
- glycerol 5 ml.

Seinhorst glycerol method

d) Place the cavity block with the nematodes in an oven (temp. 35-40ºC) for 7 h, adding 2 drops of S2 solution every 20-25 min.

e) Leave the cavity block at the same temperature.

f) Place the cavity block to desiccator with $CaCl_2$ until next day for whole dehydration of glycerol.

g) Transfer nematodes to glycerol drop in the middle of a microscopic glass. Mount nematodes as in case of lactoglycerol quick method.