

*Scottish Crop Research Institute, Invergowrie, Dundee, Scotland*

EFFECT OF DIFFERENT CONCENTRATIONS OF FORMALIN AND  
T.A.F. ON THE FIXATION OF *LONGIDORUS ELONGATUS*

by  
B. BOAG

Different types of mounting media can significantly affect the measurements of nematodes (Curran and Hominick, 1981). No comparable investigations have been made on the effect of different concentrations of the same fixative on nematode measurements although recommended levels of these chemicals can vary e.g. between 5-10% formalin (2-4% formaldehyde) (Hooper, 1970). Formaldehyde based fixatives are used extensively in nematology (Hooper, 1970) but recent reports have cast doubts on the safety of some of these chemicals (Davies, 1974, Anon, 1981). This paper reports the results of observations on the use of reduced concentrations of formaldehyde (HCHO) and T.A.F. (triethanolamine formalin) (Courtney *et al.*, 1955) and their effect on the morphometric characters used to identify nematodes.

*Materials and Methods*

Adult female *Longidorus elongatus* (de Man, 1876) Thorne *et Swanger* 1936 were extracted from soil (Boag, 1974), heat killed at 60°C for 2 mins, fixed in different concentrations of formalin and T.A.F. and stored in 10 ml 7.5 x 2.5 cm diameter tubes for 4 months. The concentrations of formalin, ranged from 0.001% to 10% in a double series and of T.A.F. from 1/1024 to the recommended strength again in a doubling series. Randomly selected nematodes from concentrations of fixatives where specimens were preserved and mounted in

glycerol by Cobbs slow replacement method (Hooper, 1970) and measured (Boag, 1981). Statistical analysis was then undertaken to correlate the effect of the different concentrations of fixatives with nematode measurements and to compare fixed specimens with those measured in water immediately after being heat killed. The concentration of formaldehyde vapour was also measured in the atmosphere above 100 ml of selected concentrations of formaldehyde solution in 500 ml polythene bags and in the laboratory using Draeger tubes (1).

### *Results and Discussion*

The results indicate that nematodes were well preserved for up to 4 months at concentrations as low as 0.039% formalin (and 1/256 the normal concentration of T.A.F.) while nematodes in concentrations below these levels decomposed rapidly. Bacteria started to accumulate on the outer cuticle at 0.039% formalin but infections were not evident in specimens in 0.078% formalin or 1/128 strength T.A.F. No significant differences were observed in the length of the odontophore or odontostyle of specimens measured in water compared with those fixed in formalin or T.A.F. and mounted in glycerol (Table I). All other parameters measured showed significant shrinkage in the fixatives with the exception of oesophageal length which had significantly lengthened. The ratio *c* was not affected by the fixatives.

The different concentrations of the fixatives significantly affected the parameters; oesophageal and odontophore length and *c* ratio in T.A.F. and *b* and *c* ratio in formalin over the wide range of dilutions used. Further statistical analysis of the results using students « *t* » tests showed that no significant differences were detected at dilutions of 1/16 T.A.F. or 0.625% formalin compared with the recommended concentrations.

Results of the measurements of formaldehyde vapour in polythene bags agreed with Henry's Law (Moore, 1962) and indicated a roughly linear relationship between the concentration of formaldehyde solution and that in the atmosphere. Consequently the use of fixatives at ca. 1/16 of the recommended levels would be expected to lower the

---

<sup>1</sup> Draeger tubes, formaldehyde 0.002, Draeger House, Sunnyside Road, Chisham, Bucks HP5 2AR.

Table I. - *Effect of fixatives on the morphometrics of Longidorus elongatus.*

| PARAMETERS<br>MEASURED | WATER | FIXATIVE                |                          |   |   |                          |   |
|------------------------|-------|-------------------------|--------------------------|---|---|--------------------------|---|
|                        |       | T. A. F.                |                          |   | FORMALIN  |                          |   |
|                        |       | Mean<br>value<br>n = 20 | Mean<br>value<br>n = 118 | Linear affect<br>of different<br>concentrations | Effect of<br>fixative<br>compared<br>with water | Mean<br>value<br>n = 117 | Linear affect<br>of different<br>concentrations |
| Length (mm)            | 5.91  | 5.16                    | N. S.                    | ***   | 5.15  | N. S.                    | ***   |
| Max. width (µm)        | 70.5  | 57.7                    | N. S.                    | ***   | 60.0  | N. S.                    | ***   |
| Tail width (µm)        | 41.7  | 38.5                    | N. S.                    | ***   | 38.2  | N. S.                    | ***   |
| Tail length (µm)       | 51.1  | 48.9                    | N. S.                    | *   | 48.1  | N. S.                    | **  |
| Oesophagus (µm)        | 394.8 | 429.3                   | *                        | ***   | 423.6   | N. S.                    | ***   |
| Odontostyle (µm)       | 93.6  | 92.1                    | N. S.                    | N. S.   | 91.5  | N. S.                    | N. S.   |
| Odontophore (µm)       | 49.9  | 48.0                    | **                       | N. S.   | 47.7  | N. S.                    | N. S.   |
| a                      | 83.9  | 90.4                    | N. S.                    | **  | 86.6  | N. S.                    | N. S.   |
| b                      | 15.2  | 12.1                    | N. S.                    | ***   | 12.2  | *                        | ***   |
| c                      | 115.8 | 106.3                   | *                        | ***   | 108.2   | *                        | *   |
| č                      | 1.23  | 1.30                    | N. S.                    | N. S.   | 1.27  | N. S.                    | N. S.   |
| V                      | 48.04 | 49.04                   | N. S.                    | **  | 49.10   | N. S.                    | **  |

N.S. - Not significant.

\*, \*\*, \*\*\*, significant at  $P = 0.05, 0.01$  and  $0.001$ .

concentration of formaldehyde vapour in the atmosphere by an equivalent amount. Laboratory tests also indicated that formaldehyde vapour can be detected by smell at 0.5 p.p.m. which is lower than the 2 p.p.m. (3 mg/m<sup>3</sup>) considered harmful by safety authorities (Muir, 1977).

This investigation has shown that both T.A.F. and formalin influence nematode measurements in a similar manner and that there is no significant difference between the effect of either fixative.

This study has indicated that plant parasitic nematodes can be stored in formalin based fixative at concentrations considerably lower than those usually recommended without any significant morphological or morphometric differences being induced. Lower concentrations would also alleviate the discomfort associated with working with preserved material and could be considered to increase safety in handling fixed material.

I am grateful to Mr T. G. E. Gillanders, Wolfson Institute of Occupational Health, Ninewells Hospital, Dundee for the use of the Draeger tubes and apparatus, Mrs. I.E. Raschke for mounting and measuring the nematodes and Dr P. B. Topham for assistance with the analysis of the results.

#### LITERATURE CITED

- ANON, 1981, No more foam insulation for American Homes. *New Sci.*, 89: 198.
- BOAG B., 1974, Nematodes associated with forest and woodland trees in Scotland. *Ann. appl. Biol.*, 77: 41-50.
- BOAG B., 1981, Measuring nematodes using a digitising tablet and microcomputer. *Syst. Para.*, 2: 145-147.
- COURTNEY W.D., POLLEY D. and MILLER V.L., 1955, T.A.F., an improved fixative in nematode technique. *Pl. Dis. Repr.* 39: 570-571.
- CURRAN J. and HOMINICK W.M., 1981, Effect of mounting methods on taxonomic characters of adult male mermithids (Nematoda: Mermithidae). *Nematologica*, 26: 455-466.
- DAVIES D.L., 1974, B.C.M.E.: possible hazard in perspective. *Chemistry in Britain*, 10: 359.
- HOOPER D.J., 1970, In laboratory methods for work with plant and soil nematodes. Ministry of Agriculture, Fisheries and Food Technical Bulletin No. 2, 39-54. Edited by J.F. Southey. Published by Her Majesty's Stationery Office.
- MOORE W.J., 1962, Physical Chemistry. Longman, London.
- MUIR G.D., 1977, Hazard in the chemical laboratory. Alden Press, London.

---

Accepted for publication on 20 January 1982.