Eur. J. Entomol. **105**: 793–795, 2008
http://www.eje.cz/scripts/viewabstract.php?abstract=1396
ISSN 1210-5759 (print), 1802-8829 (online)

A modified method for clearing, staining and mounting plant-inhabiting mites

FARID FARAJI and FRANK BAKKER

MITOX Consultants, P.O. Box 92260, 1090 AG Amsterdam, The Netherlands; e-mail: farid.faraji@mitox.org

Key words. Acari, iodine, staining, microscope slides

Abstract. A modified method for clearing, staining and mounting plant-inhabiting mites is proposed. With this method, all mites can be cleared with a single agent and mounted in a single medium. The stain added to the mounting medium enhances the clarity of the fine structures of soft-bodied mites under phase contrast and more significantly under bright field microscopy.

INTRODUCTION

Most acarines are so small that their identification is only possible after mounting of specimens on microscope slides. Numerous chemicals have been used both as clearing agents and as mountants. Also the methods of preparation vary with the kind of specimen and among researchers (Krantz, 1978). In this context, mites may be grouped in three general categories: (1) soft-bodied mites like Tetranychidae, Tydeidae, Stigmaeidae and Cunaxidae are cleared in lactophenol; (2) specimens of well-sclerotized taxa such as Gamasida are cleared in Nesbitt's fluid; and (3) Eriophyoidea are cleared and mounted in a medium such as Kono's fluid (Jeppson et al., 1975) or modified Berlese's medium (Amrine & Manson, 1996). Mites belonging to groups (1) and (2) are mounted after clearing in an aqueous medium such as Berlese's, Hoyer's or Faure's (for details of these chemicals see Krantz, 1978).

During biodiversity and faunistic surveys of plant-inhabiting mites, all of these groups of mites occur in the same samples and it would be more convenient if they could be cleared and mounted using the same agent and medium. Clearing media for the well-sclerotized specimens, could make the tiny and soft-bodied specimens, such as Eriophyoidea, too transparent. During clearing and after mounting, these mites are hard to see and easily lost. To overcome this, mites may be stained. Lignin pink (Evans & Browning, 1955), chlorazol black (Coineau, 1974), toluidine blue and metallic iodine (Amrine & Manson, 1996) are added either to the clearing agent or for Eriophyoidea, the mounting medium (Amrine & Manson, 1996). In the MITOX laboratory, we routinely and successfully use only one agent for clearing and one medium for mounting. Also, the stain we add to the mounting medium (Hoyer) significantly enhances the image and clarity of the fine structures of soft-bodied mites under both bright field and phase contrast microscopy. Here, we first describe the chemicals and the procedure of clearing and mounting and then illustrate the usefulness of the method.

MATERIAL AND METHODS

Modified clearing agent

Lactophenol	50 ml
Nesbitt's fluid	50 ml
Potassium iodide, briquettes p.a.	8 g
Iodine, resublimed p.a.	1 g

The above-mentioned chemicals are easy to mix.

Modified mountant

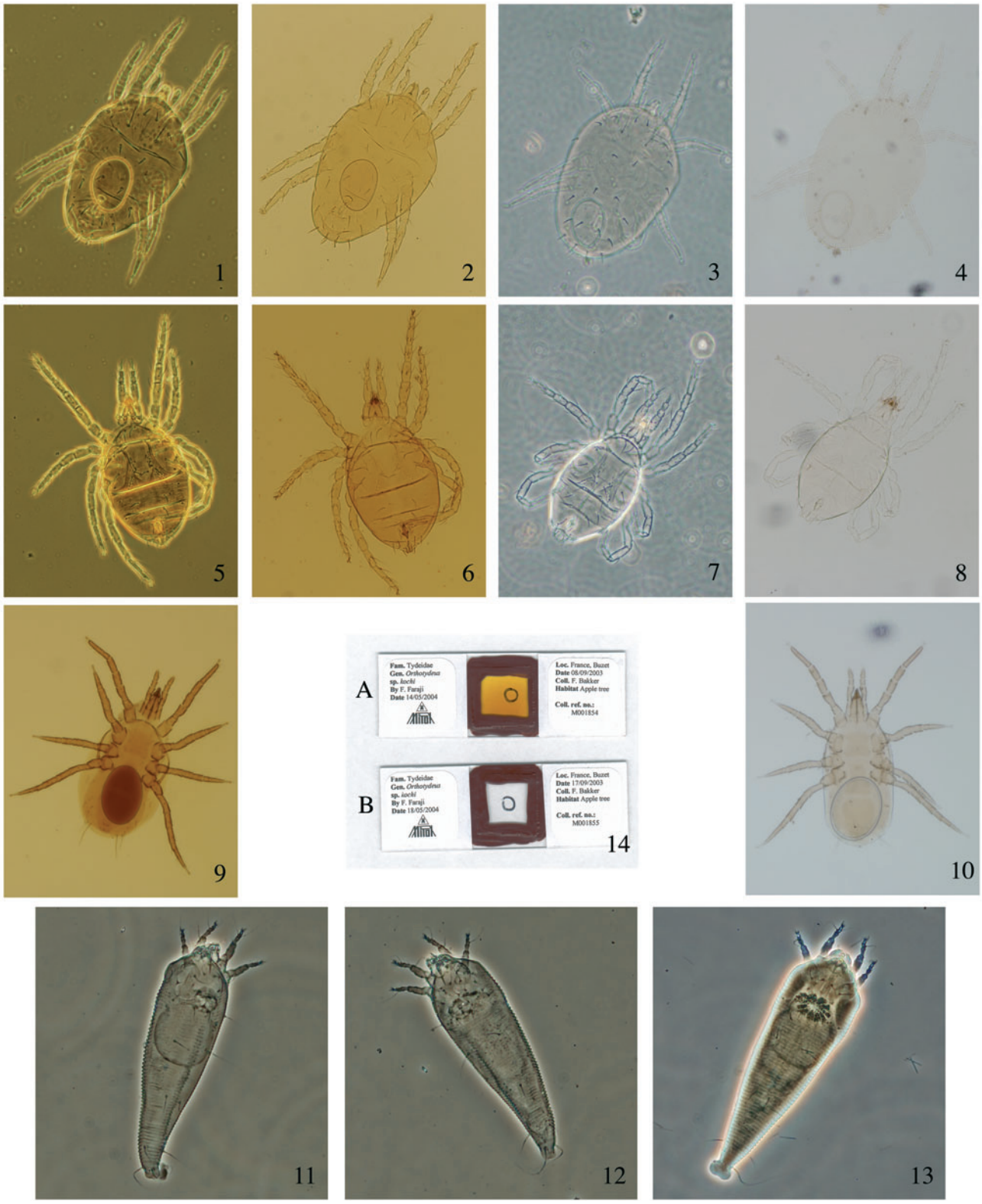
Hoyer's medium	100 ml
Potassium iodide, briquettes p.a.	1 g
Iodine, resublimed p.a.	0.3 g

Mix the above-mentioned chemicals and stir until Potassium iodide and Iodine dissolve. The mountant is ready to use when there are no small air bubbles left in the mixture (12–24 h).

All the above chemicals and those used to prepare Lactophenol, Nesbitt's fluid and Hoyer's medium were obtained from Acros Organics chemical company.

Clearing and mounting instructions

Place mites in the modified clearing agent in Syracuse watch glasses, using a flat-tip needle. Each Syracuse watch glass should be placed inside a small Petri dish, which then should be placed on a hot plate (45–50°C) for one hour. After cooling, any mites present at the surface should be immersed in the liquid. The dishes should then be placed again on the hot plate for about one hour before storing them at room temperature. The specimens are ready to mount the next day but mounting can be postponed for up to a week. Mount the mites in the modified Hoyer's medium. The colour of mountant under the coverslip should be amber [compare slide A (stained) with B (unstained), Fig. 14]. Place the slides in a warm oven or on a hot plate (45°C) for about two weeks. Before sealing, any mountant (not under the coverslip) should be removed. This can be done by placing a slide in a tray of warm water, scratching the excess mountant with a lancet or sharp-tip knife and then rubbing with a cotton bud to clean the slide. Return the slides to the oven for a further two more days. Seal the mountant that is exposed along the edge of the coverslip with Glyptal, using a small camel hair brush. Glyptal prevents moisture exchange between the mounting medium and the air (Travis, 1968). Glyptal is highly recommended as sealant because in our experience it is superior to all other sealants. Return the slides to the oven for a further 2–3 days and then repeat the sealing process. After drying the new coat of sealant in the oven, the slides are ready to be placed in slide boxes. Note: A minimum amount of mountant should be used. An excess of mountant prevents the Glyptal from drying and may result in the Glyptal spreading under the coverslip or the mountant becoming watery. Glyptal Inc. dose not sell small quantities of Glyptal, which we obtained from a retail supplier in Europe (Frost Auto Restoration Techniques Ltd., Crawford Street, Rochdale, Lancashire, OL16 5NU, England). Chloral hydrate used in Nesbitt's fluid and Hoyer's medium, phenol and Glyptal are hazardous chemicals. Therefore, the hot plate and slide warming oven should be placed under a fume hood. During mounting and sealing slides, the laboratory should be well ventilated.



Figs 1–4: *Orthotydeus kochi*, 1 – stained-phase contrast; 2 – stained-bright field; 3 – unstained-phase contrast; 4 – unstained-bright field. Figs 5–8: *Raphignathus gracilis*, 5 – stained-phase contrast; 6 – stained-bright field; 7 – unstained-phase contrast; 8 – unstained-bright field. Figs 9–10: *Neoseiulus bicaudus*, 9 – stained–bright field; 10 – unstained-bright field. Figs 11–13: *Calepitrimerus vitis* under phase contrast, 11 – cleared and mounted in medium with stain 12 – directly mounted medium with stain 13 – mounted in Berlese's modified medium. Fig. 14. Microscope slides prepared A. with the stain added to mounting medium and B. with no stain. Body (idiosoma) length of mites in Figs 1–8: 340–350 µm; Figs 9–10: 430 µm and Figs 11–13: 160–180 µm.

Photography

An Olympus digital camera (DP70 with extended focal imaging) mounted on an Olympus BX51 microscope was used for the photography. To enhance the focus of the images, five photographs of a mite were taken at different focuses. By using analySIS® docu software and application of multiple image alignment (mia) these images were then combined to produce a sharp image. Images were taken either under phase contrast or bright field, as indicated in the figure captions.

Treatments

Soft-bodied mites: *Orthotydeus kochi* (Oudemans), Tydeidae and *Raphignathus gracilis* (Rack), Raphignathidae, were cleared in lactophenol and mounted in Hoyer's medium.

Sclerotized mites: *Neoseiulus bicaudus* (Wainstein), Phytoseiidae, were cleared in Nesbitt's fluid and mounted in Hoyer's medium.

Eriophyoidea mites: *Calepitrimerus vitis* (Nalepa), Eriophyidae, directly mounted in modified Berlese's medium (Amrine & Manson, 1996).

Mites of the same species were cleared in modified agent and mounted in modified Hoyer's medium as described above. Also, some *C. vitis* were mounted directly in modified Hoyer's medium without prior clearing.

RESULTS

The images of the soft-bodied mites and the clarity of their fine structures were enhanced under phase contrast when iodine stain was added to the mounting medium (compare Figs 1 and 5 to 3 and 7, respectively). In addition, the clarity of fine structures was enhanced significantly under a bright field (compare Figs 2 and 6 to 4 and 8, respectively). For Gamasida, although the unstained specimen was clearly visible (Fig. 10), the stain enhanced the clarity of the image (Fig. 9). The clarity of eriophyid mite mounted using our method (Fig. 11) and mites directly mounted in the modified Hoyer's medium (Fig. 12) did not differ from those mounted in modified Berlese's medium (Fig. 13).

DISCUSSION

The procedure described in this paper is based on five years of experience mounting thousands of plant-inhabiting mites. These mites belonged to different groups, and were of different ages and physiological states.

Because one chemical is used for clearing and one for mounting, this method is very efficient if many mites belonging to different groups have to be mounted and identified. Added stain provides extra clarity to the fine structures of mites, especially soft-bodied mites. This difference is more pronounced under bright field microscopy.

One concern is the longevity of microscopic slides and the possible adverse effects of some of the compounds used in this method. We have mounted many mites using both the above and conventional methods for about five years and have not observed any difference in the result. However, at the moment, we recommend this method for semi-permanent microscope slides and those who want to draw figures or to take photographs of mounted mites. This method is also suitable for training students in acarology or entomology laboratories since the majority of microscopes use bright field illumination. The heating used to clear mites (one hour, 45–50°C) did not cause any loss of setae of the mounted mites. Moreover, the modified mounting medium does not crystallize, as occurs sometimes in Hoyer-mounted slides.

ACKNOWLEDGEMENTS. We are indebted to J. Beekman and G. Bouw (Paes Nederland bv) for providing the equipment and helping us with the photography. We also wish to thank G.W. Krantz for his valuable comments on this method. Our special appreciation goes to T. Bosse who patiently provided the chemicals used in this study.

REFERENCES

Amrine J.W.Jr. & Manson D.C.M. 1996: Preparation, mounting and descriptive study of Eriophyoid mites. In Lindquist E.E., Sabelis M.W. & Bruin J. (eds): *Eriophyoid Mites – Their Biology, Natural Enemies and Control. World Crop Pests, Vol. 6.* Elsevier Science Publishers, Amsterdam, pp. 383–396.

Coineau Y. 1974: Nouvelles techniques pour l'étude de la morphologie des formations chitineuses des acariens. *Acarologia* **16**: 4–10.

Evans G.O. & Browning E. 1955: Techniques for the preparation of mites for study. *Ann. Mag. Nat. Hist.* **8**: 631–635.

Jepsson L.R., Keifer H.H. & Baker E.W. 1975: Mites injurious to economic plants. Univ. California Press, Brekeley, 614 pp.

Krantz G.W. 1978: Collection, rearing, and preparation for study. In: *A Manual of Acarology*. Oregon State University Bookstore, pp. 77–98.

Travis B.V. 1968: Glyptal-a useful slide ringing compound. *J. Med. Entomol.* **5**: 24.

Received November 28, 2007; revised and accepted March 19, 2008