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# SPIDER MITES THEIR BIOLOGY, NATURAL ENEMIES AND CONTROL

# Volume 1A

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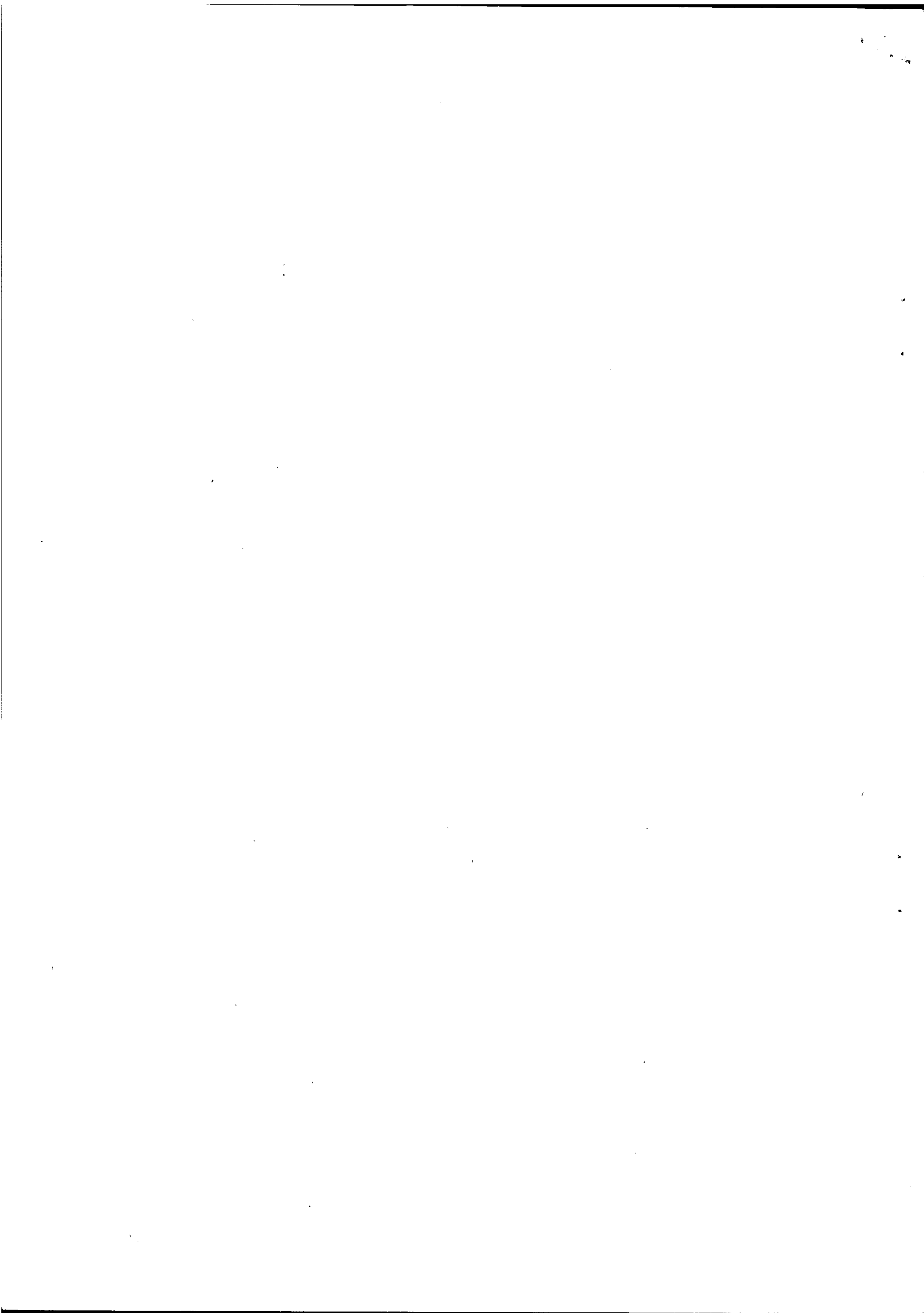

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Titre en Français :
(si le document est en langue étrangère)

Mots-clés matières : Technique préparation - Technique conservation - Acariens - Tetranychidae.
(10 au plus)

Résumé en Français :
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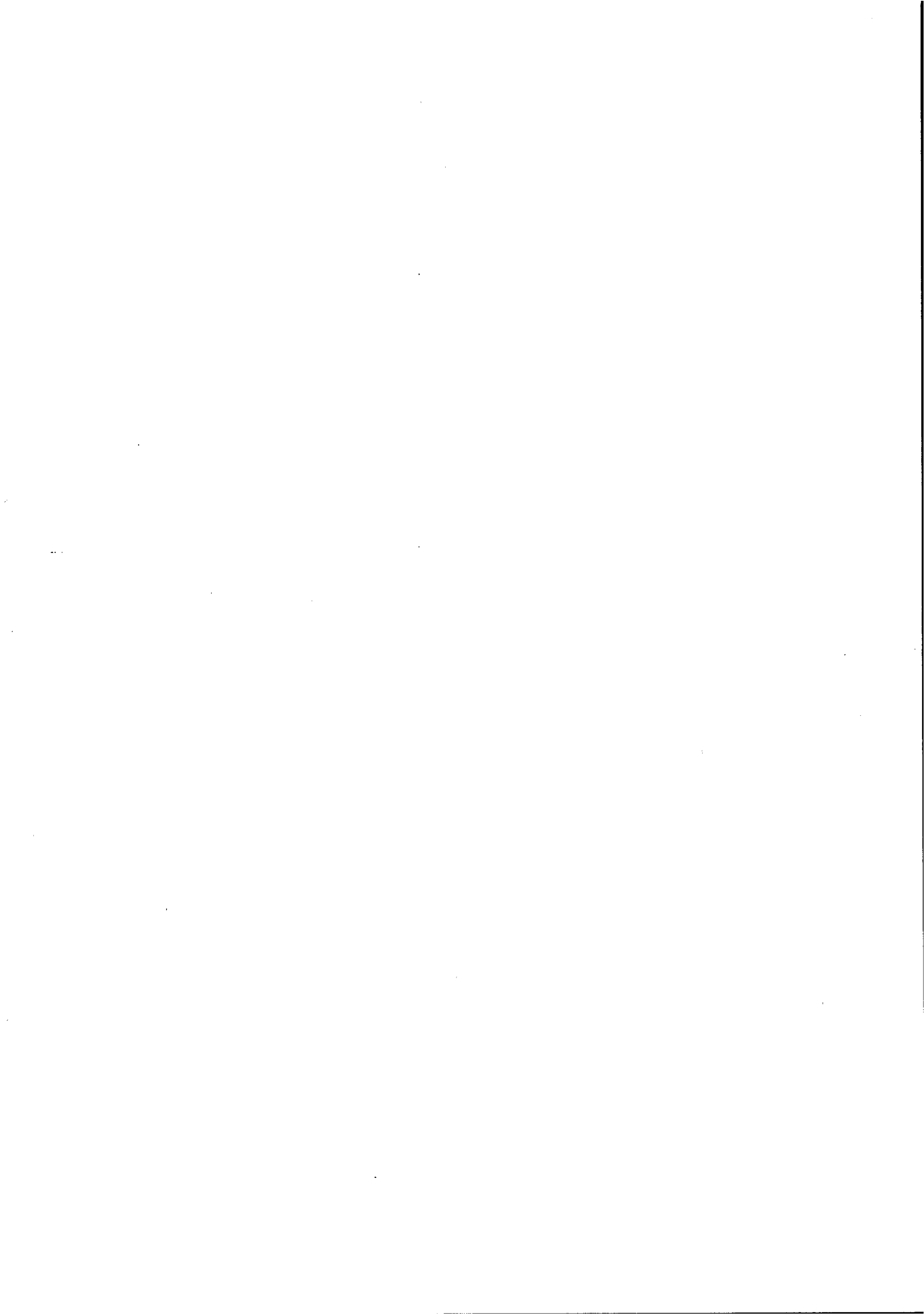
Les titres, mots-clés matières et résumés en Anglais sont indispensables pour les documents destinés à entrer dans les Bases AGRIS et ASFA (Aquatic Sciences and Fisheries Abstracts)



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# 1.5.3 Mounting Techniques

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## PRESERVATION

Tetranychidae removed from the leaves may be killed in 70% ethyl alcohol. They can be maintained for several years in this liquid, in small tubes about 45 mm long with a diameter of 10 mm which are hermetically sealed with three-winged polyethylene caps. This is also the simplest way of sending specimens to specialists for identification. Labels written in Indian ink are fixed onto the outside and placed inside each tube.

According to some authors, alcohol hardens the mites after a long period of preservation. Evans et al. (1961) recommend 70—80% industrial methyl alcohol with up to 5% glycerol.

Tuttle (cited in Jeppson et al., 1975) prefers the AGA solution (alcohol, glycerol and acetic acid), which is a modification of Oudemans' fluid and has the following composition:

8 parts 70% isopropyl or ethyl alcohol;
1 part glacial acetic acid;
1 part glycerol;
1 part sorbitol (or sugar) may be added to AGA.

## CLEARING

Tetranychidae kept in alcohol should be removed and dipped in a clearing agent, in order to examine morphological features under a microscope. Pure lactic acid coloured with lignin pink, as indicated by Evans and Browning (1955), seems the best agent. Lactic acid inflates the body and extends the legs, it attacks soft tissues while chitinized cuticles remain. Lignin pink, while keeping the bodies translucid, concentrates slightly in the mites and enables them to be clearly distinguished under the stereomicroscope.

In practice, the preliminary period in alcohol should last a minimum of 1 day (some authors recommend 2 weeks), to avoid quick disintegration of mites dipped in lactic acid. The contents of the small alcohol vial (2 $cm^3$) are poured into a thick glass cupel (4 $cm^3$) with a flat bottom. About 8—10 drops of stained lactic acid (cherry-coloured) are then added. The cupel is deposited on an electric slide warmer maintained at 40—60°C. Alcohol quickly evaporates and the clearing generally lasts 1—2 h. As bodies may burst, it is necessary to be most attentive and to monitor the operation with frequent controls under a stereomicroscope.

In certain cases, for very dark specimens, it may be useful to dip the mites for several minutes in André's fluid, which has the following composition:

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water, 30 $cm^3$;
chloral hydrate, 40 g;
glacial acetic acid, 30 $cm^3$.
This operation weakens the integument and should be carried out under a stereomicroscope.

## MOUNTING

### Temporary mounting

All specimens are best studied in temporary preparations according to the principle described by Grandjean (1949) and modified by Evans et al. (1961). One uses a cavity ground in glass slides of thickness 1.0—1.2 mm. The cavity is made with a small tungsten carbide grindstone mounted on a drill used by dental technicians and is shaped like a segment of a sphere with a diameter of about 3 mm. One may adjust the depth of the cavity to the size of the mite under study; for instance males need smaller cavities than the females of the same species.

A drop of coloured lactic acid is placed in the cavity, the mite is deposited and pushed into the liquid with a curl-tipped minutien pin fastened to a glass rod. A square cover glass (22 x 22 mm, extra thin), with a minute droplet in its centre is set on its edge and lowered slowly by means of a needle. If there is not enough lactic acid, a few more droplets should be added at the edges of the cover glass, if there is too much, it should be absorbed with a piece of filter paper. If the specimen is not clear enough, the slide may be heated gently on the electric warmer. The use of ordinary cover glasses would stop the objective of the microscope and would not allow focusing on every part of the mite.

Under the stereomicroscope, or even under a microscope at low magnification, the specimen may be orientated in any direction inside the cavity, by sliding the cover glass back and forth. The mite is not distorted and retains the appearance of a living tetranychid.

A series of such temporary mounts allows the examination of several characteristics for each specimen. With such large cover glasses, slides may be studied with caution under the oil-immersion objective. This technique is very useful for quick identification of samples, even with few specimens.

Temporary mounts sealed with Glyceel or Euparal may be transformed into semi-permanent preparations and kept for 2 or 3 years.

### Permanent mounting

None of the mounting media are fully satisfactory. Pritchard and Baker (1955) have attempted the use of different media and concluded that the best one seems to be Hoyer's solution, consisting of
distilled water, 50 $cm^3$;
gum arabic, 30 g;
chloral hydrate, 200 g;
glycerol, 16 $cm^3$.
The ingredients are mixed in the above sequence with a stirring rod. It is necessary to heat the liquid slowly at about 50°C to accelerate the dissolution of the gum arabic. Use of purified gum in fine powder form simplies the process and makes filtration superfluous.

Jeppson et al. (1975) proposed a reduction in the amount of distilled water to 40 $cm^3$. Females are orientated dorsoventrally or in profile, males

preferably are mounted in profile to see the aedeagus. It is best to put only one specimen in each slide.

Permanent mounting may be done with cavity slides or with simple slides. Mitchell and Cook (1952) also recommended the double cover-slip technique but the present author has never used it.

With cavity slides, one should use square cover glasses as for temporary mounting. The technique requires time to remove the air bubbles from the medium. Slides should be heated on the electric warmer at 50°C for at least 2 days. The slides are fragile but the specimens keep their live appearance and may be easily reprocessed if necessary (using water as solvent). They are suitable for ordinary microscopic examination only.

With simple slides, it is preferable to use small, round cover glasses (12 mm in diameter). Slides are easier to prepare and are dried in 1 day, but the specimens are squeezed, often crushed and deformed, and very difficult if not impossible to remount. They are suitable for ordinary and phase-contrast microscopes.

When dried, all permanent slides should be ringed with Glyceel or Euparal because the medium is hygroscopic and particularly unstable in a humid climate.

Two labels should be affixed to each slide. One should indicate the host plant, locality, date of collection and collector's name. The other should give the scientific name of the species, the sex of the specimen and its position (dorsal, ventral or profile), and the name of the scientist responsible for the determination.

Slides should be laid flat and stored in a dark dry place.

## REFERENCES

Evans, G.O. and Browning, E., 1955. Techniques for the preparation of mites for study. Ann. Mag. Nat. Hist., 8(12): 631—635.

Evans, G.O., Sheals, J.G. and Macfarlane, D., 1961. The Terrestrial Acari of the British Isles. An Introduction to their Morphology, Biology and Classification. Trustees of the British Museum, London, 219 pp.

Grandjean, F., 1949. Observation et conservation des très petits Arthropodes. Bull. Mus. Hist. Nat. Zool., Ser. 2, 21(3): 363—370.

Jeppson, L.R., Keifer, H.H. and Baker, E.W., 1975. Mites injurious to economic plants. University of California Press, Berkeley, CA, 614 pp.

Mitchell, R.D. and Cook, D.R., 1952. The preservation and mounting of water-mites. Turtox News, 30(9): pages not numbered.

Pritchard, A.E. and Baker, E.W., 1955. A revision of the spider mite family Tetranychidae. Mem. Pac. Coast Entomol. Soc., 2: 1—472.