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Original article

An artificial membrane for *in vitro* feeding of *Varroa jacobsoni* and *Acarapis woodi*, mite parasites of honey bees*

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Summary — A synthetic membrane through which *Varroa jacobsoni* Oudemans, a mite parasite of honey bees, can be fed was modified in such a manner that seams could be made virtually leak-proof and sacs designed to almost any configuration. These modifications thus eliminated problems encountered in previous feeding studies. In addition, another parasitic mite of honey bees, *Acarapis woodi* (Rennie), the tracheal mite, was also able to penetrate this membrane and ingest an artificial medium. Membrane modification and sac preparation are discussed in detail.

Varroa jacobsoni* / *Acarapis woodi* / rearing medium / feeding / *in vitro

INTRODUCTION

Researchers who wish to rear, *in vitro,* ectoparasitic blood-sucking arthropods are very often thwarted by lack of adequately developed rearing systems. A strategy often used is to feed ectoparasites through a membrane. However, suitable membranes have not always been found. Various materials have been used for different organisms with varying degrees of sucess and have included silk bolting cloth (Wharton and Cross, 1957), silicone sealer (Butler *et al,* 1984), plastic films such as Handiwrap (Bruce, 1989), microencapsulated plastics (Anon, 1971), Parafilm® (Bruce *et al,* 1988) and paraffin wax (Hagan and Tassan, 1965), as well as others (Crystal, 1986).

Each material has advantages and disadvantages depending on use and these will, in some respect, differ with each organism. Ideally, a material should be: (i) easy to handle; (ii) inexpensive; (iii) avail-

* This article reports the results of research and mention of a proprietary product and does not constitute an endorsement or a recommendation for its use by the USDA.

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able; (iv) strong, but penetrable; (v) adaptable to the desired configuration; and (vi) heat sealable.

In 1985 it was decided by the European Economic Community Research Committee on Varroatosis and the United States Department of Agriculture, Agricultural Research Service to determine whether *Varroa jacobsoni* Oudemans, a blood-sucking mite parasite of honey bees, could be reared *in vitro* on an artificial diet developed for another mite, *Pyemotes tritici* (Lagreze-Fossat and Montane) (Bruce, 1989). The rationale for such a project was that, if the mite could be reared *in vitro*, certain aspects of its behavior could be more easily studied and various strategies for control, based on toxicants or hormonally active chemicals added to the diet of the bee might be realized. Initially, several membranes were tested with no success. Subsequently, stretched Parafilm® was determined to be most satisfactory (Bruce *et al*, 1988). The results of this research, conducted during a 4-month period at the University of Udine (Udine, Italy) were quite successful; all feeding stages of the mite were able to penetrate the membrane and feed on an artificial diet. However, the major concern was an inability to seal the membrane without causing leaks and tears.

Since that time Italian researchers (personal communication; N Milani, Udine) have tested many other membranes, none of which proved more statisfactory than the stretched Parafilm®. With the discovery of *V jacobsoni* in the United States in September 1987, work began in late 1988 at the US Department of Agriculture, Beneficial Insects Laboratory, Beltsville, to continue the original effort to rear *V jacobsoni in vitro*. The present study was initiated to determine if the Parafilm® could be modified or improved such that its routine use would be assured for *V jacobsoni* as well as another blood-sucking ectoparasite of honey bees, *Acarapis woodi* (Rennie). Because *A woodi* imbibes very little hemolymph and does not exhibit physogastry, it was anticipated that a different kind of assay might have to be developed to demonstrate that penetration and feeding activity had taken place. Various assays are available. For example, *P tritici*, upon uptake of hemolymph, exhibits physogastry within 8–24 h, with the abdomen eventually swelling up tremendously (0.5–1.0 mm diameter) (Bruce, 1989). Diets can be dyed and, in a relatively large mite such as *V jacobsoni*, the gut (or feces) can be easily observed for color differences. However, to determine whether or not food uptake had occurred in a mite as small as *A woodi* (150 μm length), a mite that only takes up minute quantities of hemolymph, an indirect assay was used, which employed a microbial indicator.

MATERIALS AND METHODS

Source of honey bees

Brood combs from honey bee colonies known to be heavily infested with *V jacobsoni* were transported under quarantine from an apiary near Cocoa Beach, FL, to the ARS quarantine facility at the Bee Research Laboratory, Beltsville, MD. Combs were kept in an incubator at 27 ± 1 °C, 60% RH.

Honey bees were removed from colonies infested with *A woodi*, maintained at the Bee Research Laboratory, Beltsville. Approximately 50 bees were kept in the laboratory on a diet of water and sugar syrup.

Preparation of parafilm membranes

Membranes were prepared using rolls (10 cm wide) of Parafilm® (American Can Co, Neemah,

WI). A 5-cm strip (5 x 1 cm) was cut and laid on 2 glass microscope slides positioned at the ends of the film. Two additional microscope slides were placed over the bottom slides forming a "sandwich" with the Parafilm® (fig 1A). With one set of slides in each hand, the film was stretched slowly until the tension stopped the pull, at a Parafilm® length of ≈ 30 cm. Next, the film between the slides was cut from one end and removed from the strip. A glass slide was then positioned perpendicular to the strip and the previously cut end of film was folded over this slide 1–1/2 x the width of the film and cut (fig 1B). The slide could now be used as a holder for the Parafilm® to facilitate the heat-sealing of the edges. An Accu-Seal Corporation (San Diego, CA) bar-type heat sealer (Model 10-121), at a timer setting of ≈ 1 (1.0 s), was used to seal 2 of the 3 open sides (fig 1C). Next, a wax paper strip was inserted between the glass slide and the Parafilm® sac. Glass slides were again placed at the ends of the sac and the film stretched in the unstretched direction as before (fig 1D). The glass slides at the open end were removed, a pipette was inserted in front of the wax paper strip, and a quantity of medium introduced into the sac. Prior to the placement of the medium, the sac could be further expanded in all directions, by the careful introduction of a small quantity of compressed gas (EFA-duster, EF Fullam Inc, Schenectady, NY). The remaining open side of the sac was then heat sealed. Care should be taken not to introduce air bubbles into the medium or to spill medium along the sides of the sac which may result in an inadequate heat-seal being made.

Diet medium and feeding of mites

The medium used was modified insect tissue culture medium, Medium M1D (Whitcomb, 1983). For feeding trials, the medium contained a microbe (bacterium *Spiroplasma melliferum* Clark *et al*) which, if imbibed by the mites, could be reisolated from the mites by crushing them into sterile M1D medium (without bacteria). If the mites had in fact imbibed the bacteria, the diet would change color in 2–3 days as a result of a change in pH as nutrients were depleted by the bacteria.

Adult female *V jacobsoni* were removed from the brood cells as needed and were kept in Nitex stoppered glass tubes. The tubes were placed in desiccators over a saturated salt solution of 85% RH in an incubator at 27 ± 1 °C for 2–4 h. Six to 8 mites were then placed on a M1D-filled sac in a closed Petri dish for 3 days.

Adult female *A woodi* were removed from honey bee tracheae in a manner described previously (Smith, 1987) and placed directly onto an M1D-filled sac and placed in a closed Petri dish for 3 days.

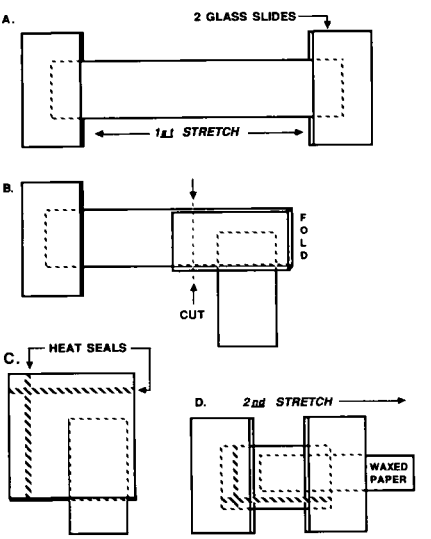

Fig 1.A. A 5 x 10 cm strip of Parafilm® is placed between glass microscope slides and pulled or stretched until tension stops the pull or to ≈ 30 cm. **B.** One end is released from the slides and folded over a glass slide and cut with scissors. **C.** 2 of the 3 sides are heat-sealed. **D.** The glass slide is removed and a length of waxed paper inserted into the sac. The sac is placed between glass slides and stretched in the previously unstretched direction. The sac can now be filled with medium or stretched further "balloon" fashion with compressed gas, filled with medium and then heat sealed.

RESULTS AND DISCUSSION

Microscopic examination of the stretched Parafilm® to determine thickness revealed that a thickness of ≈ 10 μm could be obtained routinely. While the gnathosomas of these mites differ considerably, the effective length of the mouthparts necessary for penetration is about the same, or 15 μm.

Although the number of mites of each species tested was small (5 each of 2 species), all tested positive for the microbial indicator upon microscopic examination (Zeiss, dark field microscopy 1250x), of the squashed mite preparations, and upon inoculation of these preparations into sterile M1D medium. A more complete and detailed discussion of the laboratory use and some possible biological implications of the microbe in both natural populations of honeybees and mites is given elsewhere (Bruce *et al,* 1991). These results clearly demonstrate 2 important points: 1), artificial diets can now be contained within a membrane that can be made suitably thin, in almost any configuration, and with heat-sealed seams that are extremely strong and virtually leak-proof; and 2), *A woodi* can penetrate a synthetic membrane and feed on an artificial medium. In addition, it is quite probable that this modified membrane could be used for the *in vitro* feeding of other arthropods as well (*eg Ornithonyssus sylviarum* (Canestrini and Fanzago), northern fowl mite; J Carroll, personal communication, USDA, Beltsville, July 1990). At this point little can be said about the nutritional requirements of either *V jacobsoni* or *A woodi*, but one fact is clear: a membrane and mechanism are now available to test various artificial diets and to develop strategies for the eventual control of these mite parasites of honey bees.

Résumé — Une membrane artificielle pour le nourrissement *in vitro* de *Varroa jacobsoni* et *Acarapis woodi*, acariens parasites de l'abeille. Une membrane synthétique, au-travers de laquelle l'acarien parasite des abeilles *Varroa jacobsoni* peut être nourri, a servi à fabriquer des sacs de différentes formes, et totalement étanches. Ces membranes ont été réalisées en utilisant du Parafilm®. Une bande de 5 cm a été coupée et déposée sur 2 lames de verre pour microscope, chacune à l'une des 2 extrémités du film. Deux autres lames identiques ont été déposées sur les 2 précédentes, et avec les 2 lames dans chaque main, le film a été étiré lentement jusqu'au maximum. Le film situé entre les lames a alors été coupé à une extrémité et enlevé de la bande. Une lame de verre a été ensuite positionnée perpendiculairement à la bande, et l'extrémité préalablement coupée du film a été pliée sur cette lame, d'une fois et demie la largeur du film, et coupée (fig 1B). Un appareil chauffant, pour sceller les sacs, a été utilisé pour fermer 2 des 3 côtés ouverts (fig 1C). Une bande de papier ciré a été déposée dans le sac et le film a été étiré, à nouveau en utilisant des lames de verre, dans la direction non encore étirée (fig 1D). De plus, le sac a été davantage étiré grâce à de l'air comprimé. Après introduction de milieu nutritif, le dernier côté ouvert du sac a été scellé par la chaleur.

Varroa jacobsoni* / *Acarapis woodi* / milieu d'élevage / alimentation / *in vitro

Zusammenfassung — Eine künstliche Membran zur experimentellen Fütterung der parasitischen Bienenmilben *Varroa jacobsoni* und *Acarapis woodi*. Eine synthetische Membran, durch welche die

parasitische Milbe *Varroa jacobsoni* Oudemans ernährt werden kann, wurde derart verändert, daß wasserdichte Nähte angelegt und Säcke in fast jeder Form gebildet werden konnten. Als Membran wurde Parafilm® benutzt. Es wurden Streifen von 5 cm Länge geschnitten und mit ihren Enden über zwei Mikroskop-Objektträger gelegt. Dann legte man zwei weitere Objektträger über die unteren (mit der Membran dazwischen), faßte jedes Paar der Glaspatten mit einer Hand und dehnte den dazwischengespannten Film so weit aus als möglich (Abb 1A). Der Film zwischen den Objektträgen wurde dann an einem Ende abgeschnitten und von dem Streifen abgetrennt. Als nächster Schritt wurde ein Objektträger rechtwinklig über den Streifen gelegt und das vorher abgeschnittene Ende des Films 11/2 mal der Filmbreite um diesen Objektträger gefaltet und abgeschnitten (Abb 1B). Ein stabförmiges Versiegelungsgerät wurde zur Versiegelung von zwei der drei offenen Seiten benutzt. Dann wurde ein Streifen eines Wachspapiers in die Tasche geschoben und der Film wieder mit Hilfe von Objektträgen ausgedehnt, diesmal in der anderen Richtung (Abb 1D). Diese Tasche kann jetzt zusätzlich durch Einblasen von Preßluft weiter gedehnt werden. Nach Einfüllen des nötigen Quantums der Nährlösung wird die verbliebene offene Seite der Tasche hitze-versiegelt. Die mikroskopische Untersuchung des gedehnten Parafilms ergab eine durchschnittliche Dicke von 10 µ. Das ist ausreichend dünn, um von Mundwerkzeugen der Milben durchstochen zu werden. Als Nährflüssigkeit wurde des Medium M1D benutzt, das als Kulturmedium für Insektengewebe verwendet wird. Zum Nachweis der Aufnahme der Nährflüssigkeit durch die Milben durch die Membran hindurch wurde diese vorher mit Bakterien versetzt. Nach drei Tagen Fütterung konnten diese Bakterien in den Milben nachgewiesen werden. Dieser Test verlief sowohl bei *Varroa jacobsoni* wie bei *Acarapis woodi* positiv.

Varroa jacobsoni* / *Acarapis woodi* / Zuchtmedium / Fütterung / *in vitro

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