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Rhizoglyphus robini Claparède (Acari, Acaridae)

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The cuticle of *Rhizoglyphus robini* Claparède is about 1.6 μm thick in the adult stage and has a lamellated procuticle and a thin, complex epicuticle. Pore canals pass through the cuticle from the epidermis. Muscles are attached directly to the cuticle or are secured by a complex system of extracellular fibers and septate junctions. The myo-integumental attachment sites lack the oriented microtubules that exist in myo-cuticular junctions in insects. The skeletal muscles of *R. robini* have Z, I, and A bands, but lack the H and M bands that are found in other arthropods. The opisthonotal glands consist of a lipid-filled sac underlain by several specialized cells which differ from the epidermal cells beneath the cuticle.

The digestive system has a basic acaridid form that is characterized by a well developed ventriculus, a pair of caeca, a colon and rectum, and a pair of Malpighian tubules. The male

reproductive system is characterized by a pair of testes and a large accessory gland while the female system consists of a pair of ovaries, receptaculum seminalis, and accessory glands.

The central nervous system is comprised of a supra- and sub-oesophageal ganglia from which nerve trunks emerge to supply the mouth parts, legs, digestive and reproductive systems. The peripheral nervous system consists of mechanoreceptors and chemoreceptors. The solenidion and famulus are chemoreceptors which have a thin cuticular wall with many pores and are innervated by six and four neurons respectively. The mechanoreceptors are innervated by one bipolar neuron.

The alarm pheromone of *R. robini* has been identified by means of gas-liquid chromatography and mass spectrometry as citral. This monoterpene is a mixture of two aldehydes, neral and geranial. The compound is almost identical to an alarm pheromone produced by other acaridid mites. When 100 ppm of commercial citral was added to the normal Kelthane treatment for bulb mite control in greenhouse lily plants, it resulted in significant reduction of the mite population when compared to the Kelthane treatment alone.

R. robini is capable of ingesting fungal material and passing it through the digestive tract in a viable state. Temperature and food substrate were important factors in the termination of the hypopodial stage, while relative humidity had no significant effect. Temperature also influenced the rate of post-embryonic development in *R. robini*.

Observations on the Morphology and Biology of *Rhizoglyphus robini* Claparede (Acari, Acaridae)

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Observations on the Morphology and Biology of *Rhizoglyphus robini* Claparede (Acari, Acaridae)

General Introduction

Mites are among the smallest and least studied of the arthropods. Many are predaceous on other small invertebrates in a diversity of ecological niches. Others may be phytophagous and cause considerable damage to crops and stored food products while others are animal parasites capable of transmitting a variety of pathogens.

Rhizoglyphus spp. are members of the family Acaridae, a genus with a world-wide distribution. Its members often are associated with plants or plant products (Manson 1972), commonly occurring with various types of bulbs in the field, in the greenhouse, and in storage. Little information exists on life histories of *Rhizoglyphus* spp. except for the brief reports by Hodson (1928) and Garman (1937) on *R. echinopus* Fumouze and Robin. The taxonomy of the genus has been clarified by Eyndhoven (1961, 1968) and Manson (1972, 1977), but there is a paucity of information on morphology and behaviour. The problem with the lily bulb mite, *R. robini* Claparede, in the lily growing areas of southwestern Oregon and north-western California prompted an in-depth study of this species in order to better understand its role in the bulb substrate and to more effectively deal with its management.

The major objectives of this thesis are: 1)to clarify some of the morphological aspects of *R. robini*, with emphasis on the internal morphology and sensory structures, and 2)to investigate the biology and behaviour of *R. robini*, paying special attention to the demonstration and identification of a pheromonal substance produced in response to disturbance.

CHAPTER I

Morphology of *Rhizoglyphus robini*

Introduction

This chapter describes the internal anatomy of the various body systems and the fine structure of the cuticle, muscle, sensory receptors and opisthonotal gland of *Rhizoglyphus robini*. Most of our knowledge of internal organs of acaridid mites stems from the works of Nalepa (1884, 1885) and Michael (1901).

The acaridid digestive system consists of a pharynx, oesophagus, ventriculus, two or more caeca, colon, rectum, and Malpighian tubules. Variations in the digestive system involve the number of caeca associated with the midgut, the number of Malpighian tubules and the morphometrics of various parts of the system (Rohde and Oemick 1967; Boczek et al 1969; Kuo and Nesbitt 1970; Baker 1975). The only comparative ontogenetic study on the anatomy of the digestive system of an acaridid mite was published by Hughes and Hughes (1939) on *Glycyphagus domesticus* (DeGeer).

Studies on the female and male reproductive systems of acaridid mites have revealed a basic structural plan for members of the suborder. The female system consists of paired ovaries and oviducts, accessory glands, oviporus, receptaculum seminalis, and terminal bursa copulatrix. The male reproductive system is composed of a pair of testes, vasa deferentia, sperm sac, ductus ejaculatoris, accessory gland, and penis.

The nervous system of all mites studied to date consists of an unsegmented central nervous mass from which nerves run to the various parts of the body. The nervous mass is pierced by the oesophagus which separates it into the supra- and sub-oesophageal ganglia, which are surrounded by a thin, uniform layer of connective tissue. Beneath the connective tissue layer are the cortical and neuropilar regions of the nervous mass. The central nervous mass of acaridid mites that have been studied thus far illustrate this basic design (Hughes and Hughes 1939; Kuo and Nesbitt 1970; Vijayambika and John 1975).

Mites, spiders, insects and other arthropods have an exoskeleton comprised of cuticular layers that are produced by a layer of epidermal cells. The cuticle of mites plays an important role in their physiology, since cuticular structure is associated with the state of nutrition, stage of moulting and with waterproofing or absorption of water from the atmosphere.

Arthropod cuticle consists of two main regions: the inner procuticle and the outer epicuticle (Locke 1964, 1967). Histochemical studies have demonstrated that only the procuticle contains chitin. In addition, the procuticle has a fibrillar aspect that is not seen in the epicuticle. The epicuticle usually has an outer wax and lipid layer overlying the cement layer and cuticulin layers.

All three layers exist in *Laelaps echinidus* Berlese (Wharton et al. 1968) but the cement layer is absent in the rabbit tick, *Haemaphysalis leporispalustrus* (Packard) (Nathanson 1967).

A series of fine channels or pore canals extend from the underlying epidermal layer through the procuticle to the epicuticular surface. These canals provide a pathway for waxes and lipids to reach the surface and also are involved in water uptake in ticks and insects (Locke 1967; Wharton et al. 1968). Branching pore canals occur in the cuticle of spiders (Barth 1970), insects (Locke 1967), and mites (Wharton et al. 1968; Brody 1969). They are absent in the cuticle of the mites *Acarus siro* L. (Hughes 1959) and *Tetranychus urticae* Koch (Gibbs and Morrison 1959).

Arthropod muscle fibers may be of the skeletal, visceral or cardiac type. These muscles perform tasks that are analagous to those carried out by muscles of vertebrates, but there are notable differences in structure and physiology. Arthropod skeletal muscles are inserted directly on the exoskeleton or on invaginated apodemes and move articulated cuticular plates. The visceral muscles of the gut and other organs are always striated, whereas the visceral muscles of vertebrates are unstriated. Both arthropod and vertebrate heart muscles are striated, but the muscle cells of the arthropod heart are arranged to form a simple dorsal tube. While there have been many studies on the fine structure of muscles in insects (Smith 1962, 1965, 1966), the fine structure of muscle tissue of acaridid mites or of mites in general has received little attention. The gross internal musculature of trombidiid mites is well known, based on the works of Mitchell (1962) and Mathur and Le Reoux (1965). Kuo and Nesbitt (1970) published the only complete description of the gross internal musculature of an acaridid mite.

Acarines generally posses a complex of external sensory receptors and gland openings. Cuticular sensory setae are located on the palpi and legs and are involved in the perception of a wide spectrum of chemical and mechanical stimuli. The sensory receptors on the tarsi of legs I and the palpi of _Macrocheles muscaedomesticae_ (Scop.), for example, respond to olfactory stimuli from adult house flies (Jalil and Rodriquez 1970). The sensory receptors on the first pair of legs on ticks respond to odors from their hosts and are also involved in the perception of pheromones (Foelix and Axtell 1971; Foelix 1972). Morrison (1973) described the sensory receptors on the two-spotted spider mite, _Tetranychus urticae_ and the possible role these receptors play in host-plant location. Setal sensory receptors are well developed in _R. robini_.

The paired "oil glands" or opisthonotal glands are located on the dorso-lateral surface of many acariform mites, including _R. robini_. Many functions have been attributed to the opisthonotal glands. Furstenburg (1861) and Pagenstencher (1862) considered them to be the stigmata of the respiratory system. Claparède (1868) wrote that the glands were part of the excretory system. Nalepa (1884, 1885) noticed an oily substance in the glands and suggested the oil was secreted to prevent the mites from sticking to objects. In a series of experiments, Kuwahara (1976) demonstrated that the opisthonatal gland was the site of alarm pheromone production in acaridid mites. It was this important work that provided the background for the alarm pheromone studies reported later in this work.

Materials and Methods

R. robini was maintained on an artificial medium (Bot and Meyer 1967) in petri dishes that were placed in a culture chamber at 24°C. For light microscopy, mites were fixed in 10% acrolein in sodium cacodylate buffer pH 7.2 for 24h at 4°C. The specimens were dehydrated in ethanol and embedded in glycol methacrylate. A modified rotary microtome with a glass knife holder was used to cut 2-3 m sections that were affixed to glass slides. Aqueous toluidine blue O, and haematoxylin and eosin were employed for staining the sections. Photomicrographs were recorded on Panatomic X film using a Zeiss photomicroscope.

Several fixatives were tried for transmission electron microscopy; the most useful one consisted of 2.5% glutaraldehyde 2 % paraformaldehyde, and 1% acrolein in 0.1M sodium cacodylate buffer pH 7.2, at 4°C for 24h. The specimens were washed three times in buffer (15 min for each change) and then post fixed in 2% OsO_4 in sodium cacodylate buffer pH 7.2 for 1 h at room temperature.

Dehydration of the specimens was carried out in a graded series of acetone or in acidified 2-2-dimethoxypropane, (2 changes, 7 min each). There was no observable difference between the two dehydration schedules except that the second required considerably less time. All of the specimens were embedded in Spurr's low viscosity embedding medium and the resin was polymerized at 70°C for 18 h.

Sections were cut with a diamond knife on a Sorval MT-2 ultramicrotome. Silver and gold sections on grids were placed in saturated uranyl acetate for 10 min and lead citrate for 5 min (Reynolds 1963). Sections were examined with a Philips 201 or 300 electron microscope at 60 kV and the images were recorded on Du Pont Ortho S Litho film.

The fixation procedure used for the TEM work also was employed for the scanning electron microscopy work. Specimens were dehydrated in ethanol and then critical point dried. The mites were attached to aluminum stubs with silver paint and then coated with carbon and gold. An AMR 1000 scanning electron microscope was used to record the images on Polaroid P/52 or P/N 55 film.

Results and Discussion

Cuticle

As seen in a whole mount, the cuticular covering of R. robini is a pale yellowish colour while the appendages are dark brown due to the heavy sclerotization. The integument is relatively smooth with leg and body chaetotaxy typical for the free-living Acaridae (Hughes 1976). There are no discernable separations between the dorsum and venter, but a sejugal suture divides the idiosoma into an anterior and posterior region. Light microscope sections demonstrate that the integument of R. robini is made up of cuticle and a layer of epidermal cell but the various component strata that comprise the cuticle can not be distinguished without the transmission electron microscope.

The epidermis of R. robini is a single layer of columnar cells lying beneath the cuticle; a basement membrane separates the epidermal cells from underlying body tissues (Fig. 1). Each epidermal cell has a large nucleus, many oval shaped mitochondria, and rough endoplasmic reticulum and ribosomes. The epidermal cells secrete the cuticle and the enzymes which digest the cuticle during ecdysis. These cells also are responsible for repairing wounds in the cuticle. The presence of a large nucleus and of many cytoplasmic ribosomes in the epidermal cells indicates that they synthesize large amounts of protein, a major component of cuticle.

The inner portion of the procuticle lies on the plasma membrane of the epidermal cells. I have shown that the procuticle of R. robini, like that of Acarus siro (Hughes 1969), cannot be divided into an endo-and exo-cuticle as in insects (Figs. 1, 2). It is composed instead of a series of laminated filaments, each of which is 350-450 A in diameter. The lamellae are constructed of layers of chitin-protein microfibrils that are situated parallel to the surface of the cuticle (Fig. 2). The layers of microfibrils are oriented slightly through a total of 180°.

The various stases of R. robini, except for the hypopus, have a soft cuticle that expands during feeding and, in the female, during egg maturation. Differences in the thickness of the cuticle occur in various regions of the body. These differences are due to varying thicknesses of the procuticle. The thickness of the epicuticle remains constant over the entire body surface.

The epicuticle consists of an outermost wax and lipid layer, an underlying cement layer and an innermost cuticulin layer. The wax and lipid layer is 300-350 A° thick and is composed of a fine granular material. At times this layer is not seen because dehydrants used in processing the materials dissolve it. The cement and cuticulin layers of R. robini resemble those of other mites and insects. The epicuticle has been shown to play an important role in water and, regulation to a lesser extent, in temperature regulation (Devine and Wharton 1973).

Pore canals in the cuticle of R. robini are not distinguishable with the light microscope, but they may easily be seen with the aid of the transmission electron microscope. The pore canals appear to be twisted in the procuticle and straight in the epicuticle. The canals originate at the interface with the epidermal cells and extend through the cuticle as far as the cement layer (Fig. 1).

Pore canals have been observed in the cuticle of many other mites (Nathanson 1967; Wharton et al. 1968) but they are absent in *Acarus siro* and in *Tetranychus urticae*.

A variety of functions have been attributed to the pore canals of arthropods (Locke 1961). According to Locke (1964), they may be involved in the transport of one or more epicuticular components through the procuticle, such as phenolic substances and wax precursors for the deposition and repair of the wax layer. Lees (1947) and Winston (1969) felt that pore canals may be involved in water loss and uptake. Hughes (1959) considered that respiration may take place by diffusion of gases through the cuticle by way of the pore canals.

Muscle and Muscle-Cuticle Attachment

Muscle fibers in *R. robini* are ovoid in transverse section, and a plasma membrane surrounds each cell (Fig. 3). The tightly packed microfibrils almost fill the cell and run the length of the fiber. The nucleus and mitochondria are located along the periphery of the fiber between the muscle fibrils and sarcolemma.

Longitudinal sections of muscle fibers show the striations of the various bands that occur in this tissue. The Z lines, A bands and I bands are clearly discernible (Fig. 3). The A band is composed of thick filaments while the I band is made up of thin filaments. I have discovered that the H and M bands which are found in other arthropods and vertebrates do not occur in *R. robini*.

In cross section the thick filaments of the A band are 200 A in diameter and the thin filaments are 60 A. A cross section view at the periphery of the A band shows that, each thick filament is surrounded by 12 thinner filaments. At high magnification cross bridges may be seen between the thick and thin filaments. Muscular organization in *R. robini* indicates that the muscles contract by means of the thick and thin filaments sliding past each other, conforming with the sliding filomenthypothesis proposed by Huxley (1965) for vertebrate muscle contraction. The occurrence of cross bridges also appear in vertebrate muscle. In vertebrates these bridges break down and reform during the contraction. I found that the muscles of *R. robini* differ from the striated muscle in other arthropods and vertebrates in sarcomere banding, length and substructure. The A band is 4 m long, which is 2 to 3 times as long as in vertebrates. The A band appears to be of uniform density throughout its entire length. The occurrence of 12 thin filaments around a thick filament also occurs in slow acting muscle of other invertebrates (Hagopian 1966; Anderson and Ellis 1967).

Muscles of *R. robini* may either be inserted directly into the cuticle (Fig. 5). or they may be attached to apodemes. At the point of the muscle-integumental junction, the muscles are attached to the adjacent epidermal cells by desmosomes, which are connected to the cuticle by extracellular fibrils. The epidermal cells in the muscle-cuticle junction are deeply invaginated and may extend as deep as 0.5 m into the muscle bundle (Figs.6, 6a). The extra cellular fibrils extend from the septate junction through the invagination to the cuticle.

I have discovered that, while microtubules exist in the muscle-integumental region in other arthropods, they are lacking in *R. robini*. Lai Fook (1967) showed that epidermal cells in insects have numerous oriented microtubules that extend from the muscle-integumental junction on the inner surface to the outer surface adjacent to the cuticle. The microtubules are attached to hemidesomosomes that in turn are attached to the cuticle by extracellular fibrils. The same type of muscle-cuticle attachment also is found in spiders (Smith et al. 1969). The lack of microtubules in the muscle-cuticle attachment sites in *R. robini* is a unique morphological feature.

Central Nervous Mass

The ganglia of *R. robini* are fused into a central nervous mass that is pierced by the oesophagus which marks the boundary between the supra- and a sub-oesophageal ganglia (Fig. 17). Two nerves proceed from the dorso-anterior portion of the supraoesophageal ganglion to the chelicerae and, from the lateral area, two more nerves run to the palpi. Four pairs of nerves extend from the lateral portion of the elongate suboesophageal ganglion to the legs (Fig. 9). A single nerve extends from the posterior portion of the suboesophageal ganglion to the genital region, and another runs from the dorso-posterior area of the ganglion to the digestive tract.

The transmission electron microscope reveals the structural complexity of the central nervous mass of R. robini. The entire mass is surrounded by a thin extracellular sheath of uniform thickness that is composed of collagen fibers. A complex layer of glial cells lies beneath this sheath. The cells bordering the extracellular sheath have irregular shapes which create many intercellular spaces. The inner cells of the perineurium, on the other hand, are elongate and regularly shaped cells that have few intercellular spaces between them. The perineural cells often appear vacuolated and contain mitochondria and rough endoplasmic reticulum.

The glial cells of the endoneurium are situated between the neuronal cell bodies and the periphery of the neuropile (Fig. 11). Some glial cells investing the neurons extend into the invagination on the perikarya surface. These cells contain many mitochondria, microtubules, smooth endoplasmic reticulum and glycogen deposits (Fig. 8).

I have found that the cortical region in the nervous mass of R. robini contains three types of neurons. Type I neurons have a large nucleus and the cytoplasmic area around the nucleus is reduced. Mitochondria, rough endoplasmic reticulum and a Golgi apparatus occur in the perikarya of type I neurons. Type II neurons are neurosecretory cells with distinctively large cell bodies, and a large cytoplasmic area around the nucleus. Type II cells contain many membrane-bound neurosecretory vesicles that are closely associated with the cisternae of the Golgi apparatus. Type III neurons are similar to the type I neurons, but they contain fewer mitochondria and less rough endoplasmic reticulum and ribosomes. They are the most common of the three neuron types. The cell bodies of the neurons are incompletely sheathed by the glial cells, and contact between the cell neuron bodies and nerve fibers occurs in the central nervous mass.

The neuropile region (Fig. 8) consists of glial cells and nerve fibers. The glial cells generally extend from the cortex into the neuropile region although some glial cells originate in the neuropile. The nerve fibers make contact with each other in various ways. They may cross each other at right angles, they may run parallel and make a longitudinal contact, or the ends of fibers may be so arranged as to come in contact with one another. Some of the nerve fibers in the neuropile contain neurosecretory granules (Fig. 10).

The central nervous mass of *R. robini* differs in organization from that of insect ganglia in several ways. Insects have a more complex neurolamella than the thin sheath of collagen fibers found in *R. robini*, and there is more glial ensheathment of the perikarya in insects (Maddrell and Treherne 1967).

Female Reproductive System

The female reproductive system of R. robini consists of a bursa copulatrix, receptaculum seminalis, paired ovaries and oviducts, oviporus and a pair of accessory glands (Fig. 22). The bursa copulatrix is situated on the posterior margin of the opisthosoma, behind the anal opening (Fig. 12). A short duct runs dorsally to the receptaculum seminalis, a sperm storage organ which illustrates its ectodermal origin in having a cuticular lining. The receptaculum seminalis occupies a large portion of the female opisthosoma, and is connected to the ovarial region by a short pair of ducts.

The ovary wall consists of the peritoneum and tunica propria. The tunica propria projects into the ovarial lumen and forms a stalk from which primary oogonia bud. The mature oogonia are arranged in rows on the periphery, while the maturing oogonia are located closer to the stalk (Fig. 13). The ovarial stalk eventually ruptures and releases the mature oogonia into the lumen of the ovary.

The ova are fertilized by sperm from the receptaculum seminalis, and the fertilized ova pass into the oviduct. The epithelium of the oviduct may be involved in secreting material for yolk deposition. I have observed that the eggs further down the oviducts in the sections have more yolk granules. The distal portion of the oviduct secretes the chorion of the egg. The eggs then pass to the oviporus which consists of two pairs of cuticular valves (Figs. 15, 16, 17). The labia are thin cuticular folds which extend from the oviporal valves. The labial folds are not well sclerotized which enables them to expand as the eggs pass out the oviporus (Figs. 17, 18). There are several pairs of muscles associated with the oviporus region and these muscles probably aid in the extrusion of the eggs. Two pairs of genital acetabula are associated with the oviporus region (Fig. 17); the function of these structures is unclear. Vercammen-Grandjean (1975) considered the genital acetabula in oribatid and actinedid mites to be involved in respiration, while Alberti (1980) showed that the acetabula are involved in osmotic regulation in water mites.

A pair of spherical accessory glands are located in the dorso-posterior portion of the opisthosoma. They lie against the body wall and are surrounded by a thin membrane (Fig. 14). The glands are divided into many sections and a nucleus is located in the base of each cell. The cytoplasm of the cells is strongly basophilic. The ducts from the accessory glands open into the system just above the opening of the oviducts.

Male Reproductive System

The male reproductive system of *R. robini* consists of a pair of testes, vasa deferentia, an ejaculatory duct, a penis, and an accessory gland that is also called the "chamber organ" (Fig. 22). The testes lie on either side of the rectum and occupy the posterior portion of the opiosthosoma. The testes are bound by a thin membrane and in the central portion, each contains many small cells with deep staining, small nuclei (Figs. 19, 20). The nuclei are larger at the periphery of the testes than at the center. These spermatocytes divide, forming smaller, tear-drop shaped mature sperm.

A vas deferens arises from the anterior portion of each testes and extends anteriorly for a short distance, the vasa deferentia join together at the level of legs IV, forming a common duct. The duct runs anteriorly and unites with the duct from the acessory gland, forming the ductus ejaculatoris. The walls of the vasa deferentia are composed of flattened cells with dark staining nuclei. The male accessory gland is located in the lateral dorsal area of the opisthosoma (Fig. 21). From laboratory observations of mating _R. robini_, I have observed that males secrete a fluid in which the sperm are found. This fluid may be a product of the accessory gland.

The ductus ejaculatoris terminates at the base of the penis which is a hollow, double-walled, cone-shaped chitinous structure (Figs. 22, 23). Several pairs of muscles associated with the male genital area are responsible for the extension and retraction of the penis. A pair of copulatory suckers are located on the opisthogaster on either side of the anal opening (Figs 22, 25). These suckers and the modified setal suckers on tarsi IV (Fig. 26) enable the male to hold the female during copulation.

Digestive System

The digestive system consists of a pharynx, oesophagous, ventriculus, a pair of lateral caeca, a pair of Malpighian tubules, colon and rectum. The pharynx is a short, invaginated ectodermal tube lined with a thin sheet of cuticle. Several groups of muscles are attached to the roof of the pharynx and insert on the labrum. These muscles act as a pharyngeal pump that pushes the food posteriorly into the oesophagus and ventriculus.

There is a valve at the junction of the pharynx and oesophagus that prevents the re-entry of food into the pharynx. A similar type of valve occurs in Glycyphagus domesticus (Hughes and Hughes 1939) and in Caloglyphus mycophagus (Kuo and Nesbitt 1970).

The oesophagus, like the pharynx is lined with a cuticular sheath and passes through the central nervous mass. As the oesophagous leaves the central nervous mass, it begins to widen until it reaches the ventriculus.

The ventriculus of R. robini is an oval-shaped pouch about 250 μ m long and 100 μm wide. I found that two types of cells which form the lining of the stomach (Fig. 27). The squamous cells have a dark staining nucleus and a basophilic cytoplasm. The cuboidal cells are heavily vacuolated and project into the lumen of the stomach. The cytoplasm is faintly basophilic and contains many crystalline granules. The cell nucleus is located in the base of the cell. The vacuolated portion of the cuboidal cells buds off during feeding, and maybe found in the lumen of the stomach (Fig. 27).

The caeca arise from the lateral walls of the ventriculus and are approximately 275 μm long and 75 μm wide. The caecal lining consists of a single layer of cells of the three cell types. Two types, i.e. squamous and cuboidal, are similar to those found in the ventriculus (Fig. 28). The third type of cell is globular, with the cytoplasm and nucleus confined to the basal portion of the cell, and the globular portion projecting into the caecal lumen. The globular portion contains one large membrane-bound vacuole that extends into the caecal lumen (Figs. 28, 29). Baker (1975) showed that the cuboidal and globular cells in Histiogaster carpio (Kramer) contain alkaline and acid phosphatase, and that these enzymes are involved in the breakdown of ingested food particles.

The colon of R. robini averages 75 μm long and 40 μm wide and extends from the posterior of the ventriculus to the rectum. The colon lining consists of tall simple columnar and simple squamous cells. The nuclei of these cells are located in the basal portion; the cytoplasm is strongly basophilic. The columnar and squamous cells have a brush border which increases the surface area. Hughes (1959) and Baker (1975) considered the colon of H. carpio to be the location of water absorption. The rectum is an oval shaped entity which is about 120 μm long and 40 μm wide in the adults of R. robini. The columnar cells of the rectum are more conical than those found in the colon (Fig. 30). The nucleus of each cell is located basally and the cytoplasm is basophilic as is the case with the columnar cells in the colon. The squamous cells are similar to but the brush border of the rectal cells is more dense than that of the cells in the colon (Figs. 30, 31). The rectum terminates at the anal opening on the posteroventral surface of the opisthosoma.

R. robini has a pair of Malpighian tubules that arise from the dorsolateral surface of the ventriculus near the junction of the stomach and colon. The tubules are approximately 225 μm long and 30 μm wide and consist of small squamous cells with a brush border (Fig. 32). The Malpighian tubules function as an excretory organ (Hughes 1959).

Cuticular Sensory Receptors

The legs and palpi of *R. robini* have several types of cuticular sensory receptors. Unlike the cuticular spines on the legs which have no neural connections, the sensory receptors have one or more neurons associated with them. Mechanoreceptors have only one bipolar neuron, while chemoreceptors have several neurons. The solenidia (macrosensetae) on tarsus I and II and the famulus (microsenseta) on tarsus I (Fig. 33) are considered to be chemoreceptors.

The cuticular portion of the solenidion of *R. robini* is approximately 10 μm long and 2.5 μm wide at its base while the smaller famulus is only 3.5 μm long and 1.5 μm wide at the base. The solenidion is slightly curved and exhibits longitudinal fluting while the famulus is uncurved and smooth (Figs. 34, 35). Both have a thin cuticular wall and minute pores cover the surface. The pores enlarge internally to form chambers, each providing an opening through which various types of molecules from the external environment may pass and stimulate the dendrites in the lumen of the sensillum.

The dendrites extend from the lumen of the sensory receptor to the distal end of the perikaryon and are enclosed in a cuticular sheath (Fig. 36). They become branched as they enter the lumen of the senillum. A tormogen cell surrounds the proximal portion of the dendrites, the trichogen cell, and the cuticular sheath containing the dendrites (Fig. 37). The nucleus is in the basal portion of the tormogen cell which contains many cytoplasmic extensions. It is the tormogen cell that secretes the cuticular portion of the sensory receptor.

The trichogen cell extends from the proximasl portion of the dendrite to the tormogen cell. Cytoplasmic extensions of the trichogen cell are involved in secreting the substances necessary to form the cuticular sheath. The cuticular sheath is a thin-walled cuticular cylinder that surrounds the dendrites from the upper part of the trichogen cell to the hair lumen.

The solenidia of _R. robini_ each have six neurons, while the famulus has only four. The neurons in both the solenidia and famulus have a perikaryon, a dendrite and an axon. The perikaryon is approximately 5 μm wide and 1 μm long and most of it is occupied by a large nucleus. There is an area of clear cytoplasm at either end of the perikaryon. The dendrites arising from the distal end of the perikaryon are protected by the surrounding neurilemma cell. This cell also forms a protective covering around the axon which joins the proximal end of the perikaryon. The glial cell surrounds the basal part of the perikaryon and the axon and provides an insulating mechanism.

The cuticular mechanoreceptors on the legs and palpi of *R. robini* each consist of a solid cuticular peg inserted on a base of flexible cuticle. A single bipolar neuron is associated with these sensory receptors. The dendrite is attached to the flexible base and, as the peg is deflected, the flexible cuticle in the base is deformed which in turn stimulates the neuron. Tormogen, trichogen and glial cells are associated with the mechanoreceptors.

Opisthonotal Gland

There are no histological studies on the opisthonotal gland of acaridid mites reported in the literature. The histology of these structures in *R. robini* is described in this section.

The opisthonotal glands are located dorsolaterally on either side of the opisthosoma in all stases. The external opening to the gland is approximately 4 μm in diameter, and is surrounded by a cuticular ridge (Figs. 39, 40). The internal structure consists of a cuticular sac, a cuticular flap, and several specialized cells which lie beneath the sac. The cuticular extensions in the duct of the gland (Fig. 44) serve as a flap or valve to close the gland opening.

The sac portion of the gland is composed of a porous cuticular lining about 0.1 μm thick. This area of the gland contains the oily substance that often is visible as a dark subsurface patch in *R. robini*. Unfortunately, most of the contents of the gland are lost during the dehydration process and the cuticular sac is greatly compressed during the embedding procedure. However, some sections show clearly that lipid droplets are present near the cuticular lining; it is likely that the entire sac must be occupied by these lipid droplets prior to the dehydration process (Fig. 43).

The cells which lie beneath the sac differ from other epidermal cells in that they contain more mitochondria and rough endoplasmic reticulum than the regular epidermal cells. The number of lipid droplets also is greater (Figs. 41, 42, 44). The lipid material probably is secreted into the sac via the pores in the cuticular lining.

Kuwahara et al. (1980) have demonstrated that the opisthonotal glands in several acaridid mites are sites for the production of alarm pheromones. Baker and Krantz (1982) have shown a similar function for these glands in *R. robini*. When the mites are disturbed, the gland is opened by hydrostatic pressure caused by the contraction of certain dorso-ventral body muscles. The lipid material in which the pheromone is dissolved flows out of the gland on to the dorsal surface of the mite and, as the lipid material comes in contact with the air, the pheromone contained in the lipid volatilizes out into the atmosphere. The sex pheromone of the ticks, Dermacentor andersoni (Say) and D. variablis (Styles) is stored and released in a similar manner (Sonenshine et al. 1981).

Conclusions

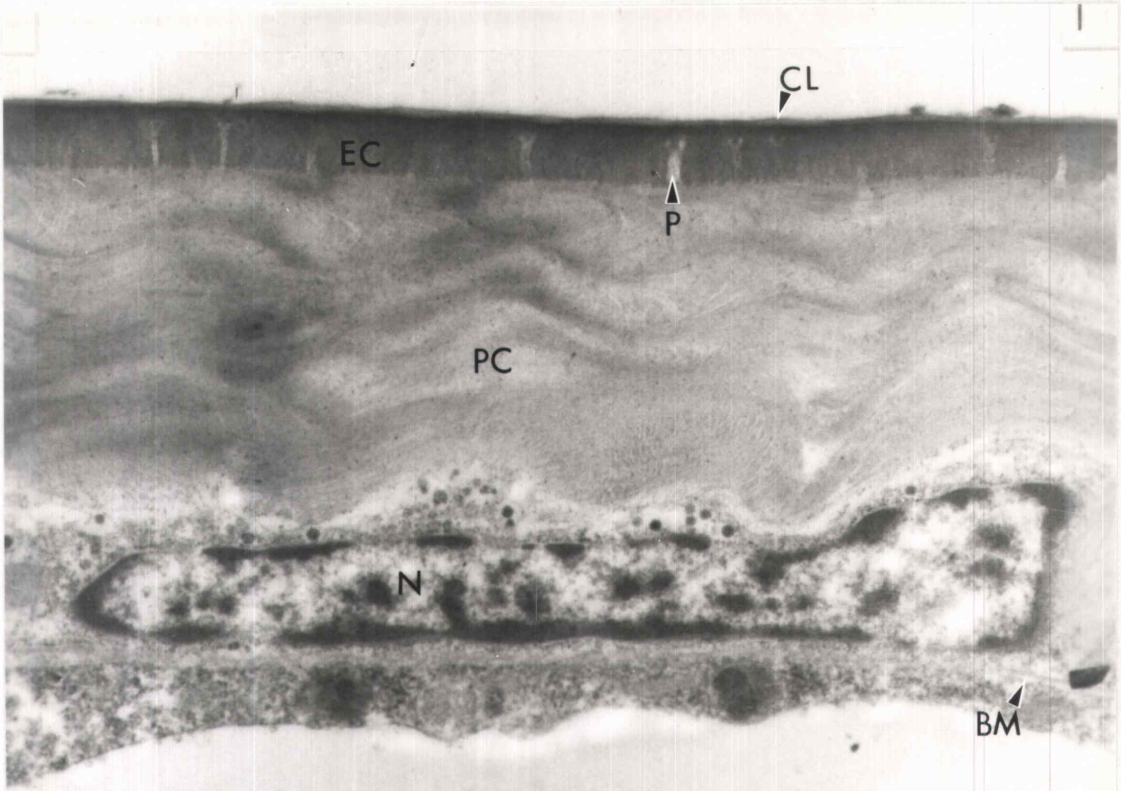
The cuticle of R. robini differs from that of other arthropods in that the procuticle cannot be separted into endocuticle and exocuticle. However, as with other arthropods, an epicuticle composed of a lipid and wax layer, a cement layer and a cuticulin layer compose the epicuticle. The pore canals in the cuticle of R. robini deposit materials on the surface of the epicuticle. The Z line, A band and I bands are found in the muscles of R. robini while the H and M bands present in the muscle of other arthropods are not discernible in R. robini. In addition, the muscle insertions on the cuticle differ from those reported for insects and spiders in that the muscle-cuticle attachment lacks microtubules.

The nervous system of R. robini is a fused central mass, with nerve trunks extending to the oral region, legs, digestive, and reproductive systems. The female and male reproductive systems follow the basic acaridid plan for these systems.

The solenidion (macrosenseta) and the famulus (microsenseta) each have a thin cuticular wall with many pores and are innervated by six and four neurons respectively. The mechanoreceptors are innervated by one bipolar neuron that is attached to the flexible base around the solid cuticular peg. The opisthonotal gland of _R. robini_ is composed of a cuticular sac, cuticular flap, an opening surrounded by a cuticular ridge, and a group of specialized epidermal cells which secrete the lipid droplets normally associated with the gland. An alarm pheromone produced at the gland site is presumed to be carried in these droplets.

Figure 1. Transverse section of R. robini integument. Note the elongated nucleus (N) of the epidermal cell which lies on the basement membrane (BM). The cuticle is divided into the procuticle (PC) and epicuticle (EC); the cement layer (CL) is the outmost layer seen in the micrograph. Pore canals (P) may be seen in the cuticle. X16,000

Figure 2. Transverse section of cuticle at a higher magnification. The various component parts can be seen but the lamellar arrangement (numbers 1 to 60) of the procuticle (PC) is clearly visible. X28,300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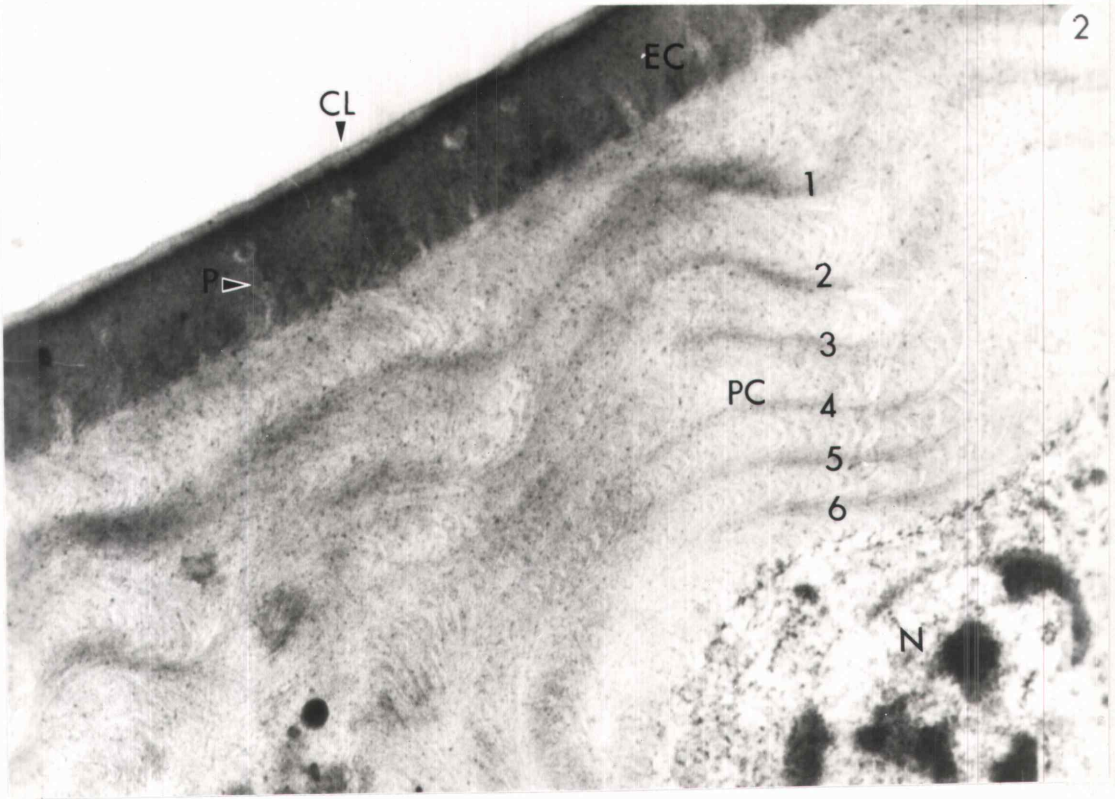
BM


2
EC
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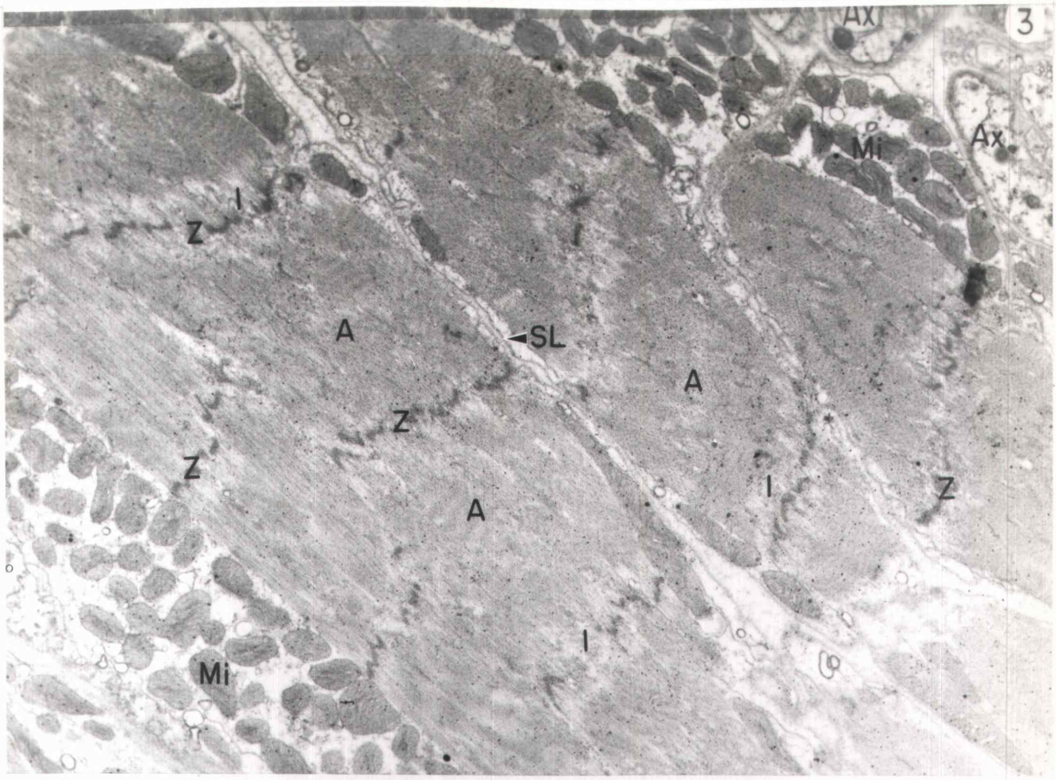
1
P
2
3
PC
4
5
6
N

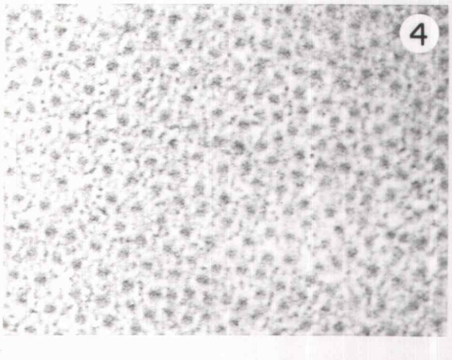
Figure 3. Longitudinal section of R. robini muscle fibers showing the Z, I and A bands, the mitochondria (Mi) along the periphery of the fiber, and a sarcolemma (SL) surrounding each fiber. Several axons (Ax) may be seen in the upper right portion of the micrograph. X17,500

Figure 4. Transverse section showing the thick-thin filament relationship. The thick filaments (large dots) have a diameter of 250 A and the thin filaments (small dots) surround each thick filament and are 60 A in diameter. X39.600

Figure 5. Longitudinal section the desmosones (D) of a muscle fiber inserted directly into the cuticle (c) of R. robini. X31,300

Figure 6. Transverse section of a epidermal (EI) invagination into the muscle (M) of R. robini. The desmosomes (D) and extracellular (EF) are associated with the muscle-epidermal complex. X33,500


3
Ax
Ax
Mi
Z
I
A
SL
A
Z
Z
A
I
Z
I
Mi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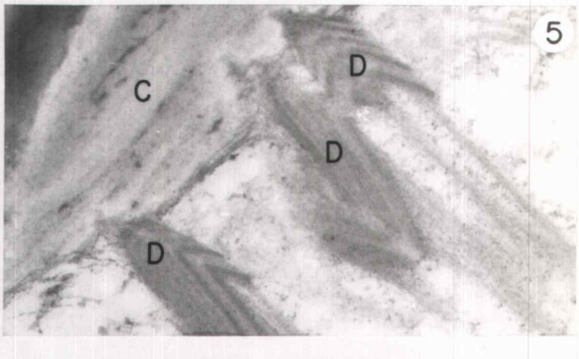
5
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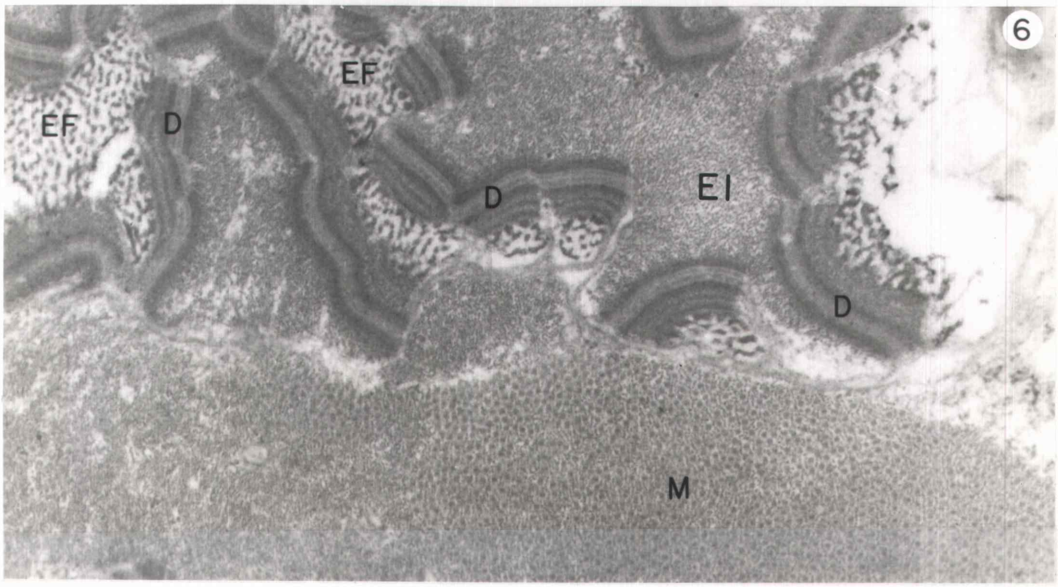
D
D


6
EF
EF
D
D
EI
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M

Figure 6a. Diagram showing the relationships between the cuticle (c) and epidermal and muscle cells at a myo-cuticle junction. The specialized tight junction region (D) between the muscle and epidermal cell is indicated by cross hatching. The desmosome forms where invagination of the plasma membrane on the outer side of an epidermal cell (1) lies close to an invagination of the plasma membrane on the inner side of the epidermal cell (2). Plasma membrane of muscle cell designated (3). The extracellular fibrils which link the desmosomes to the cuticle lie in the invaginations (E1, E2, E3) of the outer side of the epidermal cells.

Figure 6a

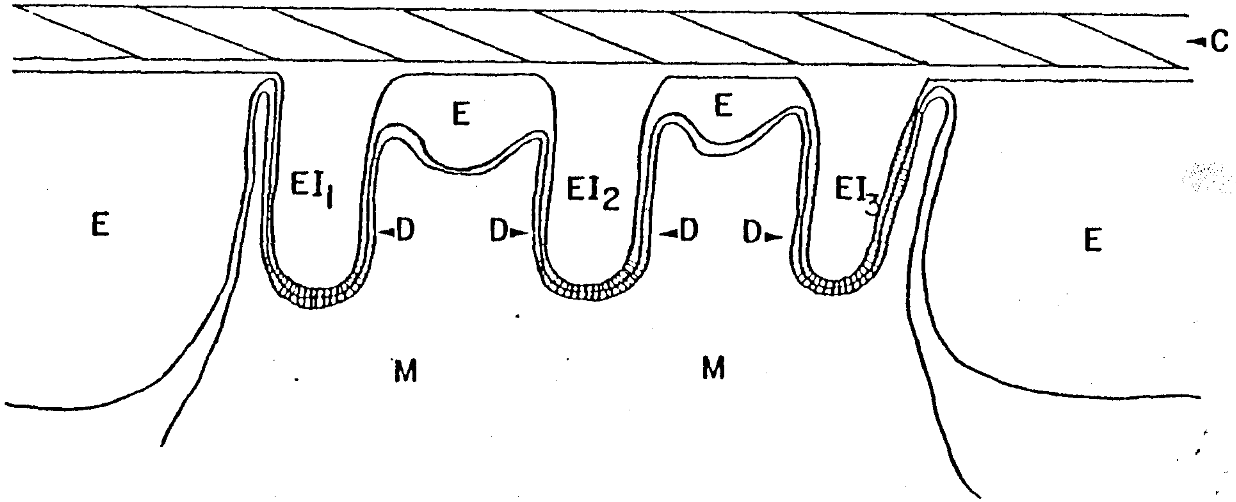

C
E
E
EI$_1$
EI$_2$
EI$_3$
E
D
D
D
D
E
M
M

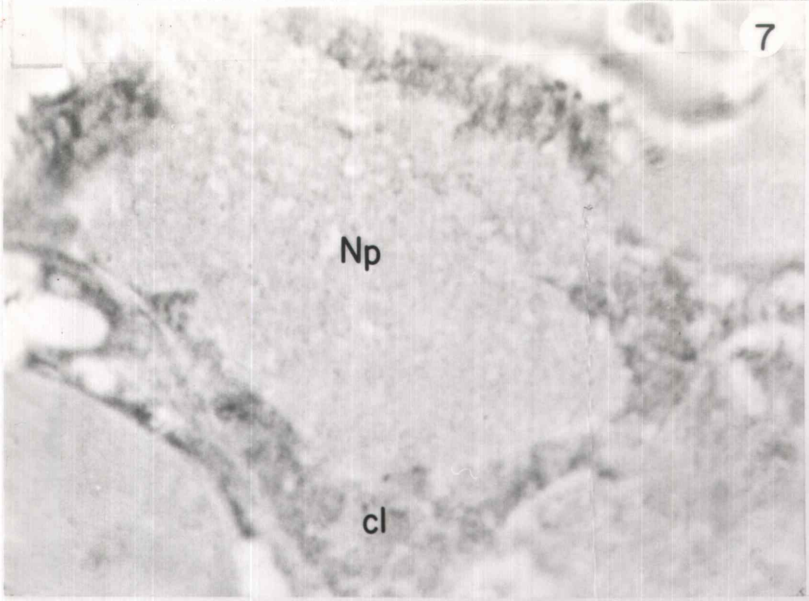
Figure 7. Longitudinal section of the supraoesophageal ganglion showing the neuropilar region (p) surrounded by the cortical layer (cl). X4,200

Figure 8. A TEM micrograph of the supraoesophageal ganglion showing the glial cells (arrows) and axons (Ax). X24,000

Figure 9. Transverse section of the suboesophageal ganglion showing the cortical (cl) and neuropilar (Np) regions. The numbers 1 to 4 on the corical area indicate where nerves leave the ganglion to the legs. X1,300

Figure 10. A section of the neuropilar region of the suboesophageal ganglion showing axons (Ax) and axons containing neuro-secretory granules (nsg). X26,400

Figure 11. A section of the suboesophageal ganglion the arrow points to the glial ensheathment of axons in the neuropilar region (np) and nuclei of the corical region (cl). X13,800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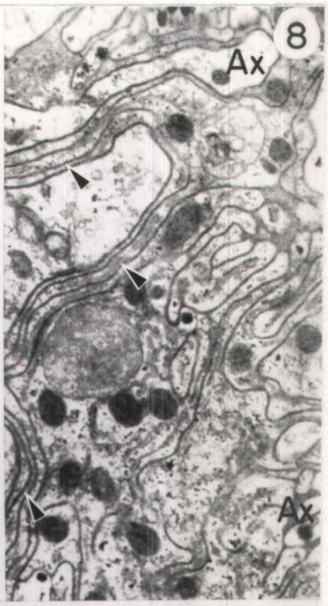
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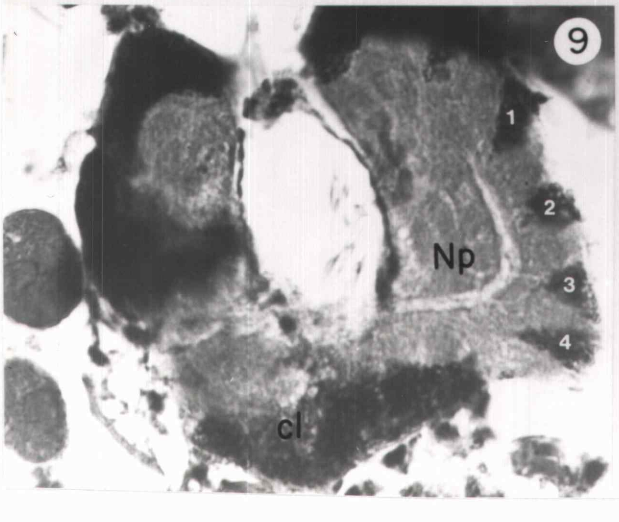
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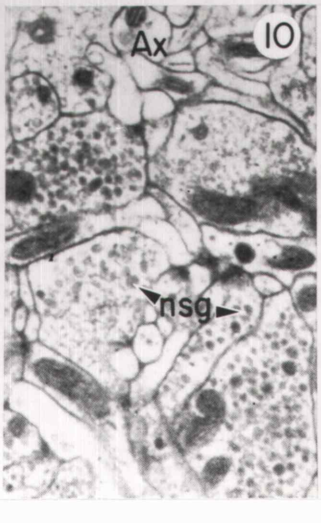
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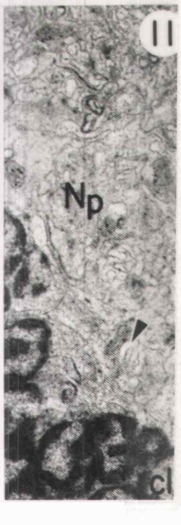
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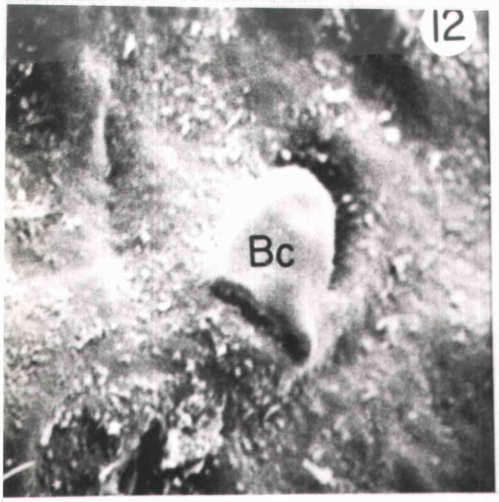
Figure 12. The cuticular flap covering the opening to the bursa copulatrix of the female of R. robini. X875

Figure 13. Developing ova in the female reproductive system; the star indicates immature ova while the arrow shows a mature ovum. X1,150

Figure 14. Accessory gland (Ag) and ovary (OV) of the female. The rectum (R) appears in the upper right. X1050

Figure 15. Section of the oviporus area of the female reproductive system; the two triangular valves an outer pair (Ovl) and inner pair (Ivl). X1250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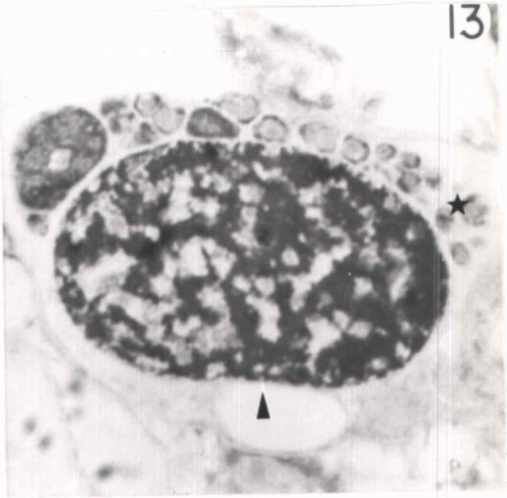
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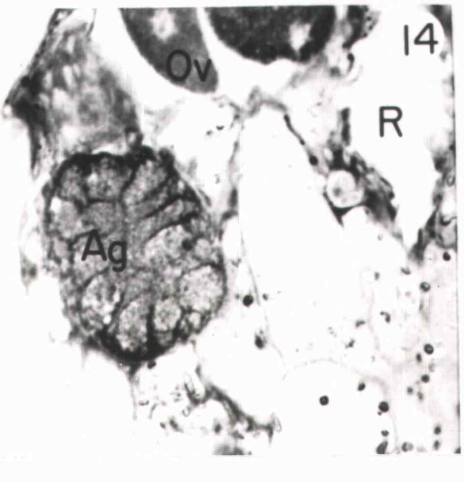
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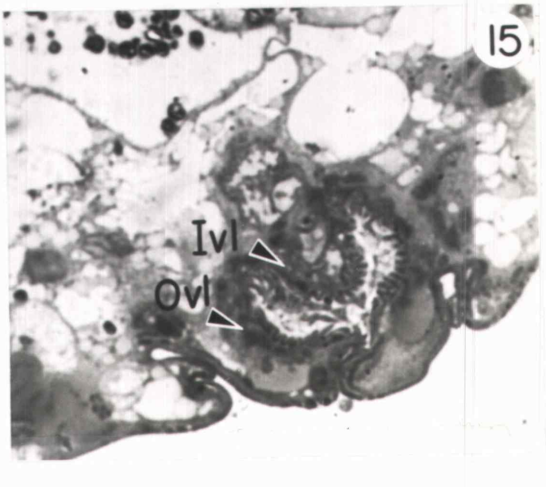
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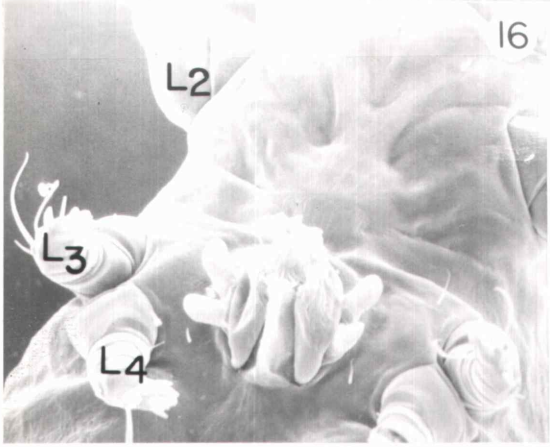
Figure 16. Ventral surface of the female with extended oviporus apparatus lying between legs III and IV. X650

Figure 17. Higher magnification of Figure 16, showing the triangular outer valves (Ovl) and inner valves (Ivl) and the pleated cuticular extension of these valves known as the labia (L). There are two pairs of genital acetabula (Ga). X1025

Figure 18. TEM micrograph of the pleated article of the labia. X10,600



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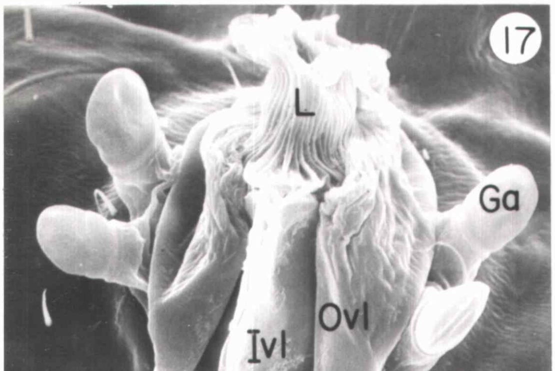

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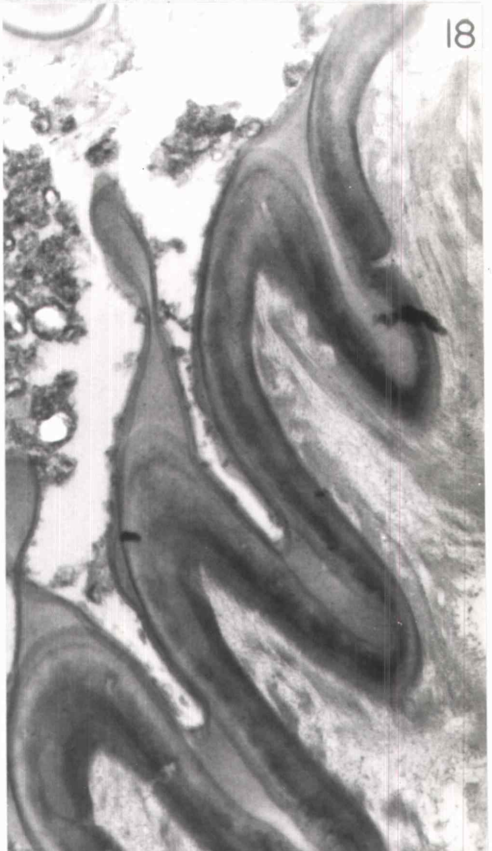

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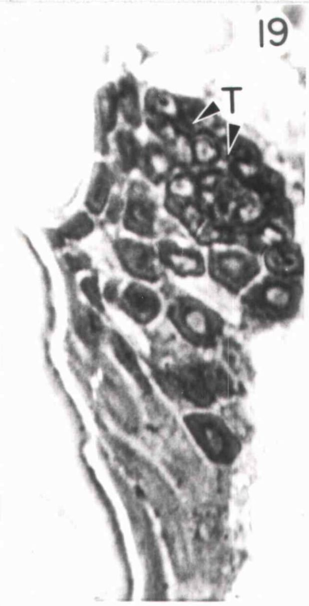
Figure 19. A phase-contrast micrograph of a transverse section of the male testes (T). X675

Figure 20. Higher magnification of Figure 19 showing the variation in the size of the cells in the testis (T). X1950

Figure 21. A phase contrast micrograph of the male accessory gland or chamber organ. X1800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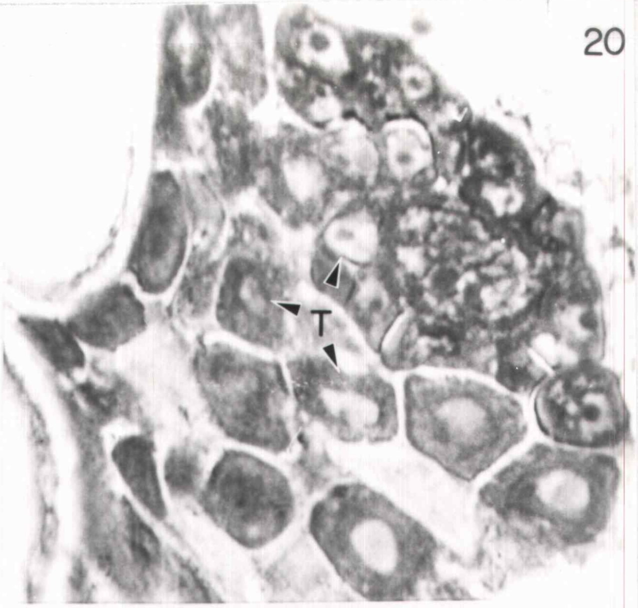
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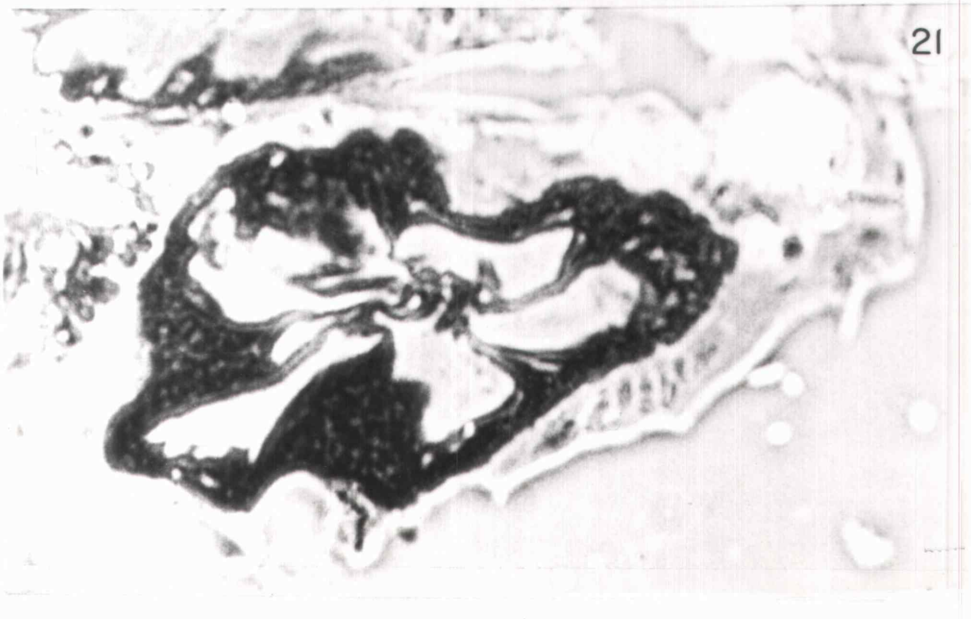
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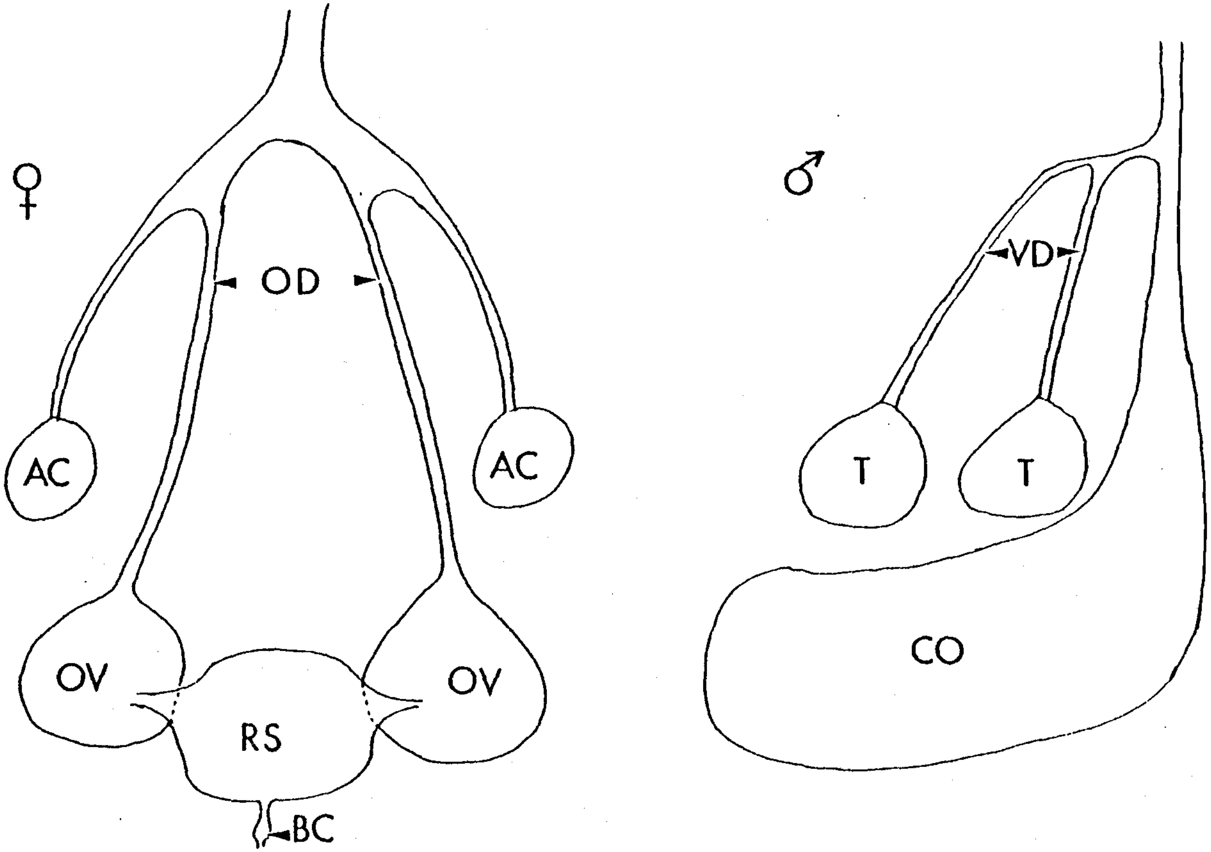
Figure 22. Diagrams showing the main features of the female and male reproductive systems. The female system consists of a pair of accessory glands (AG), a bursa copulatrix (BC), paired oviducts (OD) and ovaries (OV), and a receptaculum seminalis (RS). The male system consists of a pair of testes (T), vasa deferentia (VD) and the chamber organ (CO).

22


♀
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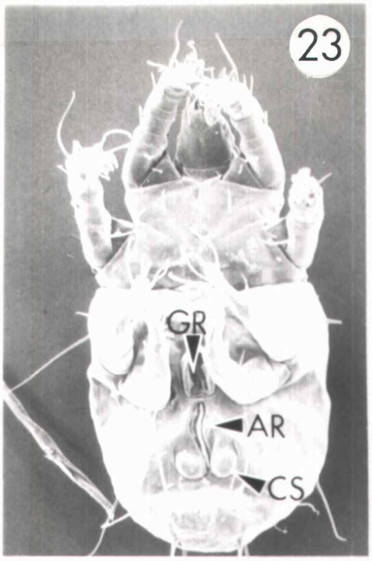
CO

Figure 23. SEM micrograph of the ventral surface of the male of R. robini showing the genital region (GR) lying between legs IV, the anal region (AR) posterior to the genital region, and the copulatory suckers (CS). X350

Figure 24. The penis (Pn) of the male lying in the genital sheath. X1225

Figure 25. Higher magnification of Figure 23 showing the genital region (GR), the anal region (AR) and the copulatory suckers (CS). X975

Figure 26. The modified setae on legs IV that are used as suckers for clasping the female during copulation. X1750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GR
AR
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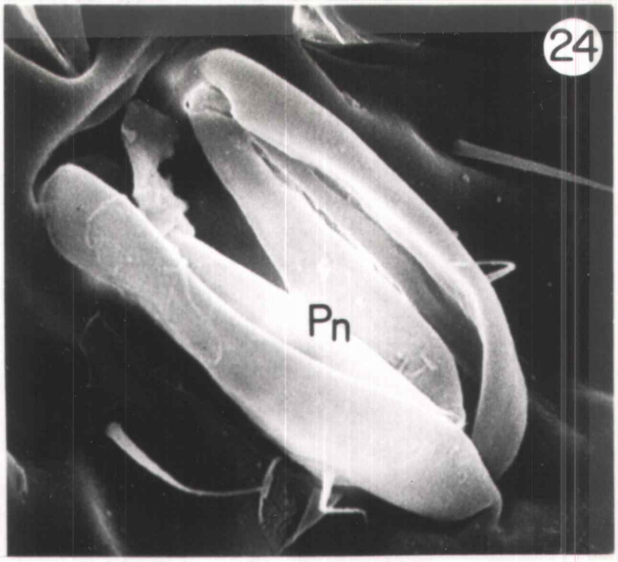
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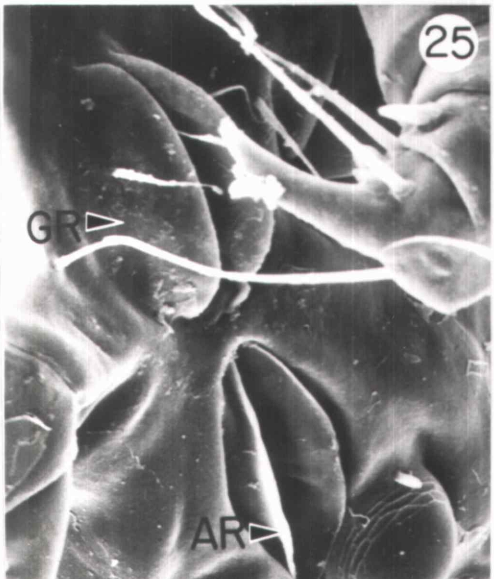
Pn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GR
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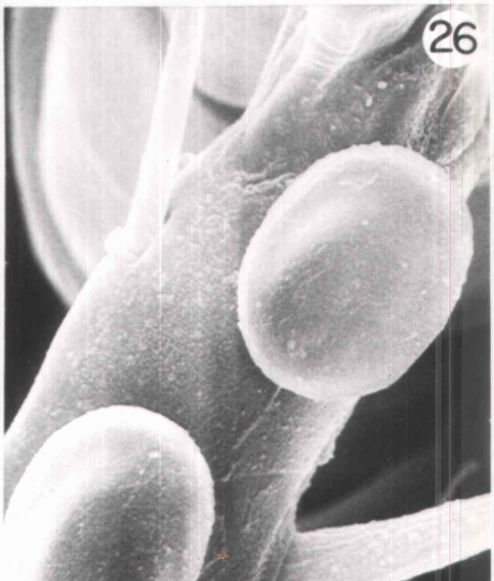
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Figure 27. A section of the midgut showing the cuboidal cells (Cc) and squamous cells (Sc) composing the lining of the midgut. The star is a budded off cuboidal cell. X2050

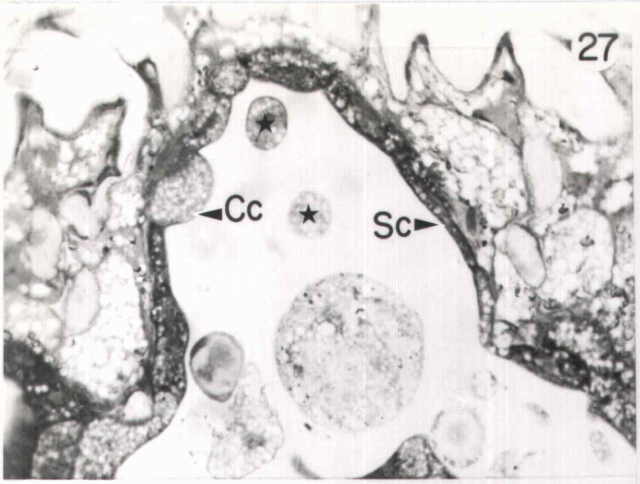
Figure 28. A section of the caecum with three types of cells making up the caecal lining, cuboidal cells (Cc), squamous cells (Sc) and globular cells (Gc). The cuticle (C) may also be seen. X1876

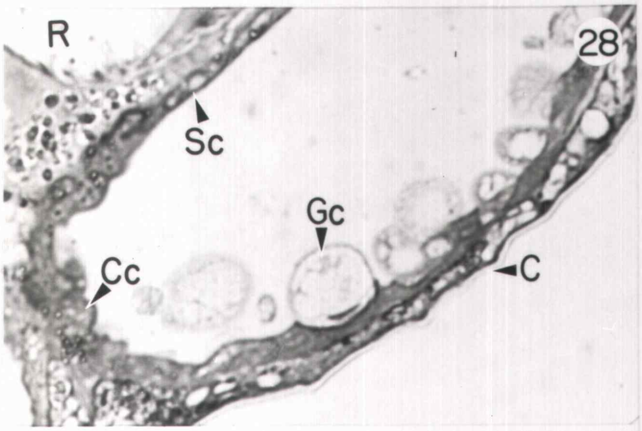
Figure 29. A phase-contrast micrograph of the globular cells (Gc) with their large vacuole. X2950

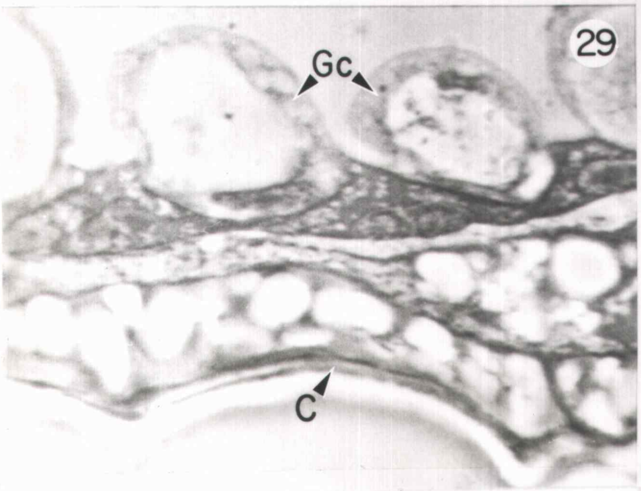
Figure 30. A section showing the cells lining the rectum; squamous cells (Sc) and conical cells (Cnc) have a brush border (BB) and the food bolus (FB) can be seen in the rectal lumen. X1300

Figure 31. A high magnification phase contrast micrograph of the rectal squamous cells (Sc) with their brush border (BB). The caecal squamous cells (Csc) may also be seen. X3100

Figure 32. The lining of the Malpighian tubules (MT) showing the brush border (BB). The rectum (R) appears on the right side of the photograph. X1425


27
Cc
Sc
★
★


R
28
Sc
Gc
Cc
C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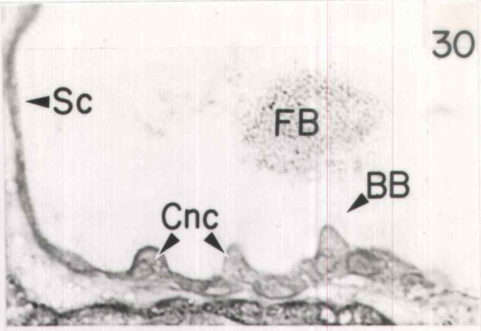
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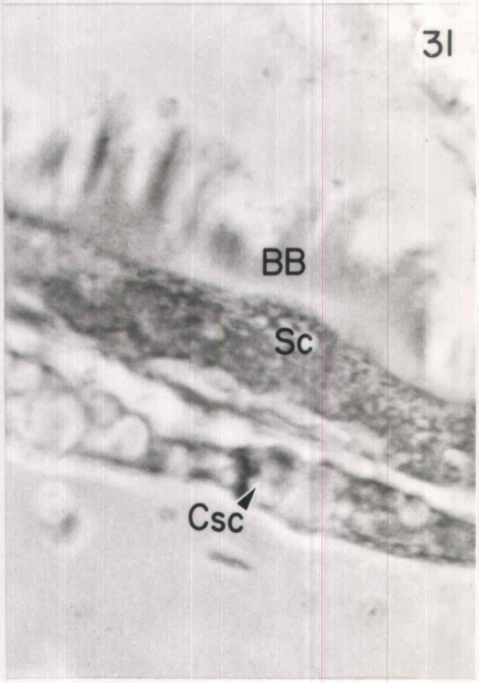
BB
Cnc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Sc
Csc

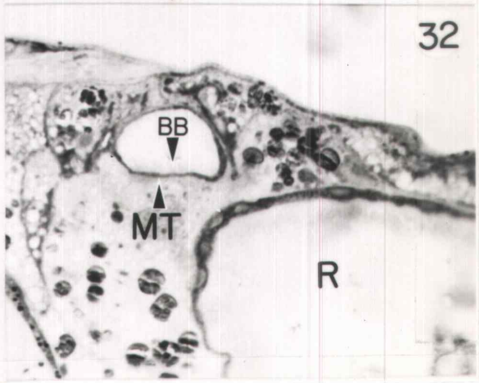

32
BB
MT
R

Figure 33. SEM micrograph of *R. robini* showing the long mechanoreceptors on tibac I II (the large arrows) and the olfactory receptors (the small arrows) on tarsi I-II. X500

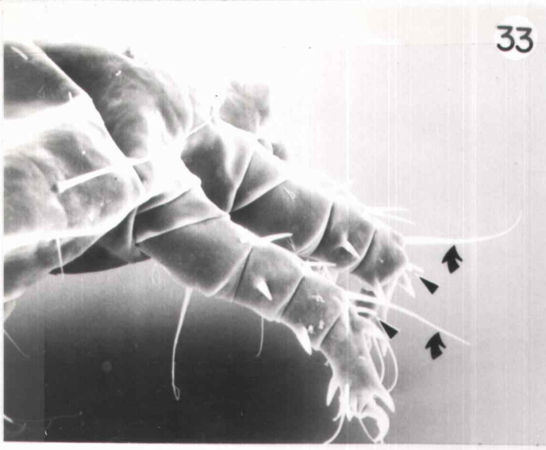
Figure 34. Higher magnification of Figure 33. The long, slightly bent solenidion and the short, straight famulus on tarsus I. X4300

Figure 35. Solenidion on tarsus II. Note the fluting in the peg surface. X7450

Figure 36. Transverse section of the famulus near the base of the peg. The dendrites (D) are surrounded by a cuticular sheath (CS). X19,500

Figure 37. Transverse section of the solenidion beneath the base of the cuticular peg. The dendrites (D) are surrounded by the cuticular sheath (CS) and extension of the trichogen cell (Tr) may be seen in the receptor lumen. X27,300

Figure 38. Cross section of the solenidion. The cuticular portion has many pores (p) and the dendrites (d) are in the lumen of the peg. X26,400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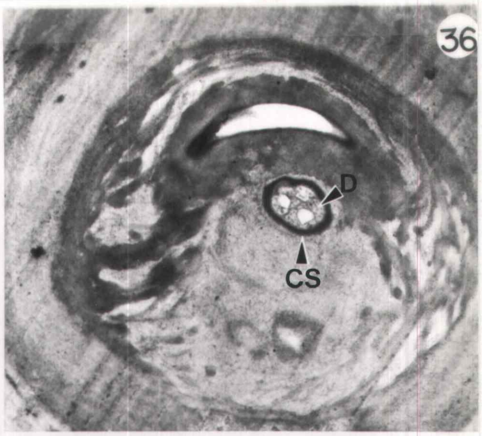
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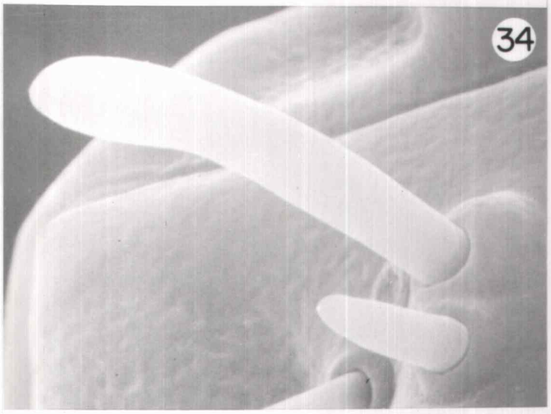
D
CS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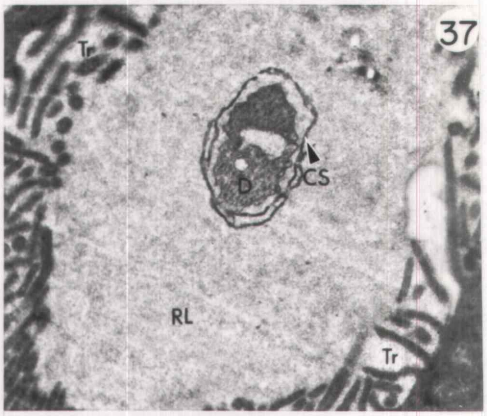
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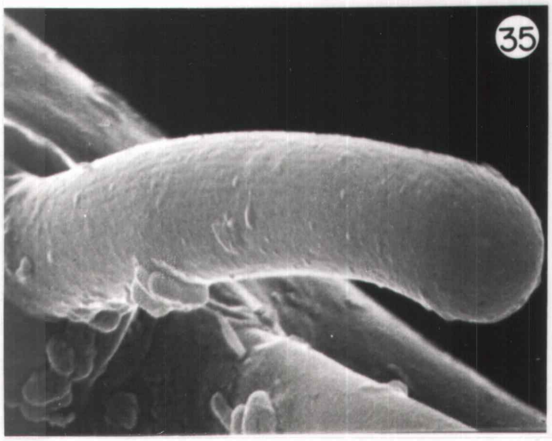
Tr
D
CS
RL
Tr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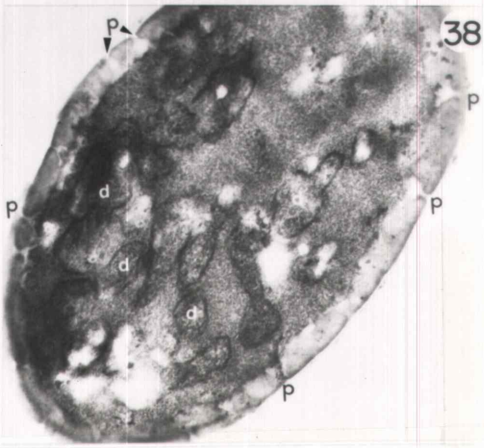

38
P
P
P
P
P
d
d
d
P

Figure 39. The opisthonotal gland (OG) and gland opening (O) of *R. robini*. X800

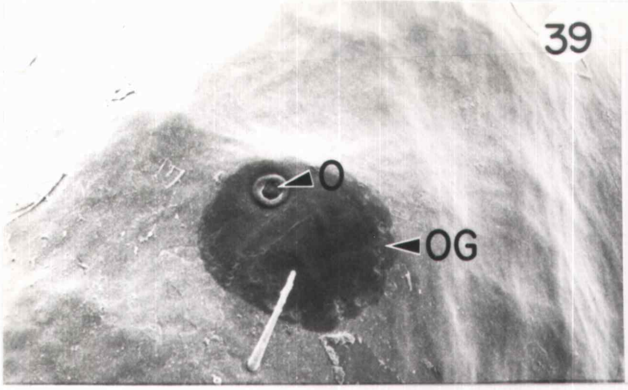
Figure 40. The cuticular ridge (R) around the opening of the opisthonotal gland. X1475

Figure 41. The nucleus (N) and mitochrondria (Mi) of the cell lying beneath the cuticular sac of the opisthonotal gland. X8500

Figure 42. Lipid droplets (LD) in the gland cell. X16,800

Figure 43. Lipid droplets (LD) in the cuticular sac. X14,900

Figure 44. Drawing of the opisthonotal gland, the opening is surrounded by a cuticular ridge (CR); the cuticular flap (CF) prevents the lipid droplets (LD) from escaping the cuticular sac (CS). The gland cells contain mitochondria (Mi), rough endoplasmic reticulum (RER), lipid droplets (LD), and a nucleus (N). These cells lie on a basement membrane (BM).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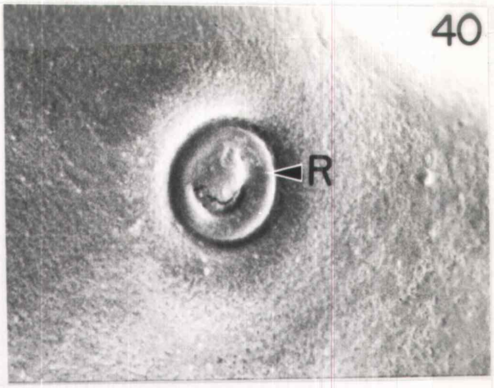
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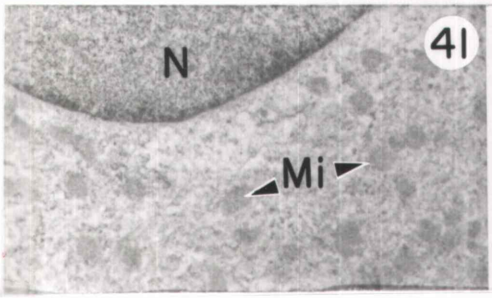
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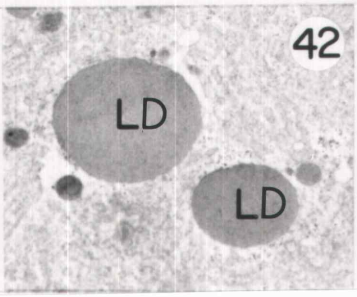
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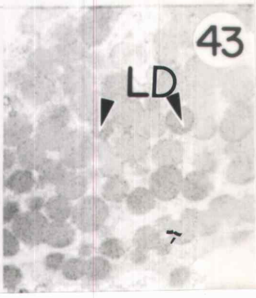
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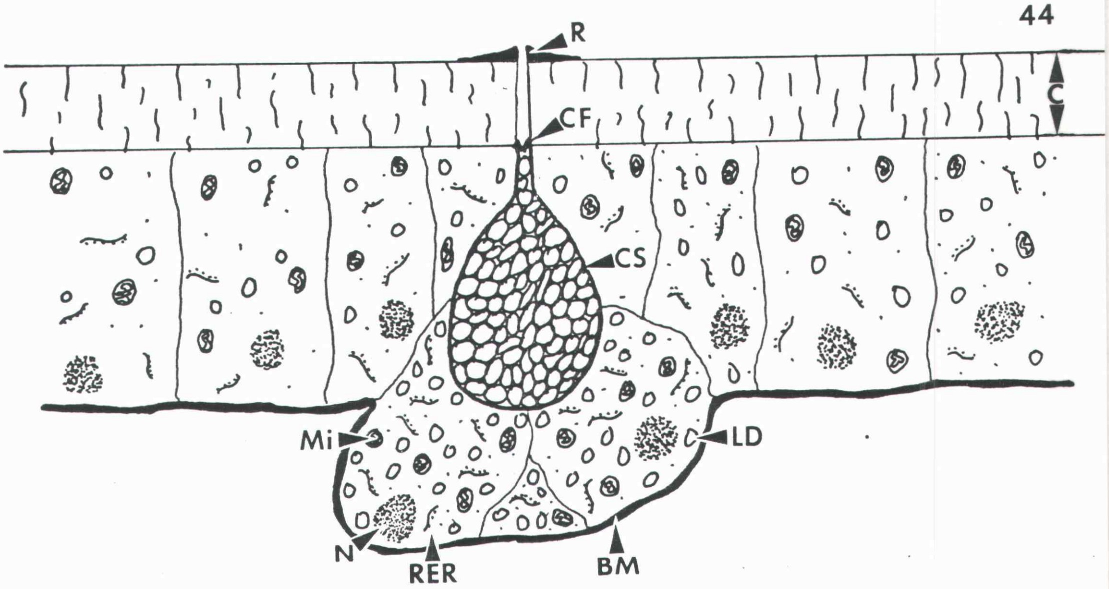

44
R
C
CF
CS
Mi
LD
N
RER
BM

CHAPTER II

Alarm Pheromone of Rhizoglyphus robini and Some of Its Effects

Introduction

Pheromones are chemical messengers which may induce a response either in individuals of the same species that produces the pheromone, or in other species with close ecological ties. Pheromones are responsible for coordinating activities such as mating, dispersal, and aggregation. They may be volatile substances which may be detected over considerable distances or they may be ingested by the recipient. Different types of pheromones have been isolated and characterized from species representing most of the major phyla of invertebrates and vertebrates.

Until recently, studies on mite pheromones dealt largely with those produced by ixodid ticks. Berger et al. (1971) were the first to report the existence of tick pheromones and since that time, sex pheromones (Sonenshine et al. 1974; Chow et al. 1975), and assembly pheromones (Graf 1975; Rechav et al. 1976) have been described from these acarines. Leahy et al. (1973) demonstrated the presence of an aggregating pheromone in an argasid tick, Argas persicus (Oken). Since the initial discovery, aggregating pheromones have been found in other argasid ticks as well (Leahy et al. 1975a,b). During the same period, Cone et al. (1971a,b) and Regev and Cone (1975, 1976,

1980) isolated a sex attractant pheromone from females of the two spotted spider mite Tetranychus urticae (family Tetranchyidae). Similarly, a sex pheromone was demonstrated in the predatory phytoseiid mites Typhlodromus fallacis (Oudemans) (Rock et al. 1970) and T. occidentalis Nesbitt (Hoy and Smilanick 1979).

Dispersal of organisms in a population may be triggered by a special pheromone produced in response to a physical or chemical disturbance. Alarm pheromones have been identified from invertebrates as diverse as sea anemones (Howe and Sheikh 1975; Howe 1976), opisthobranchs (Sleeper et al. 1980) and insects (Bergstrom and Lofqvist 1970; Blum and Bohart 1972; Montgomery and Nault 1977; Maschvitz and Gutman 1979, Wohler 1980). Kuwahara et al. (1980a,b) and My-Yen et al. (1980) isolated an alarm pheromone from several species of acaridid mites including Tyrophagus putrescentiae (Schrank) and Aleuroglyphus ovatus (Troupeau).

Laboratory observations at Oregon State University on R. robini exposed to extracts of disturbed individuals suggested that disturbed mites produce a pheromone that causes mites to disperse from the area of the extract application. Identification of the pheromones is discussed below, along with the results of greenhouse experiments testing the efficacy of this substance as an adjuvant in controlling R. robini in Easter lily bulbs, Lilium longiflorum.

Materials and Methods

Gas-Liquid Chromatography and Mass Spectrometry

Stock cultures of *R. robini* were maintained in closed 10 cm petri dished on an artificial diet developed by Bot and Meyer (1969). Cultures were kept at 23° C in a temperature cabinet. Samples for gas-liquid chromatography (GLC) and mass spectrometry were prepared both from whole mite extracts and from mite volatiles.

Two grams of *R. robini* were washed in 5 ml of pentane for 24 h, and then centrifuged for 10 min at 3000 rpm. A 5 1 aliquot of the pentane supernatant was injected into a Hewlett-Packard 5710A gas chromatograph equipped with a 12 m x .02 mm ID flexible silica capillary column coated with SP2100. Mite volatiles were collected from ~3500 live mites placed in a 500 ml Erlenmeyer flask and allowed to acclimate for 4 h. A stream of air was passed over the mites for 1 h, after which time a Porapak Q® column was introduced into the system and the air was passed through the column for 30 min (Byrne et al. 1975). An equivalent number of mites in a second flask were disturbed by shaking the flask briskly for 30 sec before the Porapak Q® column was introduced into the system. Columns containing volatiles from undisturbed and disturbed mites were washed twice with 5 ml of pentane. A 1µl aliquot from each supernatant was then analyzed with a Finnigan 4023 automated gas chromograph and an EI-CI mass spectrometer system.

Purpald® Test

Based on the results of Kuwahara et al. (1980a,b), it was assumed that the alarm pheromone produced by R. robini might be an aldehyde similar to citral. Purpald®, 4-amino-3 hydrazino-5 mercapto-1,2,4-triazol, (Aldrich Chemical Co.), an extremely sensitive aldehyde-detecting agent that produces a red-purple colour in the presence of an aldehyde, such as citral, under basic conditions (Dickinson and Jacobson 1970). Two groups of 20 mites each, one undisturbed, and one disturbed (by shaking the container for 30 sec) were placed in 5 cm petri dishes containing a solution of 3% agar and 1% Purpald at 50° C. The solutions with the mites were allowed to cool to room temperature and a cotton plug with 4 drops of 28% aqueous ammonia was placed in each dish. The dishes were then covered and held at room temperature overnight. The mites were removed from the agar medium in each dish and examined under a dissecting microscope.

Bioassay

Mites were taken from the culture dishes and washed thoroughly in two changes of distilled water. A moistened 4.5 cm filter paper disc was placed on the bottom of a 5 cm petri dish and the mites were spread over the surface and allowed to stand for 3 h. A 0.6 cm paper disk was used as the treatment substrate in bioassay tests. The discs were treated by saturating them in the following solutions for 60 sec: pentane (control), pentane extract of undisturbed mites, pentane extract of disturbed mites, pentane plus citral @10 ppm, @10^2 ppm, @10^3 ppm, @10^4 ppm, and @10^5 ppm. Each disc was air-dried for 30 sec and then placed in the center of a petri dish in which test mites had previously been placed. Observations were made every 30 sec, up to 5 min on each treatment. The observations were recorded and a photograph of the results taken immediately following the 5 min observation.

Greenhouse experiments

Kelthane®, or dicofol (4,4'-dichloro-alpha-(trichloromethyl benzhydrol) has been shown to be effective against *R. robini* when used as a drench on lily bulbs (Ascerno et al. 1981). Based on the preliminary success of Griffiths and Pickett (1980) in using an alarm pheromone adjuvant for control of the aphid *Myzus persicae* on Chinese cabbage, *Brassica pekinensis* Rupr., we postulated that the addition of citral to the Kelthane treatment for lily bulb mite control might increase the activity of mites sequestered between the bulb scales, improving the likelihood of mite contact with the Kelthane component. Accordingly, various amounts of commercially available citral (32° neral, 68° geranial, Aldrich Chemical Co.) was used in conjunction with Kelthane in a series of experiments described below.

Uninfested Easter lily bulbs, "Nellie White" variety, were seeded with 140 *R. robini* (110♀, 30♂) and stored in the laboratory for a period of 3 weeks. Bulbs were removed from storage and drenched for 30 min in the following treatments: Kelthane (31.5 g of 35% WP in 15 ℓ H_2O); citral @ 100 ppm; Kelthane (as above) plus citral @ 100 ppm; Kelthane (as above) plus citral @ 1000 ppm; and water. The bulbs were then individually potted and maintained on a greenhouse bench. Six bulbs were taken from each treatment on days 1-7 following treatment in test II. Bulbs were carefully taken apart and subjected to Berlese funnel extraction for 5 days. The soil in which the bulbs were planted was extracted by means of flotation in a super-saturated solution of NaCl.

Results and Discussion

Analyses of *R. robini* extracts and volatiles revealed the presence of citral (cis- and trans 3,7-dimethyl-2,6-octadienal), a monoterpene containing the aldehydes neral and geranial. While the compound differed slightly in percentages of the aldehyde compounds identified from other acarid mites (Kuwahara et al. 1980a,b), the chemical similarity was striking and suggested that, like other acarids, *R. robini* utilizes citral as an alarm pheromone.

Further verification of the identity of citral was provided through a comparison of the mite volatiles and commercial citral (Aldrich Chemical Co.) by means of gas-liquid chromatography and mass spectrometry (Figs. 48, 49). The mass spectra showed that peaks 8 and 9 had an identical base ion peak m/e 41 and a molecular ion peak of m/e 152 (Fig. 49). The fragmentation pattern was the same.

The location of the pheromone production site of *R. robini* was demonstrated by employing Purpald. The undisturbed mites were stained a purple colour in their opisthonotal glands while the disturbed mites showed no reaction (Fig. 47). This difference was caused by the disturbed mites secreting most of their alarm pheromone, citral which is an aldehyde. No other aldehyde was detected by the G.L.C. or mass spec analyses was another piece of evidence to indicate that the opisthonotal gland was the site of citral production.

Filter paper discs-treated in pentane and placed in the petri dishes containing mites elicited, no reaction in the mite population. However, when a disc treated in a pentane extract of previously undisturbed mites was placed in the dish, there was a definite behavioural response. After 60 sec, there was noticeable agitation among the mites around the disc and this activity increased with the passage of time. The mites began to move away from the disc after 120 sec, and after 180 sec, there was a totally mite-free area around the disc. When a disc dipped into a pentane and disturbed mite extract was placed in the petri dish, the mites did not react (Fig. 45). Failure to react was probably due to the fact that the disturbed mite extract did not contain appreciable amounts of alarm pheromone, since most of it was volatilized when the mites were disturbed prior to extraction.

The difference in mite reactions to various concentrations of citral was a function of the amount of time that elapsed between test initiation and the start of mite movement away from the treated disc. For example, the mites did not exhibit any general movement away from the disc containing 10^2 ppm of citral until 180 sec after test initiation while at 10^4 ppm, after only 60 sec (Fig. 46).

Kelthane significantly reduced R. robini numbers when compared with the citral or water treatments (Fig. 50). Since citral showed no miticidal effects in laboratory tests on dispersal, the mite decrease noted in citral-treated bulbs was considered to be the result of a mass exodus of mites into the surrounding soil. Flotation extraction of the soil corroborated this assumption (Table 1). Kelthane-citral treatments resulted in significantly higher levels of control than did Kelthane alone (Fig. 50, 51) but there was no significant difference between treatments using citral at 100 and 1000 ppm (Fig. 51). The numbers of mites extracted during the 10 week period following treatment with Kelthane-citral were not only considerably lower at each sample interval than those noted in the Kelthane treatment, but the population build-up following treatment with Kelthane-citral lagged well behind that in bulbs treated with Kelthane alone (Fig. 51). It is likely that the intial population reductions seen during the first 7 days after treatment with Kelthane-citral (Fig. 50) were instrumental in delaying mite buildup during the subsequent 9 week observation period. The magnitude of mite population suppression in lily bulbs treated with Kelthane -citral suggests that the need for additional treatments for mite control during plant maturation may be substantially reduced or eliminated.

Conclusions

The Purpald test indicated the presence of an aldehyde, is present in the opisthonotal gland of *R. robini*. The alarm pheromone released by the mite was identified as citral, a monoterpene containing geranial and neral. The addition of citral to the noraml Kelthane treatment for control of *R. robini* in lily bulbs significantly increased the efficacy of Kelthane in reducing the mite populations.

Figure 45. Dispersal of R. robini in test arenas in which filter paper discs treated with an extract of undisturbed R. robini (a) and disturbed R. robini (b).

45

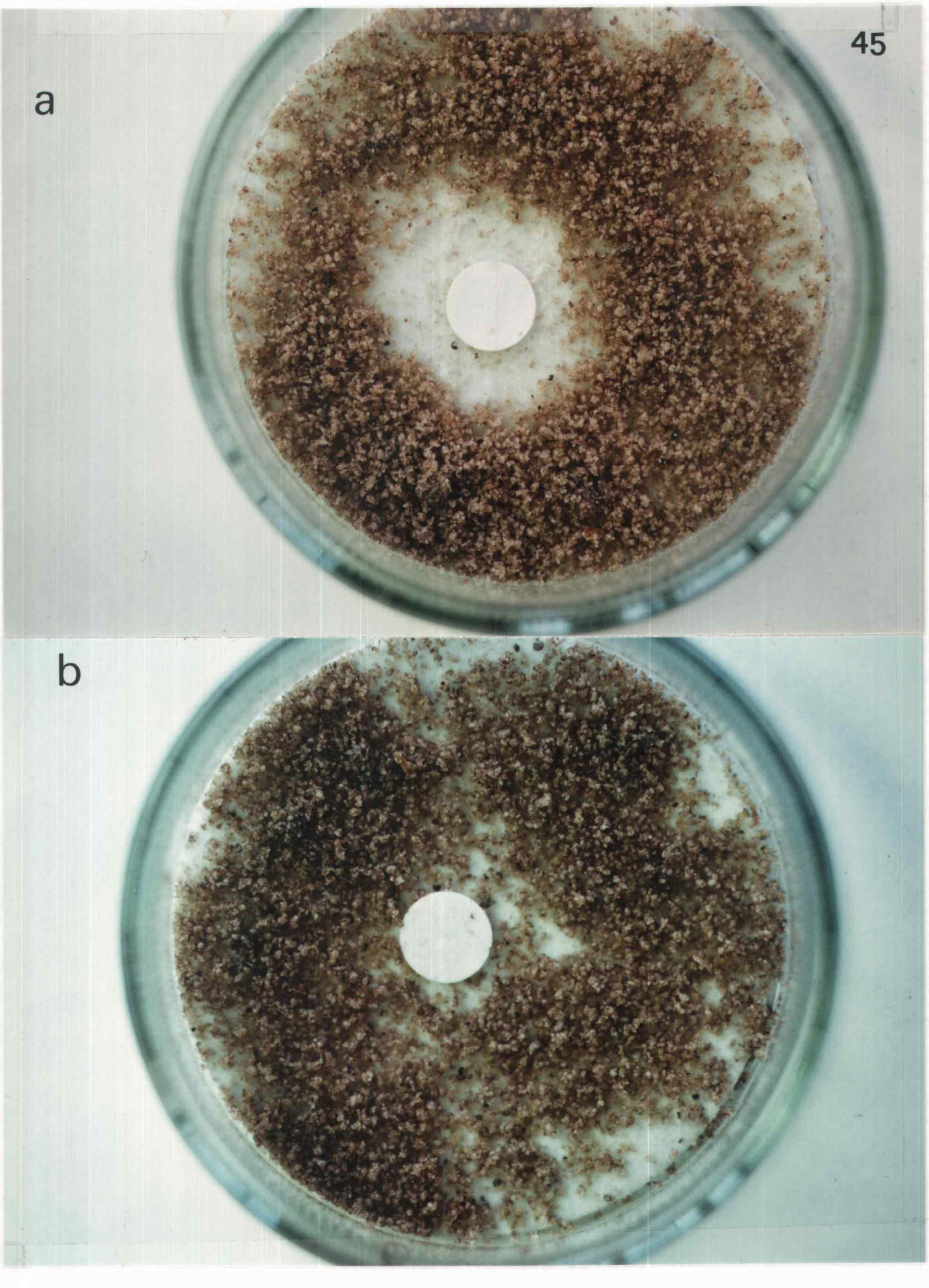
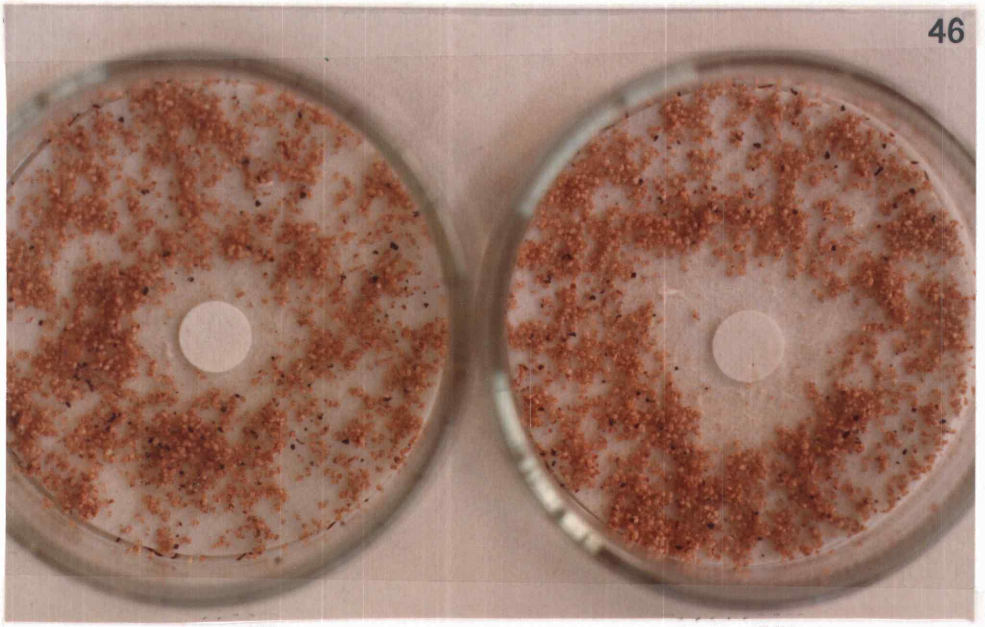


Figure 46. Dispersal of *R. robini* in test arenas in which discs treated with 100 ppm and 10,000 ppm of commercial citral have been placed.



100ppm 10,000ppm

Figure 47. Purpald® reaction with undisturbed (a) and disturbed (b) mites, the undisturbed mites are stained in the area of the opisthonotal gland.

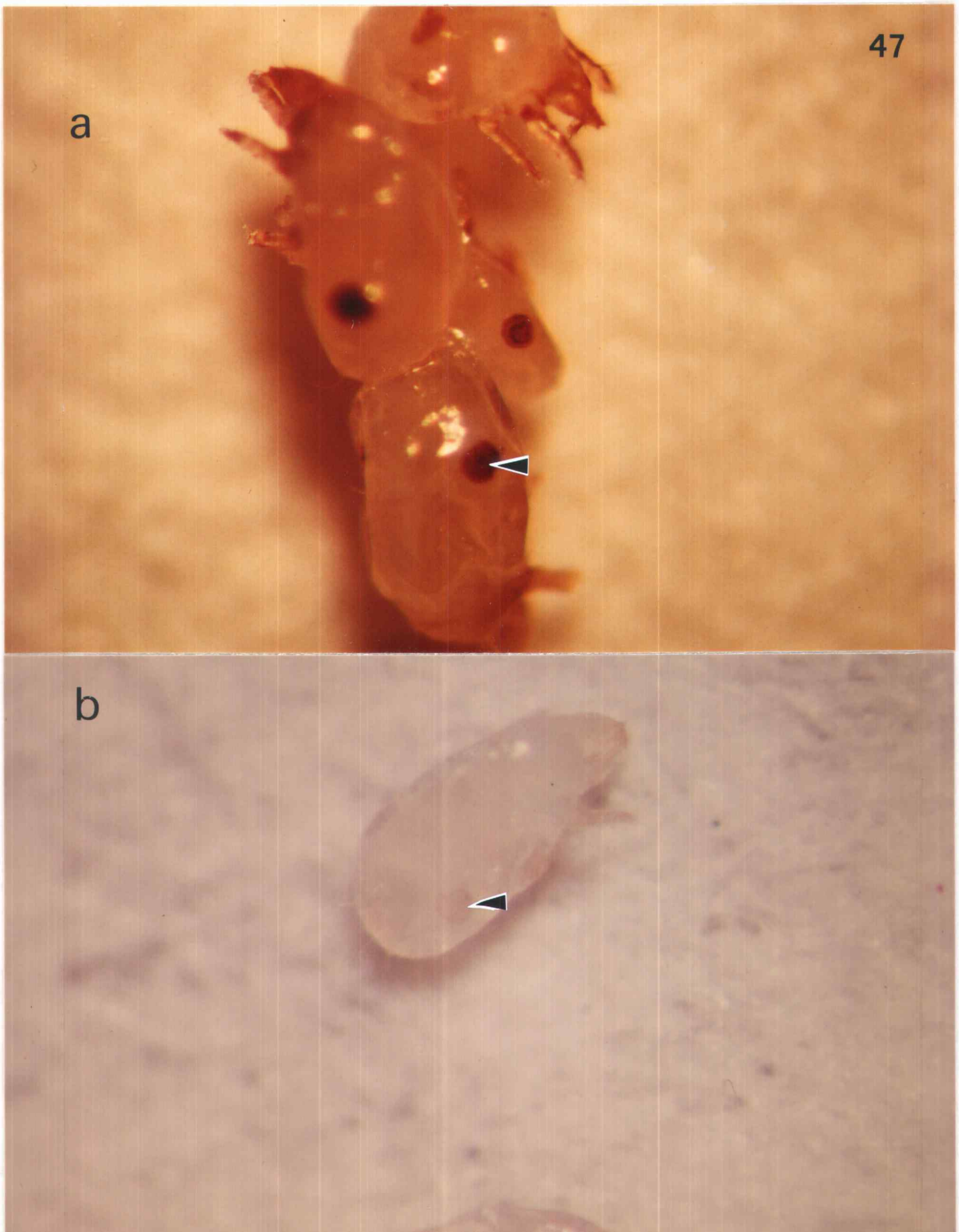

47
a
b

Figure 48. Gas liquid chromatographs of volatiles from undisturbed (a) and disturbed (b) mites.

Peak 1 = tridecane, 2 = tridecene, 3 = tetradecane, 4 = tetradecene, 5 = pentadecane, 6 = pentadecane, 7 = pentadecadiene, 8 = neral, 9 = geranial.

Figure 48

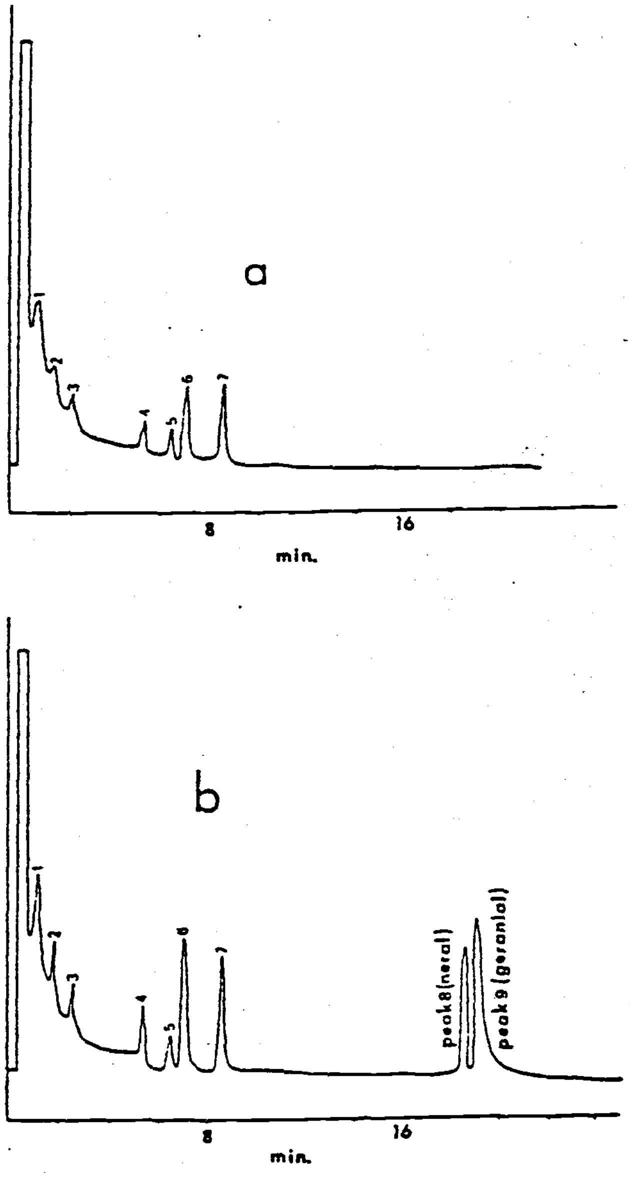
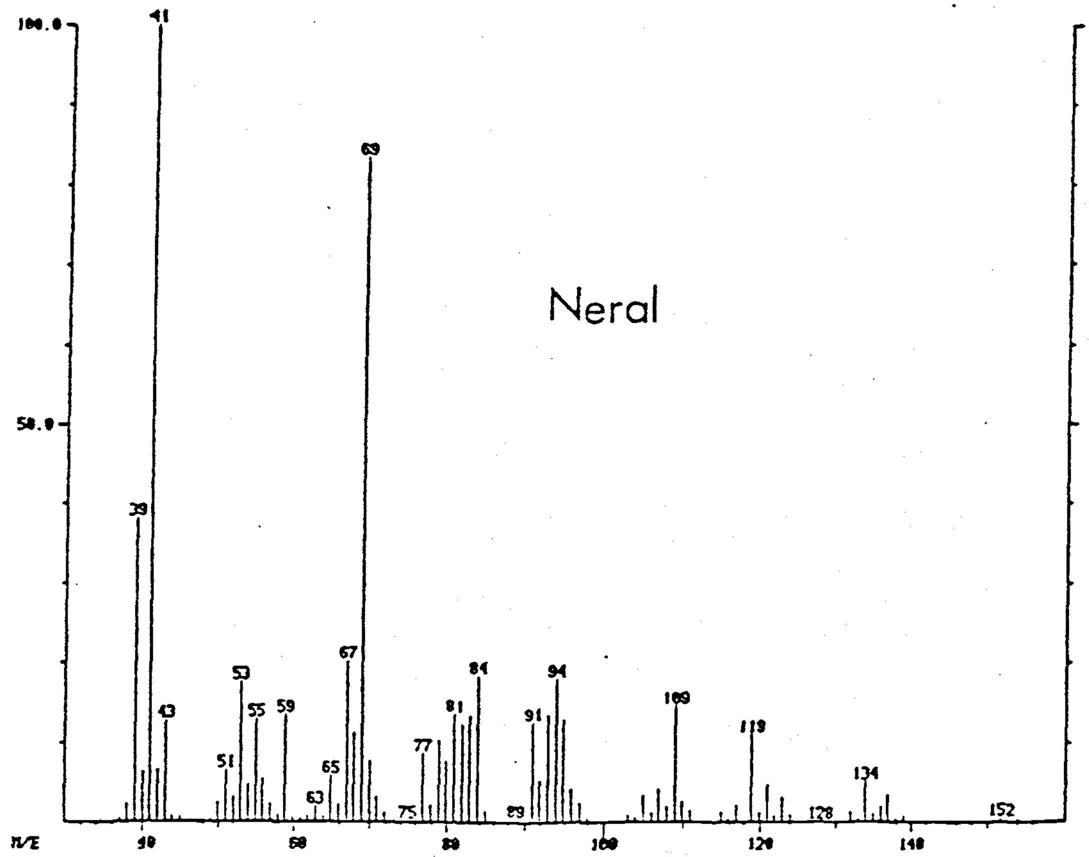

a
1
2
3
4
5
6
7
8
16
min.
b
1
2
3
4
5
6
7
peak8(neral)
peak9(geranial)
8
16
min.

Figure 49. Mass spectra of peak 8 (neral) and peak 9 (geranial).

Figure 49


Neral
100.0
50.0
M/E
40
60
80
100
120
140
41
69
39
43
51
53
55
59
63
65
67
75
77
81
84
89
91
94
109
119
128
134
152

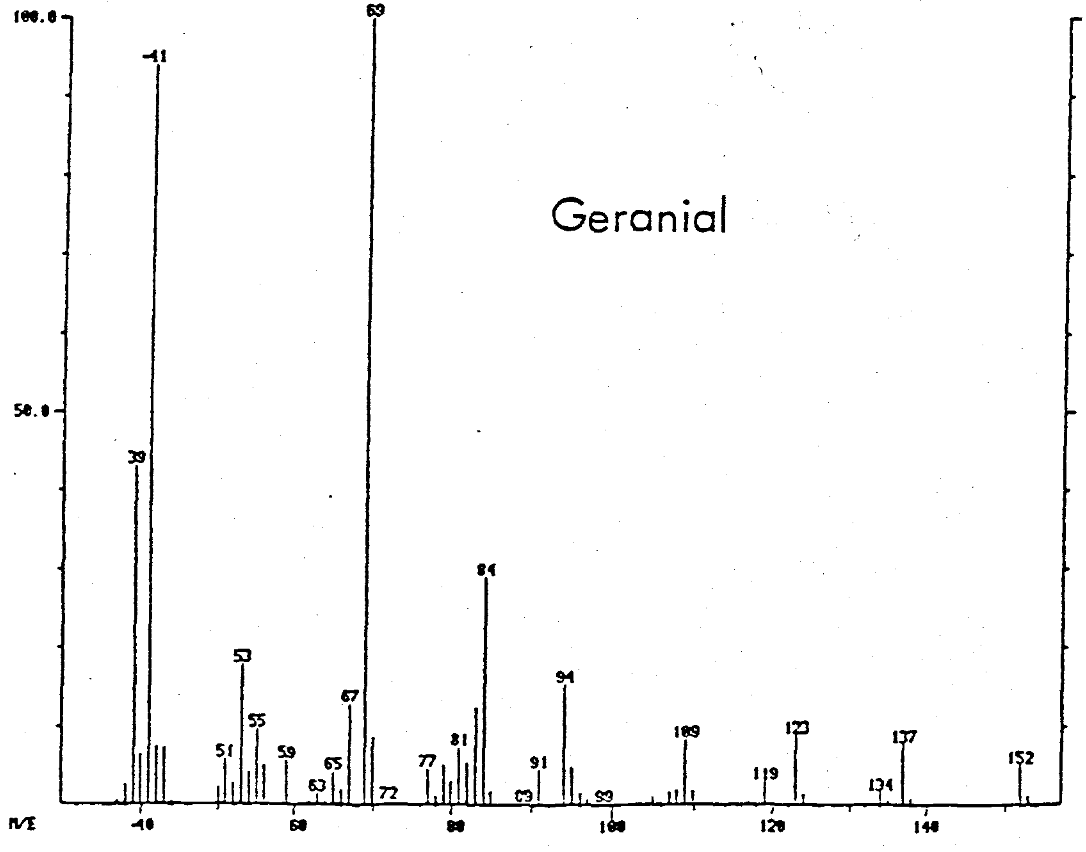

Geranial
100.0
50.0
M/E
40
60
80
100
120
140
69
41
39
51
53
55
59
63
65
67
72
77
81
84
89
91
94
99
109
119
123
134
137
152

Figure 50. A comparison of water, citral and Kelthane treatments on mite populations in lily bulb. Number of mites at inception is indicated by star.

Figure 50

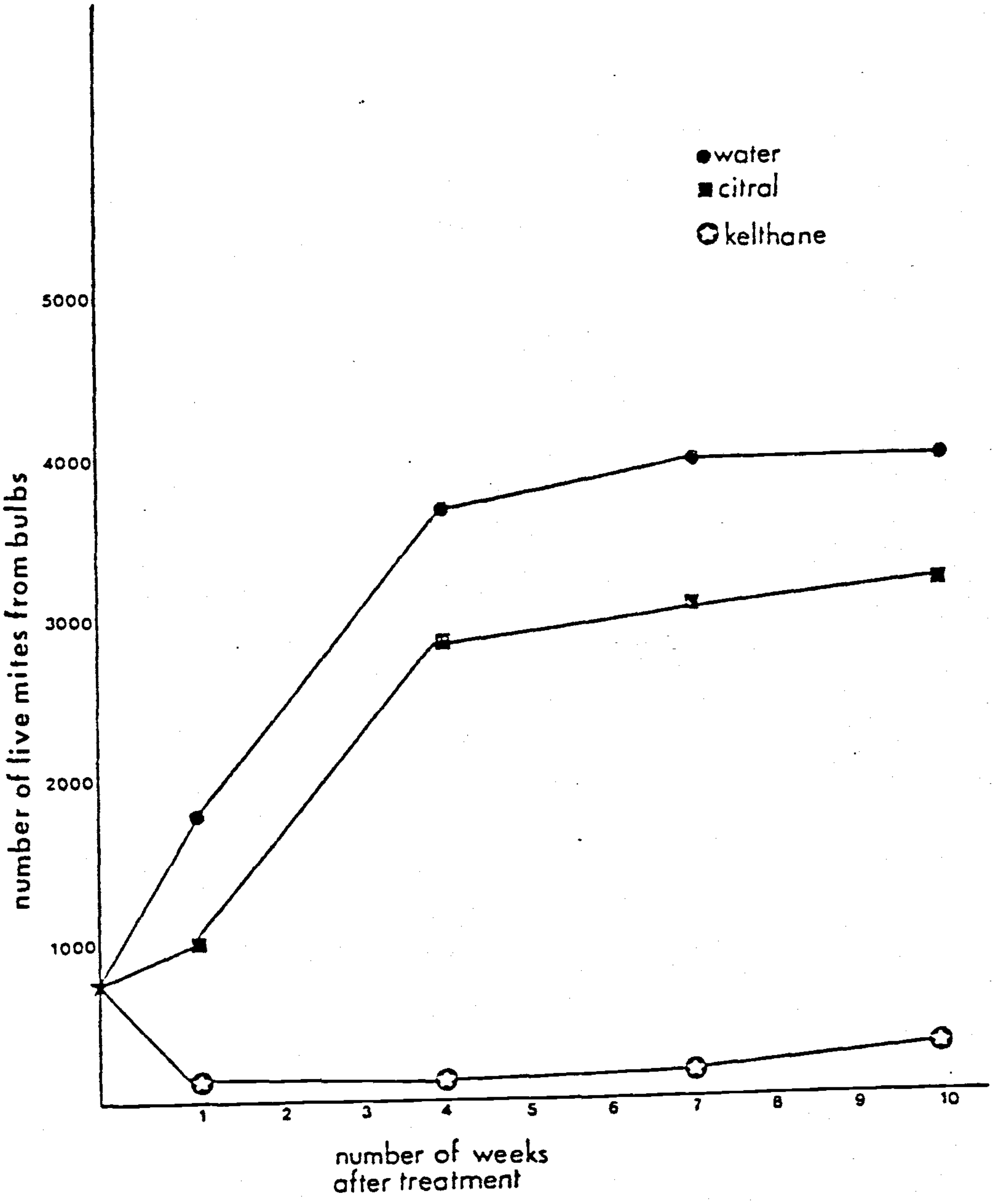

water
citral
kelthane
number of live mites from bulbs
5000
4000
3000
2000
1000
1
2
3
4
5
6
7
8
9
10
number of weeks
after treatment

Figure 51. A comparison of the effects of Kelthane and Kelthane-citral treatments on mite populations for the first seven days.

Figure 51

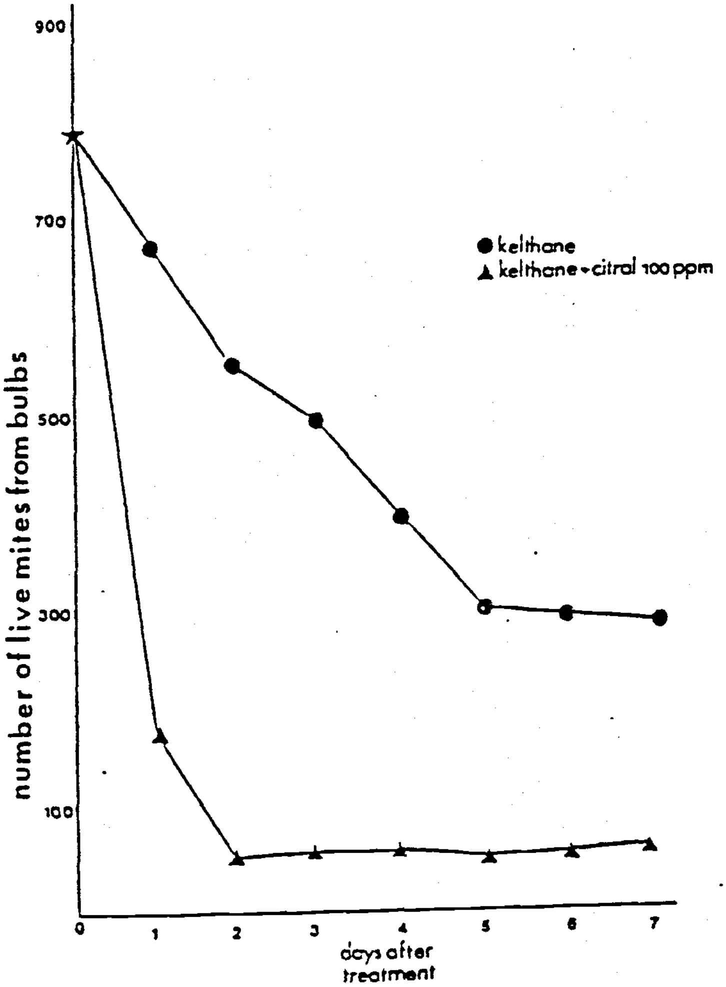

900
700
500
300
100
number of live mites from bulbs
0
1
2
3
4
5
6
7
days after treatment
● kelthane
▲ kelthane+citral 100 ppm

Figure 52. A comparison of the effects of Kelthane and Kelthane-citral treatments on mite populations over a 10-week period following treatment.

Figure 52

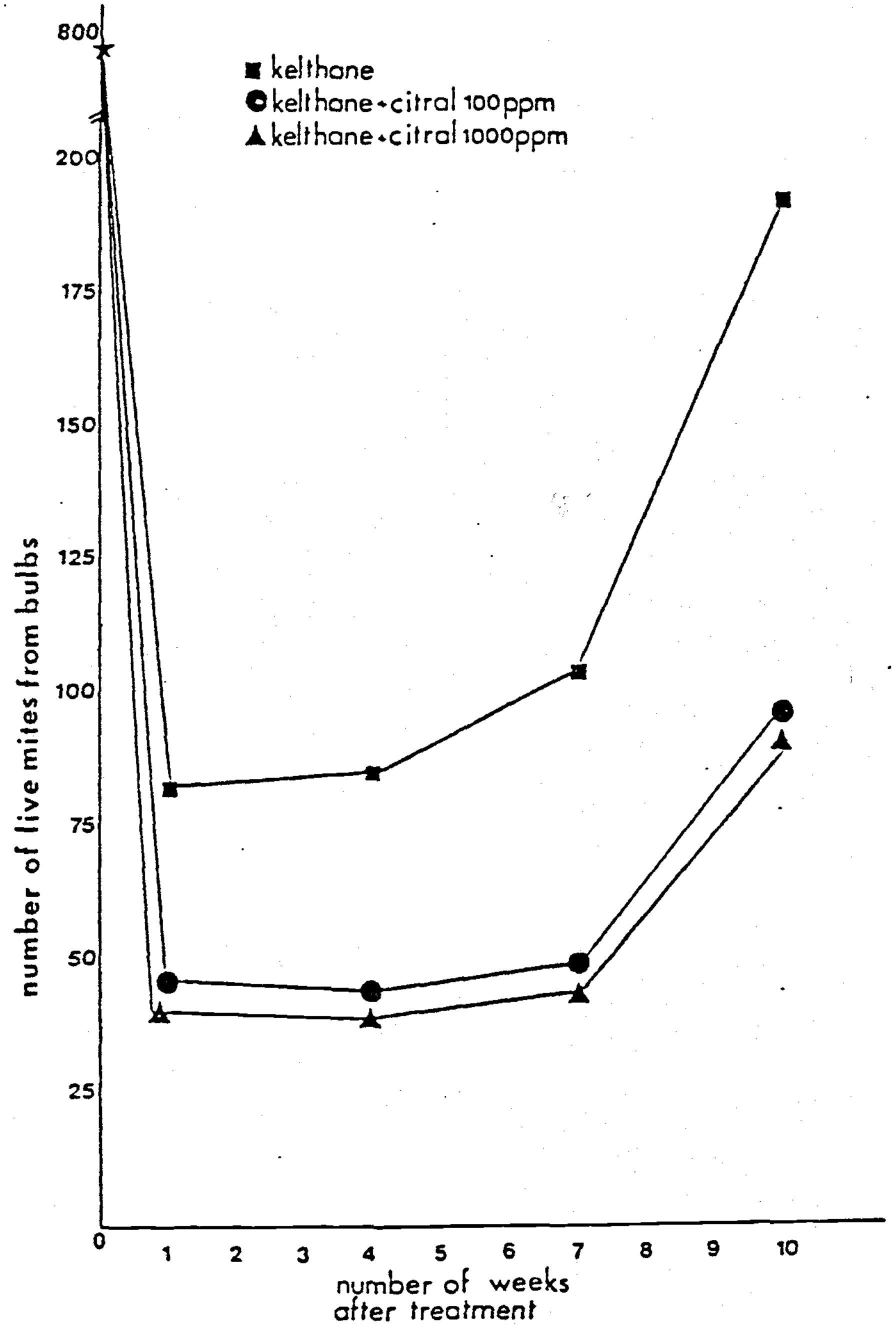

■ kelthane
● kelthane+citral 100ppm
▲ kelthane+citral 1000ppm
number of live mites from bulbs
800
200
175
150
125
100
75
50
25
0
1
2
3
4
5
6
7
8
9
10
number of weeks after treatment

Table 1. Effect of water, citral, and Kelthane treatments on numbers of *R. robini* extracted from the soil in which treated bulbs were placed.

Table 1

Treatment Weeks after Treatment	Citral	Water	Kelthane	Kelthane plus citral @ 100 ppm	Kelthane plus citral @ 1000 ppm
1	526	63	27	25	23
3	246	56	17	18	14
7	110	55	15	12	12
10	71	65	20	19	20

CHAPTER III

Some Observations on the Biology of _R. robini_

Introduction

There are several phytopathogens that may damage the bulb portion of lilies, gladioli, and narcissi (Garrett 1956). Mites have been implicated in the dissemination of several of these plant pathogens. Forsberg (1959) and Englehard (1969) listed several acaridid mites as potential vectors of fungal pathogens on gladiolus and narcissus corms. Poe et al. (1979) demonstrated the acquisition and retention of _Pseudomonas marginata_ by _Histiostoma feroniarum_ (DuFour) and _R. robini_. _Fusarium_ spp. and _Cylindrocarpon_ spp produce root rots in lily bulbs often causing severe economic losses for commercial lily growers. Based on the possibility that _R. robini_ may be involved in spreading these fungal pathogens, experiments were devised to determine whether _R. robini_ feeds on _Fusarium_ and/or _Cylindrocarpon_, and to determine if the fungal material can pass through the digestive system of _R. robini_ in a viable state.

A non-feeding faculative deutonymph or hypopus, may appear between the protonymphal and tritonymphal stages during the life cycle of _R. robini_ (Michael 1884). The hypopus is heteromorphic, differing considerably from the other stages in its morphology and behaviour. Environmental factors leading to the formation of the hypopodial stage have been identified for several species of acaridid mites; the literature on the factors causing the induction of the hypopodial stage has been reviewed by Wallace (1960), Woodring (1963), Chmielewski (1967) and Griffiths (1967). However, few studies have been done on the factors influencing hypopodial termination (Schulze 1924, Baker 1964, Cutcher and Woodring 1969, Kuo and Nesbitt 1970). The effect of temperature, relative humidity and substrate on the termination of the hypopodial stage of *R. robini* are reported in this chapter.

Hodson (1928) and Garman (1937) gave accounts of the life history of *R. echinopus* at temperatures of 60° F and 70° F. Their results indicated that differences in temperature have an effect on the developmental rate. The effect of temperature on the post-embryonic developoment of *R. robini* is presented in this section.

Materials and Methods

Mites were removed from stock cultures and placed on colonies of *Fusarium oxysporum*, *F. solani* and *Cylindrocarpon* sp. for 24hr. The mites were then rinsed in 2 sterile water baths and placed in a third bath for 1 hr. While in the third bath, the mites deposited fecal pellets which were collected for analysis. Since the pellets were in sterile water, the possibility of surface contamination was negligible. The pellets were removed and subjected to another sterile water bath, and then transferred to the fungal culture media. The media were incubated at 21° C for 1 week and examined for possible presence of fungi.

Hypopodes of R. robini were placed on 2 cm discs of Bot and Meyer medium in zipper vials and held in incubating chambers at temperatures of 15° C to 33° C at a relative humidity of 80%. A second group of hypopodes was held at 26° C in a series of chambers at relative humidities 40% - 95%, at increments shown in Table 5. Individuals of a third group of hypopodes were placed on various plant and animal substrates and maintained at 26°C and 80% rh.

Individual eggs of R. robini were placed on Bot and Meyer medium discs and kept at temperatures from 12° C to 30° C at 78% rh. The time required from hatching to the appearance of the adult was recorded.

Results and Discussion

The results shown in Table 2 indicate that Fusarium oxysporum, F. solani and Cylindrocarpon sp. are ingested by R. robini, and that viable material of all three fungal species is able to pass through the digestive tract of the mite. Although the results do not prove that R. robini is involved in the epidemiology of root rot in lily bulbs, the passage of viable fungi in the fecal pellets suggests that R. robini may provide a means for dissemination of fungal pathogens in the field.

Observations on the termination of the hypopodial stage are summarized in Tables 3, 4 and 5. Temperature was found to be an important factor affecting termination. The percentage of hypopodes moulting to the tritonymphal stage increased as the temperature increased from 15° C to 29° (Table 4). Also the mean number of days needed for the moult decreased from 12.2 days at 15° C to 2.3 days at 29° C. At temperatures of 31° C and 33° C, the moulting rates were lower. Similar results have been demonstrated for other acardid mites (Schulze 1924, Baker 1964). A substantially greater percentage of hypopodes successfully moulted to the tritonymphal stage when held on plant substrates than when held on animal substrates (Table 4), which is contrary to the results obtained for *Caloglyphus mycophagus* (Megnin) (Kuo and Nesbitt 1970). There was no significant difference between termination rates of hypopodes placed on undamaged and damaged lily bulb tissue. Hypopodes held on the Bot and Meyer medium had the highest percentage of hypopodes moulting to the next stage (93%). Varying relative humidity had little effect on stage termination (Table 5).

There is a negative correlation between temperature and the duration of the larval and nymphal stages, and temperature and the fecundity of *R. robini* (Figs. 53, 54). There is a linear relationship between temperature and post-embryonic development until less than optimal temperatures are reached (29° C and higher) (Fig. 53).

Conclusion

Recovery of viable *Fusarium* and *Cylindrocarpon* from the fecal pellets of *R. robini* previously maintained on a substrate of these fungi showed that *R. robini* ingests fungal material which passes through the digestive system in a viable state. *R. robini* should therefore be considered a potential vector of fungal pathogens on lily.

Temperature was an important factor in the termination of the hypopodial stage. Food substrate played an important role, with more hypopodes moulting to the tritonymphal stage when placed on plant substrates than on animal substrates. The rate of post-embryonic development was significantly influenced by temperature.

Table 2. Fungal colonies present on nutrient agar plates derived from fecal pellets of *R. robini* that were fed on 3 pure fungal cultures.

Table 2

Pellets from mites reared on

F. oxysporum			*F. solani*			*Cylindrocarpon* sp.		
No. pellets recovered	No producing fungi	% production	No. pellets recovered	No producing fungi	% production	No. pellets recovered	No producing fungi	% production
23	20	87	22	19	86	19	17	89
18	15	83	23	20	87	24	22	88
26	24	92	22	20	91	24	22	92
25	23	92	20	18	90	20	18	90
21	19	90	20	18	90	22	20	90
113	101	89 (average)	107	95	89 (average)	109	98	90 (average)

Table 3. Effect of temperature on moulting of hypopodes to the tritonymphal stage of *R. robini* that were placed on Bot and Meyer's medium at 80% rh.

Table 3

Temperature (^{o}C)	No. of hypopodes[1]	No. (and %) molting	Days Required Range	Mean
15	120	24(20%)	11-13	12.2
17	90	36(40%)	7-10	9.0
19	105	54(51%)	3-6	5.1
21	90	54(60%)	1-5	3.0
23	75	51(68%)	1-5	2.8
25	75	57(76%)	1-4	2.8
27	90	82(91%)	1-4	2.5
29	90	84(93%)	1-3	2.3
31	105	87(83%)	1-4	2.5
33	105	84(82%)	1-4	2.5

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[1] Each figure represents a total of 3 equal replicates. Thus, the figure 120 signifies 3 replicates of 40 individuals each.

Table 4. Effect of food substrate on moulting of hypopodes of *R. robini* to the tritonymphal stage at 26° C and 80% rh.

Table 4

Substrate	No. of hypopodes[1]	% molting	Days Required Range	Mean
Undamaged lily bulb	95	87	2-5	2.4
Rotting bulb	95	86	2-5	2.4
Potato	90	81	2-5	2.7
Ground mealworm (_Tenebrio_)	95	60	2-5	3.3
Ground cockroach (_Leucophaea_)	95	57	2-6	3.4
Cat chow	90	62	2-6	3.6
Artificial medium (Bot and Meyer's)	90	93	2-5	2.3

[1] Each figure represents a total of 3 equal replicates.

Table 5. Effect of relative humidity on moulting of hypopodes of *R. robini* on Bot and Meyer's medium at 26° C.

Table 5

Relative humidity (%)	No. of hypopodes[1]	% molting	Days Required Range	Days Required Mean
40	99	45	2-6	3.6
50	96	47	2-6	3.6
60	90	49	2-6	3.5
70	90	53	2-7	3.5
80	96	54	2-5	3.6
90	96	54	2-6	3.3
95	96	54	2-5	3.4

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[1] Each figure represents a total of 3 equal replicates.

Figure 53. Effects of temperature on post embryonic development of R. robini.

Figure 53

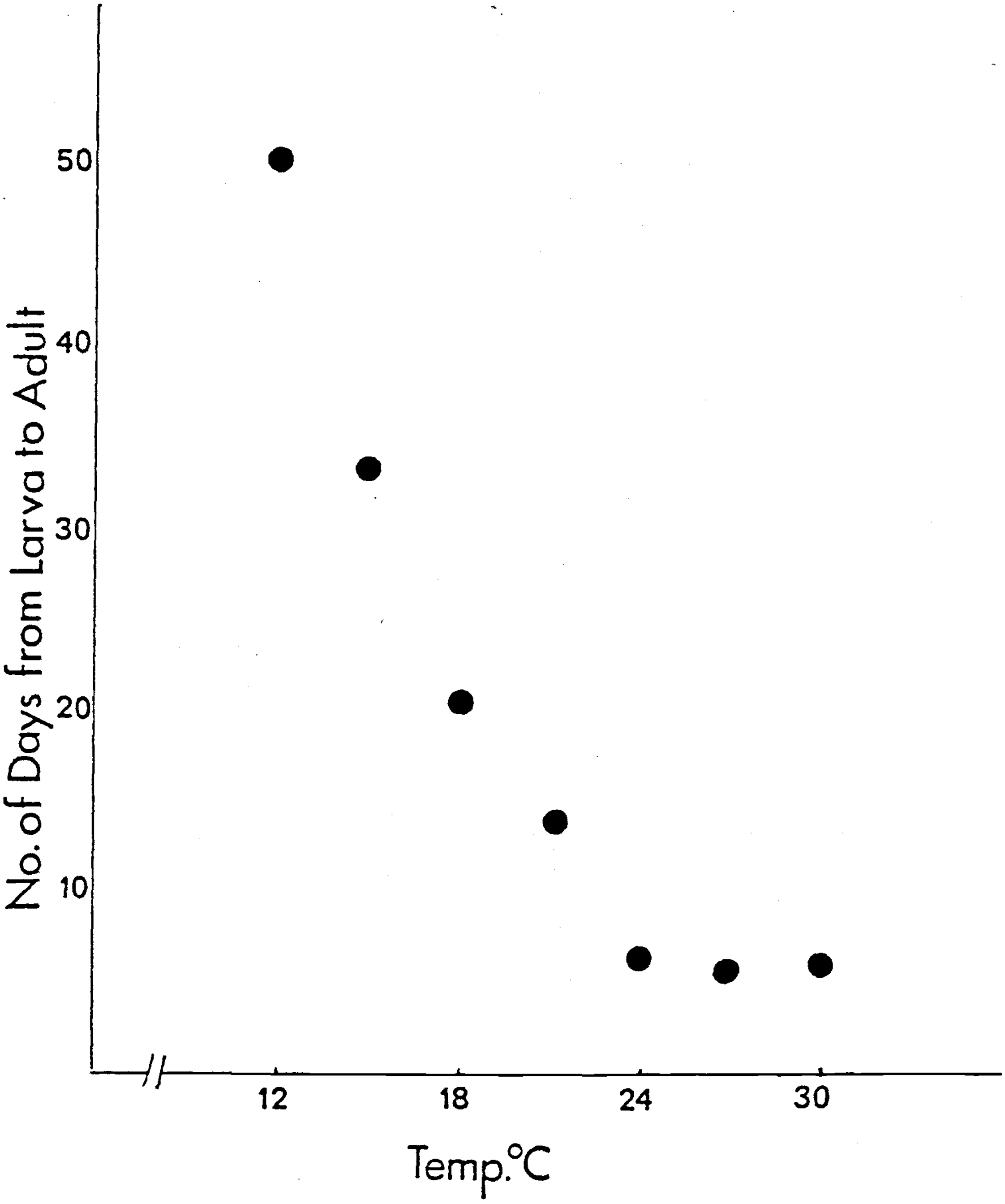
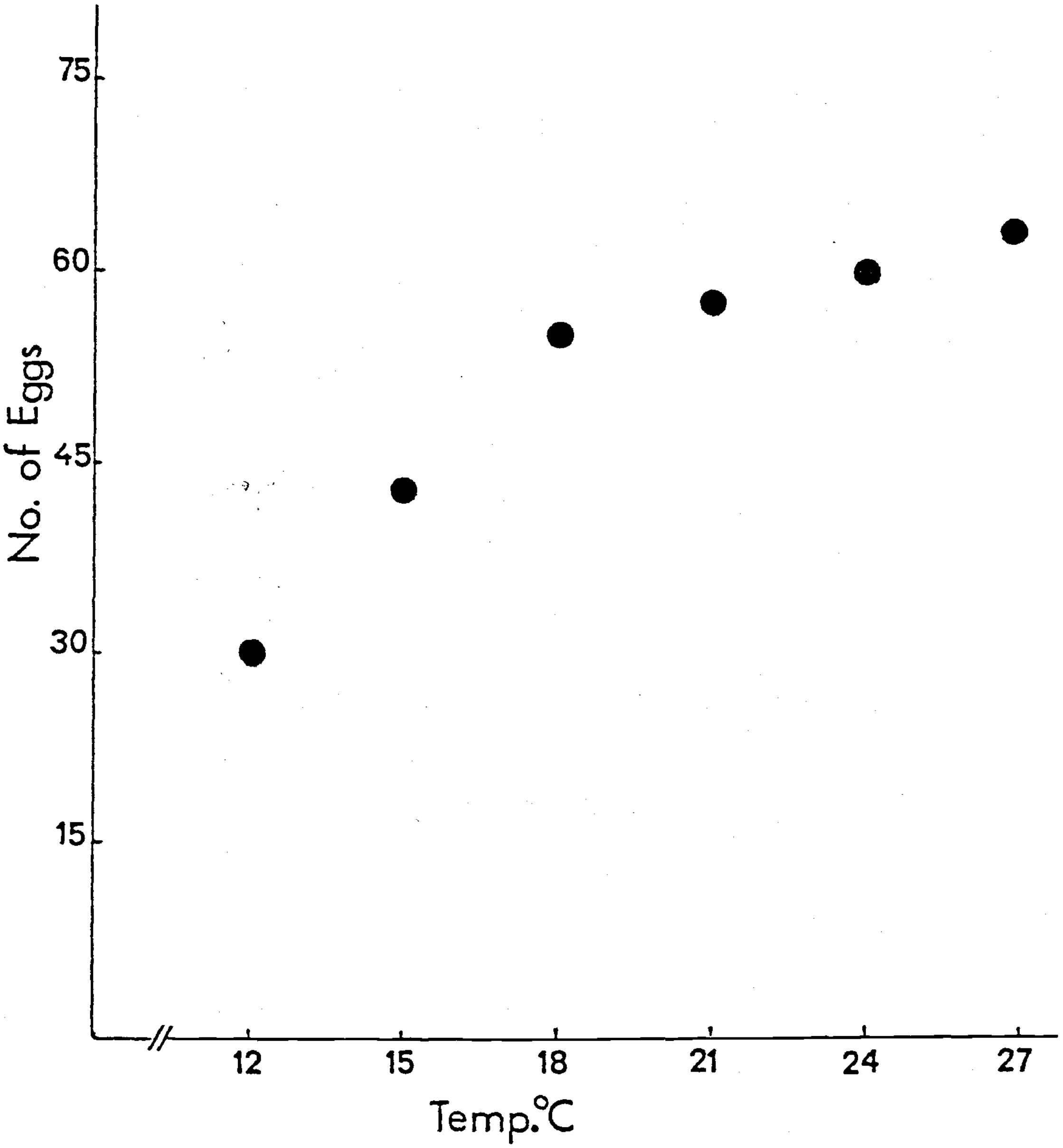


Figure 54. Effect of temperature on egg production of *R. robini*.

Figure 54



BIBLIOGRAPHY

Alberti, G. 1979. Fine structure and probable function of genital papillae and Claparede organs of Actinotrichida. *In*: Recent Advances in Acarology, 2: 501-507.

Anderson, W. A. and R. A. Ellis. 1967. A comparative electron microscope study of visceral muscle fibers in *Cambarus*, *Drosophila* and *Lumbricus*. Z. Zellforsch. 79: 581-591.

Ascerno, M., F. Pfleger and F. Wilkins. 1981. Effects of root rot and *Rhizoglyphus robini* on greenhouse-forced Easter lily development. Environ. Entomol. 10: 947-949.

Baker, R. A. 1964. The further development of the hypopus of *Histiostoma feroniarum* (Dufour). Ann. Mag. Nat. Hist. Ser. 13 7: 693-695.

Baker, R. A. 1975. The structure and function of the alimentary canal in *Histiogaster carpio* (Kramer 1881) Acari-Sarcoptiformes. Acarologia 17: 126-137.

Barth, P. G. 1970. Die Feinstrucktur des Spinnenintegument II. Die raumleche Anordung der Mikrofasern in der lamellierten Cuticula und ihre Beziehung zur Gesalt der Porekanale, *Cuprennius sale* Keys. Z. Zellforsch. 104: 87-106.

Bergstrom, G. and J. Lofqvist. 1970. Chemical basis for odour communication in four species of *Lasius* ants. J. Insect Physiol. 16: 2353-2375.

Blum, M. and G. Bohart. 1972. Neral and geranial: identification in a colletid bee. Ann. Ent. Soc. Amer. 65: 274-275.

Boczek, K., C. Jura and A. Krzysztofourticz. 1969. The comparison of the structure of the internal organs of post-embryonic stages of *Acarus farris* (Oud.) with special reference to the hypopus. Proc. Int. Congr. Acarol., England p. 265-271.

Bostanian, N. J. and F. O. Morrison. 1973. Morphology and ultrastructure of sense organs in the two spotted spider mite (Acarina: Tetranchyidae). Ann. Entomol. Soc. Amer. 66: 379-383.

Bot, J. and M. Meyer. 1969. An artifical rearing medium for acarid mites. J. South Afr. Entomol. Soc. 29: 199.

Brody, A. R. 1969. Comparative fine structure of acarine integument. J. NY Entomol. Soc. 77: 105-116.

Byrne, K., W. Gore, G. Pearce and R. Silverstein. 1975. Porapak Q collection of airborne organic compounds serving as models for insect pheromones. J. Chem. Ecol. 1: 1-7.

Chmielewski, W. 1967. The formation and role of the hypopus stage in mites. Ekol. Pol. A. 13: 325-338.

Chow, Y.,S., C. B. Wang and L. C. Lin. 1975. Identification of a sex pheromone of the female brown dog tick, Rhipicephalus sanguineus. Ann. Entomol. Soc. Amer. 68: 485-488.

Claparède, E. 1868. Studien an Acariden. Z. wiss. Zool. 18: 445-546.

Cone, W., L. McDonough, T. Maithlen and S. Borsajewicz. 1971a. Pheromone studies of the two spotted spider mite. I. Evidence of a sex pheromone. J. Econ. Entomol. 64: 355-358.

Cone, W., S. Predki, and E. C. Klostermeyer. 1971b. Pheromone studies of the two spotted spider mite. II. Behavioral response of males to quiescent deutonymphs. J. Econ. Entomol. 64: 379-382.

Cutcher, J. J. and J. P. Woodring. 1969. Environmental regulation of hypopial apolysis of the mite, Caloglyphus boharti. J. Insect Physiol. 15: 2045-2057.

Devine, T. L. and G. W. Wharton. 1973. Kinetics of water exchange between a mite, Laelaps echinidus, and the surrounding air. J. Insect Physiol. 19: 243-252.

Englehard, A. W. 1969. Bulb mites associated with diseases of gladiolus and other crops in Florida. Phytopathol. 59: 1105.

Eyndhoven van, G. L. 1961. Artunterschiede beim Genus Rhizoglyphus (Acar). Proc. IX Int. Congr. Entomol. Vol. I, p. 274-276.

Eyndhoven van, G. L. 1968. Rhizoglyphus engeli nov. sp., with notes on the genus Rhizoglyphus (Acari: Acaridida). Beaufortia 15: 95-103.

Foelix, R. F. and R. C. Axtell. 1971. Fine structure of tarsal sensilla in the tick Amblyomma americanum (L.). Z. Zellforsch 114: 22-37.

Forsberg, J. L. 1959. Relationship of the bulb mite Rhizoglyphus echinopus to bacterial scab of gladiolus. Phytopathol. 49: 539.

Furstenburg, M. 1861. Die Kratzmilben der Menchen und Thiere. Leipzig, 222 pp.

Garman, P. 1937. A study of the bulb mite. Conn. Agric. Expt. Sta. Bull. 402, pp. 889-908.

Garrett, S. D. 1956. Biology of Root Infecting Fungi. Cambridge University Press.

Gibbs, K. E. and F. O. Morrison. 1959. The cuticle of the two spotted spider mite, Tetranchyus telarius (L.) (Acarina: Tetranychidae). Can. J. Zool. 37: 633-637.

Graf, J. F. 1975. Ecologie et ethologie d 'Ixodes ricinus en Suisse (Ixodides: Ixodidae). Cinquen note: Mise en evidence d'une pheromone sexuelle chez Ixodes ricinus. Acarologia 17: 436-441.

Griffiths, D. C. and J. Pickett. 1980. A potential application of aphid alarm pheromones. Ent. Exp. and Appl. 27: 199-201.

Hagopian, M. 1966. The myofilament arrangement in the femoral muscle of the cockroach, Leucophaea maderae Fab. J. Cell Biol. 28: 545-562.

Hodson, W. E. 1928. The bionomics of the bulb mite Rhizoglyphus echinopus. Bull. Ent. Res. 19: 187-200.

Howe, N. and Y. Sheikh. 1975. Anthopleurine: a sea anemone alarm pheromone. Science 189: 386-388.

Howe, N. 1976. Behaviour of sea anemones evoked by the alarm pheromone, anthopleurine. J. Comp. Physiol. 107: 67-76.

Hoy, M. A. and J. M. Smilanick. 1979. A sex pheromone produced by immature and adult females of the predatory mite, Metaseiulus occidentalis (Acarine: Phytoseiidae). Ent. Exp. and Appl. 26: 291-300.

Hughes, A. M. 1976. The Mites of Stored Food. Min. Agric. Fish. and Food, London, U. K. 287 pp.

Hughes, T. E. 1969. Presidential address. Proc. 2nd Int. Congr. Acarol. pp. 11-14.

Hughes, T. E. and A. M. Hughes. 1939. The internal anatomy and post-embryonic development of *Glycyphagus domesticus* De Geer. Proc. Soc. Zool. B, 8: 715-733.

Huxley, H. E. 1965. The mechanisms of muscular contraction. Sci. Amer. 213: 18-27.

Jalil, M. and G. Rodriquez. 1970. Studies of behavior of *Macrocheles muscaedomesticae* (Acarina: Macrochelidae) with emphasis on its attraction to the house fly. Ann. Entomol. Soc. Amer. 63: 738-744.

Kuo, J. and H. H. J. Nesbitt. 1970. The internal morphology and histology of adult *Caloglyphus mycophagus* (Megnin) (Acarina: Acaridae) Can. J. Zool. 48: 505-518.

Kuwahara, Y., H. Fukami, S. Ishii, K. Matusomoto and Y. Wada. 1980a. Pheromone study on acarid mites III. Citral: isolation and identification from four species of acarid mites. Jap. J. Sanit. Zool. 31: 49-52.

Kuwahara, Y., K. Matsumoto and Y. Wada. 1980b. Pheromone study on acarid mites IV. Citral: composition and function as an alarm pheromone and its secretory gland in four species of acarid mites. Jap. J. Sanit. Zool. 31: 73-80.

Lai-Fook, J. 1967. The structure of developing muscle insertions in insects. J. Morph. 123: 5033-528.

Lane, N. J. 1968. The thoracic ganglion of the grasshopper, *Melanoplus differentilis*: fine structure of the perineurium and neuroglia. Z. Zellforsch. mikrosk. Anat. 86: 293-312.

Leahy, M., R. Vandehey and R. Galun. 1973. Assembly pheromone in the soft tick *Argas persicus* (Oken). Nature, London 246: 515-517.

Leahy, M., G. Karuhize, C. Mango and R. Galun. 1975a. An assembly pheromone and its perception in the tick *Ornithodorus moubata* (Murray). J. Med. Entomol. 12: 284-287.

Leahy, M., S. Sternberg and C. Mango. 1975b. Lack of specificity in assembly pheromones of soft ticks (Acari: Argasidae). J. Med. Entomol. 12: 413-414.

Lees, A. D. 1947. Transpiration and the structure of the epicuticle of ticks. J. Exp. Biol. 23: 379-410.

Locke, M. 1961. Pore canals and related structure in insect cuticle. J. Biophys. Biochem. Cytol. 10L 589-618.

Locke, M. 1964. The structure and formation of the integument of insects. In: Physiology of Insecta, M. Rockstein (ed.), Vol. 3, pp. 379-470. Academic Press, New York.

Locke, M. 1967. The development of patterns in the integument of insects. Adv. Morphogenesis 6: 33-38.

Maddrell, S. H. and J. E. Treherne. 1967. The ultrastructure of the perineurium in two insect species, Carausius morosus and Periplaneta americana. J. Cell. Sci. 2: 119-128.

Manson, D. C. M. 1972. A contribution to the study of the genus Rhizoglyphus Claparede, 1869 (Acarina: Acaridae). Acarologia 13: 621-650.

Manson, D. C. M. 1977. A new species of Rhizoglyphus from Columbia, South America (Acarina Acaridae) Int. J. Acarol. 3: 99-104.

Masada, Y. 1975. Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry. Hirokawa Publ. Co., Inc. Tokyo, Japan, 364 pp.

Maschwitz, U. and Ch. Gutman. 1970. Spur- and Alarmstoffe bei der geflecken Brutwanze Elasmucha grisea (Heteroptera: Acanthosomidae). Insectes Soc. 26: 101-111.

Mathur, S. H. and E. J. LeRoux. 1965. The musculature of the velvet mite, Allothrombium lerouxi Moss. Ann. Soc. Entomol. Quebec 10: 129-164.

Matsumoto, K., Y Wada and M. Okamoto. 1979. The alarm pheromone of grain mites and its fungal effect. Recent Advances in Acarology Vol. I, pp. 243-249, Academic Press, New York.

Michael, A. D. 1901. British Tyroglyphidae, Vol. I. Ray Society, London. 291 pp.

Mitchell, R. D. 1962. The musculature of a trombiculid mite Blankaartia acuscutellaris (Walch). Ann. Entomol. Soc. Amer. 55: 106-119.

Montgomery, M. and L. Nault. 1977. Comparative response of aphids to the alarm pheromone (E) -β farnesene. Ent. Exp. and Appl. 22: 236-242.

My-Yen, L., K. Matsumoto, Y. Wada and Y. Kuwahara. 1980. Pheromone study of acarid mites V. Presence of citral as a minor component of the alarm pheromone in the mold mite, Tyrophagus putrescentiae (Schrank) (Acarina: Acaridae). App. Ent. Zool. 15: 474-477.

Nalepa, A. 1884. Die Anatomie der Tyroglyphen I. Sitb. Akad. Wiss. Wien. 90: 197-228.

Nalepa, A. 1885. Die Anatomie der Tyroglyphen II. Sitb. Akad. Wiss. Wien. 92: 116-127.

Nathanson, M. E. 1967. Comparative fine structure of sclerotized and unsclerotized integument of the rabbit tick, Haemaphysalis leporispalustris (Acari: Ixodidae). Ann. Entomol. Soc. Amer. 60: 1125-1135.

Okamoto, M., K. Matsumoto, Y. Wada and H. Nakano. 1978. Studies on the antifungal effect of mite alarm pheromone citral. Evaluation of the antifungal effect of citral. Jap. J. Sanit. Zool. 29: 255-260.

Pagenstencher, H. A. 1882. Einiges zur Anatomie von Tyroglyphus siro. Z. wiss. Zool. 11: 122.

Poe, S. L., W. Noble and R. E. Stall. 1979. Aquisition and retentions of Pseudomonas marginata by Anoetus feroniarum and Rhizoglyphus robini. Recent Advances in Acarology, Vol. I, pp. 119-124.

Rechav, Y., G. B. Whitehead and M. M. Knight. 1976. Aggregation response of nymphs to pheromones produced by Amblyomma hebraeum. Nature, London. 256: 563-564.

Regev, S. and W. W. Cone. 1976. Evidence of farnesol as a male sex attractant of the two-spotted spider mite, Tetranychus urticae Koch (Acarina: Tetranychidae). Environ. Entomol. 4: 307-311.

Regev. S. and W. W. Cone. 1980. The monoterpene citronellol, as a male sex attractant of the two-spotted spider mite, Tetranychus urticae (Acarina: Tetranychidae). Environ. Entomol. 9: 50-52.

Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17: 208-212.

Rock, G. C., R. J. Monroe and D. R. Yeargon. 1976. Demonstration of a sex pheromone in the predaceous mite, Neoseiulus fallacis. Environ. Entomol. 5: 164-166.

Rohde, C. J. and D. A. Demick. 1967. Anatomy of the digestive and reproductive systems in the acarid mite (Sarcoptiformes). Acarologia 9: 608-616.

Schulze, H. 1924. Uber die Biologie von Tyroglyphus mycophagus (Megnin) zugleich ein Beitrag zur Hypopusfrage. Z. Morphol. Okol. Tiere 2: 1-57.

Sleeper, H., V. Paul and W. Fenical. 1980. Alarm pheromone from the marine opisthobranch Navanax inermis. J. Chem. Ecol. 6: 57-70.

Smith, D. S. 1962. Cytological studies on some insect muscles. Rev. Can. Biol. 21: 279-301.

Smith, D. S. 1965. The flight muscles of insects. Sci. Amer. 212: 76-88.

Smith, D. S. 1966. The organization of flight muscle fibre in Odonata. J. Cell Biol. 28: 109-128.

Smith, D. S. 1968. Insect Cells: Their Structure and Function. Oliver and Boyd, Edinburgh, Scotland.

Sonenshine, D. E., R. Silverstein, E. C. Layton and P. Homsher. 1974. Evidence for the existence of a sex pheromone in 2 species of ixodid ticks (Metastigmata: Ixodidae). J. Med. Entomol. 11: 307-315.

Sonenshine, D. E., D. M. Gainsberg, M. D. Rosenthal and R. M. Silverstein. 1981. The sex pheromone glands of Dermacentor variabilis (Say) and Dermacentor andersoni Stiles, sex pheromone stored in neutral lipid. J. Chem. Ecol. 7: 345-357.

Vercammen-Grandjean, P.-H. 1975. Les organs des Claparede et les papilles genitales de certains acariens sont-ils des organes respiratoires. Acarologia 17: 624-630.

Vijayambika, V. and P. A. John. 1975. Internal morphology and histology of the fish mite Lardoglyphus konoi (Sasa and Asanuma) (Acarina: Acaridae). Acarologia 16: 709-718.

Wallace, D. R. 1960. Observations on the hypopus development in the Acarina. J. Insect Physiol. 5: 216-219.

Wharton, G. W., W. Parish and D. E. Johnston. 1968. Observations on the fine structure of the cuticle of the spiny rat mite, Laelaps echidnina (Acari: Mesostigmata). Acarologia 10: 206-214.

Winston, P. W. 1969. The effect of dessication on the humidity preferences of Bryobia praetiosa Koch (Acarina: Tetranychidae) Amer. Zool. 9: 654-655.

Wohlers, P. 1980. Die Fluchtreaktionen der Erbsenlaus, Acyrthosiphon pisum ausgelost durch Alarmpheromon und zusatzleche Reize. Ent. Exp. and Appl. 27: 156-168.

Woodring, J. P. 1963. The nutrition and biology of saprophytic Sarcoptiformes. Advances om Acarology Vol. I, pp. 89-111.