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An Autoradiographic Search for the Site of Fertilization in Spider Mites.

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FERTILIZATION IN SPIDER MITES.

The Louisiana State University and Agricultural
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Zoology

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AN AUTORADIOGRAPHIC SEARCH FOR THE SITE OF
FERTILIZATION IN SPIDER MITES

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Entomology

by

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M.S., Louisiana State University, 1967
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ABSTRACT

The fate of spermatozoa after mating had not been previously investigated in tetranychid mites. Histological evidence indicated that the spermatheca connects with the outside only. Autoradiography was employed in an attempt to trace the passage of spermatozoa from the spermatheca to the egg. Labeled spermatozoa were obtained by rearing males on bean seedlings growing in ^3H -thymidine solution. Heavy labeling was found in those mites feeding on the radioactive leaves, and radioactivity was found to be passed on transovarially. By mating radioactive males to untreated females and preparing autoradiographs of these females at varying intervals after mating, the seminal material was traced.

Labeled seminal material was located in the spermatheca of recently mated females. Females mated to radioactive males showed a general body labeling several hours after copulation. Eggs in these mated females showed patterns of labeling not found in ^3H -fed or untreated females. It is hypothesized that the spermatozoa leave the spermatheca and travel through the haemolymph to the ovary where eggs are fertilized precociously.

INTRODUCTION

Terrestrial animals must have a method of internal fertilization if they are to reproduce sexually. Most aquatic organisms simply shed their gametes into the water where eggs are fertilized, and where development takes place. Because the gametes of terrestrial animals are in effect aquatic organisms they must be provided with the appropriate environment in which to function.

Terrestrial arthropods have evolved systems by which the male can transfer spermatozoa. Two main trends have resulted. One involves the transfer of a spermatophore, which is common in aquatic arthropods and many insects. The other has resulted in the elimination of the spermatophore and the development of a more or less elongated male copulatory organ which transports semen directly to a female sperm storage organ.

According to Baker and Wharton, (1952) fertilization occurs internally in the Acarina, but the methods by which the spermatozoa are introduced into the females reproductive system vary considerably. A copulatory organ is lacking in the Mesostigmata and spermatophores are introduced into the female by chelicerae. In other Acarina spermatozoa may be introduced by a penis.

Spider mites (Acarina: Tetranychidae) employ the second method of internal fertilization. The male mite has a copulatory organ called an aedeagus, which enables the male to place sperm into the spermatheca of the female.

This study is concerned with how the sperm travels to the site of fertilization after deposition in the spermatheca, and where this site is located in the female. In mating, spermatozoa are introduced into a receptacle whose only apparent opening is to the outside. The path taken by spermatozoa from the receptacle to the oocytes has not been studied.

REVIEW OF LITERATURE

Tetranychids are arrhenotokous; that is, virgin females produce eggs all of which become males, while mated females lay eggs which develop into either females or males. The males from eggs laid by mated females are haploid and all their genes originate only from their mother (Helle and Von Zon, 1966). Thus all males are uniparental. A few species of tetranychids are strictly parthenogenetic and reproduce without males (thelytoky) (Boudreaux, 1963).

The investigations made into the internal anatomy of spider mites have shed little light on the process of

fertilization. Many of the reports on the structure of the female genital system are vague if not incorrect.

Claparede (1868) gave a brief description of the development of Tetranychus telarius (Duges). This early work contained no investigation into the internal anatomy.

Michael (1896) described the internal anatomy of Bdella basteri (Johnston), an insect predator which lives along the sea coast. According to Michael, the receptaculum seminis, or spermatheca, is an azygous sac formed by an invagination of the wall of the oviduct not far from the external labia, but still not in the flexible membranous portion of the duct which adjoins the labia. Michael found that "the receptaculum seminis is but slightly seen in immature females and sometimes not at all in very old ones after the eggs are impregnated." He further stated that the receptaculum seminis in B. basteri is not hard or chitinized, but soft and flexible, with its walls composed of columnar cells, and its exterior in most parts covered by a layer of fine constrictor muscles.

Sig Thor (1904) carried out some comparative anatomy among Acarina, but did not include Tetranychus. In all the forms he investigated he found the receptaculum seminis to be a structure formed from the oviduct.

Schrader (1923) described oogenesis and spermatogenesis in Tetranychus bimaculatus Harvey, but

did not describe the reproductive organs nor suggest the possible site of fertilization.

Blauvelt (1945) completed the most comprehensive study yet done on the internal morphology of spider mites. He investigated the anatomy of one of the best known species, Tetranychus telarius (T. urticae Koch). Blauvelt's work is largely based on the study of material sectioned at 2 to 5 microns and stained with iron haematoxylin. Blauvelt gave a detailed description of the female reproductive system in T. telarius. He made no mention of fertilization. Blauvelt stated that the seminal receptacle is a small, erect, ovoid sac situated posterior to the vagina and opening into it through a short curved duct. The epithelium is a single layer of cells arranged radially around a central lumen.

Beament (1951) reported on the structure and formation of the egg of the fruit tree red spider mite Metatetranychus ulmi Koch, and speculated on the subject of fertilization. He stated that the egg is extremely resistant to penetration and to attack by chemicals or solvents, and the shell has no micropyle or other form of entrance for sperm. Because of these characteristics of the egg Beament believed the egg must be fertilized before the shell layer is produced. The shell layer is formed in the ovary, towards the end of yolk accumulation, which means the egg must be fertilized before the ovum

is completely mature. Beament believed that fertilization in tetranychid mites is a similar phenomenon to fertilization in ticks (Lees and Beament, 1948) where the eggs are fertilized precociously before they are even a quarter of their final diameter, apparently by spermatozoa which have migrated up the oviducts. Beament felt the early stage of copulation (immediately on emergence of the female from the last nymphal moult) supported this argument. The spermatozoa of spider mites possess no flagella. The actual method of locomotion of sperm is unknown.

Snieder-Borkenbosch (1955) found some slight differences in the reproductive system of M. ulmi compared with Beament's and Blauvelt's works, but neither the spermatheca nor fertilization was discussed.

Ashton (1961) reported finding a well-developed seminal receptacle in the brown wheat mite Petrobia latens Muller. Males are not known in this species. Therefore this organ must be vestigial or may have a function other than for sperm storage. Ashton suggested that it may be that sexual reproduction occurs occasionally.

Ehara (1960) reported on a comparative study of the internal anatomy of three Trombidiform Acarinids found in Japan. He did not speculate on the time or place of fertilization, but did describe the spermatheca in two species. In Neomolgus littoralis (L), a mite commonly found under sea-weeds and on rocks along the coast of

northern Japan, the spermatheca is a small blind sac arising from the terminal portion of the oviduct. The cells forming the epithelium of the spermatheca are columnar with small nuclei. In Bryobia charai Prichard and Keifer, a species feeding on chrysanthemum in Japan, the spermatheca is a very small, clavate, blind organ entering the posterior part of the vagina via a short curved duct. The epithelium of the spermatheca consists of a single layer of columnar cells surrounding the lumen.

Anwarrulah (1963) described the female reproductive system in M. ulmi, T. viennensis Zacher, Bryobia rubrioculus Scheuten and B. kissophila van Eynhoven. He found that the spermatheca of M. ulmi and T. viennensis corresponded to that reported in the works of Beament and Blauvelt, but the two Bryobia species were different. Anwarrulah stated that no receptaculum seminis was found in the Bryobia.

He suggested that because no males are known in either of these species the genital apparatus is simpler than in the bisexual forms.

Dosse and Langenscheidt (1964) reported a passage connecting the receptaculum seminis with the vagina in the Tetranychus urticae-cinnabarinus complex. Their illustrations show no such connection, and in fact show the receptaculum seminis connecting with the outside. No

attempt was made to explain how fertilization takes place.

Helle (1967) summed up the knowledge on mite fertilization and carried out several interesting mating experiments. Helle found that a virgin female, once inseminated, goes on depositing only haploid eggs for a considerable time after the conclusion of copulation. In regard to the success or failure of a second fertilization he concluded that there is no clear relationship between the time interval and the effect of the second mating. In Helle's experiment four females had successful second matings. He suggested that there is reason to assume that the sperm supply of the first mating is imperative for the success of the second one. Helle explained this by assuming that normally the sperm supply is more than sufficient for fertilization of all oocytes available for fertilization. He speculated that if the sperm supply of the first mating is insufficient, the second sperm supply becomes effective.

Boudreaux (1963) suggested that a barrier may be established by copulation which prevents further effective insemination. He also mentioned the possibility of fertilization of oocytes as found in ticks.

Eyndhoven (in Helle, 1967) has questioned the notion that in the Tetranychidae the spermatheca is connected to the female genital system. Following this

lead, I also have evidence that in the species I studied, the spermatheca is connected to the outside via a narrow passage starting as a copulatory pore behind the oviducal opening, but just ahead of the anus. If the spermatheca is provided with semen through a separate copulatory pore, the question arises concerning the path taken by spermatozoa to penetrate and fertilize the eggs. As best as can be determined, there is no special duct connecting the spermatheca with any part of the female genital system.

The present study was undertaken in order to try to discover the fate of spermatozoa, introduced by copulation into females of Tetranychus spp. and in particular, the method of passage from the spermatheca, into which they are introduced, to the egg cell to be fertilized.

MATERIALS AND METHODS

This investigation was based on attempts to incorporate ^3H -thymidine in the chromosomes of the spermatozoa of males, so that when these marked males were mated to virgin untreated females, it would be possible to locate by autoradiography the labeled spermatozoa in histological sections of these females. The thymidine, as far as is known, is incorporated only in DNA, and any label found in the experimental females may be presumed to originate from introduced labeled spermatozoa. Histological sections must be used because mites are so small (length 500 microns) that dissection is impossible.

A group of virgin female mites was reared from the egg on a bean leaf growing in a solution of ^3H -thymidine. There is reason to believe, from studies in other arthropods, that the egg contains as much as 10,000 (or more) times the DNA contained in somatic cells. Durand (1960) in reporting on studies of the eggs of gryllids, Orthoptera, stated he believes there to be a cytoplasmic storage of DNA or similar material in these insect eggs. Durand showed that an unfertilized egg contained 7,400 times the amount of DNA found in a normal diploid cell when calculated on the basis of bound deoxyribose and

100,000 times that when calculated on the basis of bound phosphorus.

Vickers (1964) reported a similar condition in the cotton boll weevil Anthonomus grandis. He found that the nucleic acids reached their highest levels during early larval development and decreased gradually until the prepupal stage.

It is believed that the DNA in eggs is used during rapid embryo-genesis in the formation of new cells, and that the germ cells will contain some of this DNA. Therefore, the haploid eggs of females reared on a labeled diet will contain labeled DNA which will be incorporated in the chromosomes of the male mite hatching from that egg. After rearing such labeled males on ^3H -thymidine treated leaves, they were then mated to unlabeled virgin females. The females were fixed at various intervals after mating and sectioned. These sections were placed on a microscope slide and covered with autoradiographic emulsion and allowed to be exposed to the tritium radiation for at least two weeks. Upon development the autoradiographs were studied for labeled semen in situ.

Species of Mites Used

A laboratory culture of Tetranychus neocaldonicus Andre, collected March 6, 1963, on banana in Florida, was used for the experiments with ^3H -thymidine. This is a

very hardy culture of mites and the deutonymphs are easily recognized, simplifying selection of virgin females.

Other mites that were investigated histologically were T. yusti McG., T. urticae Koch, T. gloveri Banks and Panonychus citri McG.

Rearing Methods

Laboratory cultures were reared on detached bean leaves placed on wet cellucotton cut to fit 3.5 inch Petri dishes. A 35 mesh plastic screen cover was constructed for each dish to permit ventilation and give protection. Mites were confined to the leaves with Tangle-foot® which was dispensed from a syringe along the margin of the leaf. Cultures were held at laboratory temperature. Illumination was supplied by three 40-watt fluorescent tubes mounted 20 inches above the cultures and automatically set for 14 hours daily illumination.

The plants in which the ^3H -thymidine was incorporated were seedlings of Henderson bush lima beans grown in the laboratory. The root area was removed and the stem washed thoroughly before immersion in the ^3H -thymidine solution (Figure 1).

The container for the ^3H -thymidine solution was a 15 ml vial inserted in a suitable hole in a number 11 rubber stopper used as a base, and with a number 3

stopper in the top. A hole in the top stopper allowed the stem to be immersed in the tritiated solution. Lanolin jelly was used to seal the space between the stem and the stopper to prevent evaporation. Only one primary leaf was left on the plant used for ^3H -thymidine up-take. These plants were held in growth chambers with 14 hours of light daily and a temperature of 80° F. for light periods and 70° F. for darkness.

Observation of the mites on these leaves was accomplished by using a special horizontal dissecting microscope.

Histological Methods

After trying several popular fixatives, Carnoy's formula 2 was found to fix tetranychid tissue satisfactorily. The histological process finally used was one furnished by Helle (personal communication). Mites were fixed for 3 hours in Carnoy's and then dehydrated in absolute alcohol for 2 periods of 30 minutes each. Specimens were then placed in toluene for 2 periods of 30 minutes each. Mites were then placed in Tissuemat® at 56-57° C. for 1-1/2 hours.

Several innovations were developed to facilitate handling the mites while preparing them for sectioning. Small baskets permit large numbers of mites to be handled simultaneously. Baskets were constructed by cutting off

the bottom half of a 10 ml vial and fitting the open bottom with nylon mesh. It was found that by using small Petri dishes for the chemicals several groups of mites could be prepared together (Figure 2).

In order to increase the possibility of obtaining good sections several mites were arranged and cut at once. To retain heat in the paraffin while the mites were being arranged in the desired positions, special molds were developed. These molds were 3/4 inch brass rods with tape forming the sides. Preheating the brass rods before the paraffin and mites are introduced considerably increases the period the paraffin remains a liquid (Figure 3).

Blocks were cut at 3 to 5 micra with an American Optical microtome. Both stainless steel razor blades and standard microtome blades were used. Serial sections were mounted on cleaned, albuminized glass slides and allowed to air dry. When stains were used Harris' hemotoxylin and eosin were employed. Slides were covered with Permount and #1 coverslips.

Autoradiographic Technique

The ^3H -thymidine used in this work was a 5.00 millicurie sample purchased from New England Nuclear Corporation. This sample had a specific activity of 15.9 curies/millimole, and a concentration of .076 milligrams in 5.0 milliliters of sterile water as assayed by the

supplier. This material was diluted to 1 millicurie in 10 ml of sterile water for storage and use.

Material of which autoradiographs were to be made was prepared according to the histological methods previously discussed. After serial sections were allowed to air dry they were placed in xylene for 5 minutes. Next the slides were placed in the following solutions for 5 minutes each; 50% xylene and 50% absolute alcohol and distilled water. The following procedure is a modification of Jcftes (1959).

After slides were ready for emulsion application the following steps must be carried out in darkness using only a Wratten series three safe light. The emulsion used was Kodak NTB-2 and it was stored in a lead box under refrigeration. The emulsions container is taken from the refrigerator and placed in a water bath to bring the temperature up to 40° or 50° C. Higher temperatures were avoided. The container was opened and gently stirred with a glass rod. Shaking or vigorous stirring resulted in bubbles. The emulsion was then poured into a special dipping tube which was designed to allow dipping in the least amount of emulsion possible. This tube was a 3/4 inch plastic tube cut 4 inches long. By placing this section of tubing in boiling water it was easily flattened and one end sealed (Figure 4).

Each slide was grasped at the label and dipped into the emulsion so that the mite sections were immersed well below the surface. The slide was immediately withdrawn and allowed to drain vertically for several minutes. The slides were placed in a slide storage box which was wrapped with aluminum foil and stored in an exposure box, which was a converted microscope case. After the exposure period, which varied from 2 weeks to 3 months, the slides were developed.

Development was done in staining jars and four slides were processed at once. The following process was used:

<u>Solution</u>	<u>Time</u>
1. Kodak D-19	5 minutes
2. Stop bath	15 seconds
3. Distilled water	30 seconds
4. Acid fixer	10 minutes
5. Distilled water	5 minutes
6. Absolute alcohol	5 minutes
7. Absolute alcohol	5 minutes
8. Absolute alcohol	5 minutes
9. Xylene	5 minutes
10. Xylene	5 minutes

Lights were used after the slides had been in the fixative for 2 minutes. The temperatures of all solutions were between 18° C. and 20° C. A cover slip was applied to each autoradiographic slide with Permount.

Grain Counting

Need for a method of quantitative interpretation of the autoradiographs was realized after visual differences in grain number were noticed in the experimental material. Counts of exposed silver grains in the autoradiographs were made at 550x magnification, within a selected constant area, using an ocular micrometer as a counting grid. On each autoradiograph 10 random counts were made inside and outside the mite sections. The outside count, being the background, was divided into the tissue count and the resulting figure used as an expression of the tissue activity. Only tissue counts twice the background were considered a valid expression of difference.

There were innumerable artifacts and pitfalls, inherent in both histologic and autoradiographic techniques that complicated the quantitative evaluation of autoradiographs. To recognize and control these problems, replications as well as parallel samples without radioactivity were processed for comparison.

Liquid Scintillation Counting

To check the amount of radioactivity reaching mites reared on a ^3H leaf, liquid scintillation counts were made on several mites and leaf discs. In order to dissolve the samples in the liquid scintillator a modified procedure of Mahin and Lofberg (1966) was used. The samples were individual mites, and leaf discs 2 mm in diameter.

The samples were placed in scintillation vials and 10 μ liter of perchloric (HClO_4) acid and 10 μ liter of fresh hydrogen peroxide were added. The capped vials were carefully heated on a hot plate set at 80°C . until the samples were completely digested (2 or 3 minutes). The vials were allowed to reach room temperature and then placed in a refrigerator for 10 minutes. After removal from the refrigerator 3 ml of (2-ethoxy-ethanol) were added. Ten ml of PPO-POPOP-toluene scintillation solution were added next to each vial. These samples were counted on a Beckman LS-250 spectrometer.

Experiment

Males used in this sperm tracking study were reared on two different bean plants growing in ^3H -thymidine solution. Each plant absorbed approximately 1 ml of $1\mu\text{C}/\text{ml}$ ^3H -thymidine solution per day. At least $2\mu\text{C}$ of this solution was taken up by each plant during the course of this experiment.

Only males whose parents had spent their entire lives on a radioactive leaf were used for mating. Mating between the radioactive males and virgin females took place on leaves containing no ^3H -thymidine. Because males copulate as soon as the females molt from the teliochrysalis, adult females were assumed to have mated. Females were placed on holding leaves as deutonymphs. By regularly observing the leaves for newly molted females, the approximate time of mating could be determined. Groups of from 4 to 10 females which were mated within 30 minutes of each other were fixed together. These groups were cut and mounted together in order to increase the possibility of obtaining preferred sections. The females were fixed at intervals from 15 minutes to 9 days after mating. Groups of mated mites which were not fixed immediately were placed on untreated leaves and held until needed. In all, 40 groups of mites including checks were cut and prepared for autoradiography.

The first 10 groups were exposed for 3 months before development. The background was very high outside the mite tissue in these autoradiographs. Therefore, the exposure time was cut from 3 months to 2 weeks for the remainder of the groups.

Several of the last autoradiographs developed were stained through the emulsion with hematoxylin to facilitate photography.

RESULTS

Detailed microscopic examination of the developed autoradiographs and standard histological sections as well as results of the liquid scintillation counts revealed the following:

1. There appeared to be no connection between the spermatheca and the vagina or oviduct in the species of tetranychid mites examined. In all five species in which the spermatheca was observed there was no evidence of a connection.

2. Histological sections revealed a direct connection to the outside from the spermatheca by way of a duct located between the "vaginal opening" and the anus. This duct can be readily observed in whole mounts of adult females. The duct, which ends in the spermatheca appears to have a cuticular lining. See Figures 1, 6, 7, and 8.

3. The liquid scintillation counts made on leaf disc show that the bean leaves do take up ^3H -thymidine. Counts for the disc 2 mm in diameter were between 750,000 and 1,000,000 counts per minute. A check vial counted at the same time had 111 cpm.

4. Liquid scintillation counts and autoradiographs both indicated that mites feeding on the radioactive leaves take up ^3H -thymidine and incorporate it into their bodies. Scintillation counts on single female mites which fed on the radioactive leaves were from 2,550 to 3,124 cpm. Autoradiographs of females from the radioactive leaves showed high ^3H levels, but because these autoradiographs were among those exposed for three months the background was too high to make meaningful comparisons. The females which had been removed from the ^3H -thymidine leaves and held on untreated leaves for 3 days did show a much higher silver grain count in the cuticle than in any other part of their body (Figure 9). Although no scintillation counts were made on males, autoradiographs showed 7.62 times the background radiation in the males reared on ^3H -thymidine treated leaves (Figure 10).

5. Autoradiographs show that ^3H -thymidine or a metabolite is passed on transovarially. Female mites reared on the radioactive leaf and removed to an untreated leaf produced progeny which were radioactive. In female offspring the tissue counts were 7.19 times that of the background counts. The males from the same parents had 14.34 times the background counts in their tissues.

6. Autoradiographs of 5 recently mated females showed what appears to be labeled material in the spermatheca. The concentrations of silver grains above

these spermathecas appear to correspond with what is thought to be sperm (Figure 11).

7. The autoradiographs showed a general body labeling of female mites several hours after mating with radioactive males. This labeling did not seem to be completely random. The silver grains appeared to relate to certain structures rather than others. Areas which were vacuolated or empty had fewer exposed grains above them (Figure 12). The tissue count of all mites mated from 4 hours to 5 days was twice that of the background count. The average exposed grain count in these mites' tissues was 5.24 times the background. Three groups of females were mated for 6 days and only one of these did not have twice the background. One group of mites which had been fixed three days after mating showed 13.42 times the background.

Some other interesting observations were made from the autoradiographs which may or may not be significant.

In two females, having been mated with radioactive males, sections through eggs in the last stages of development showed concentration of grains above an area believed to be the nucleus. There also appeared to be patterns of grains throughout the yolk area.

In one section through a spermatheca two days after copulation a concentration of silver grains over the

spermatheca seems to gradually extend forward through the mite (Figure 13).

Another interesting observation is the fact that the cuticle of the radioactive offspring of tritium fed parents does not appear radioactive as in the parents. Heavy concentrations of silver grains appeared over the tissues of the offspring, but not over the cuticle (Figures 14 and 15). Since excess ingested radioactivity may be excreted into the cuticle of older mites (see Figures 9 and 14), there is a possibility that the label transmitted in the seminal material by a radioactive male is not entirely in the nuclei of the spermatozoa, but could be in a yet unmetabolized state.

DISCUSSION

The objective in this study was to locate the site of fertilization in female tetranychid mites. From the results of this work the site of fertilization can not be definitely established, but some light has been shed on the process of fertilization. These results show the need for further investigation into mite fertilization. If fertilization takes place as the egg transcends the oviduct as in insects, a duct or a concentration of sperm should be seen leading from the spermatheca to oviduct. No such duct or concentration was ever seen.

Some part of the seminal material placed in the females' spermatheca during copulation was radioactive. Since ^3H -thymidine was the initial labeling product, there is a good possibility that nuclear DNA should be labeled. This does not rule out the possibility that the seminal fluid could contain radioactive DNA or that the ^3H -thymidine could have been metabolized into another product. Since the introduction of semen was the only difference in mites showing labeling and those which did not, some or all the semen was radioactive. From the autoradiographs it appears certain that some part or all the seminal material left the spermatheca and spread throughout the

body. Autoradiography, as it was used in this study, is not precise enough to trace individual sperm. Because of the uncertain fate of the ^3H and the background, no tracks in the emulsion could positively be identified as coming from spermatozoa.

The general body labeling found in females mated to radioactive males, and the patterns of exposed grains in developing eggs suggest that in tetranychids, the spermatozoa migrate from the spermatheca to the young ova by way of the haemolymph rather than up the oviduct. This further suggests that spermatozoa (which lack flagella) are capable of diapedesis through both the spermathecal wall and the ovarian wall.

Stripping film might well have been used instead of liquid emulsion because of the need for quantitative comparisons. Joffes (1959) pointed out that the liquid emulsion layer is not uniform in thickness, which might cause inaccuracies. There were variations in this study, but for the most part the results were consistent and were verified by non-radioactive checks.

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FIGURE 1. Seedling in which ^3H -thymidine was incorporated.

FIGURE 2. Baskets in which mites were handled while being prepared for sectioning.

FIGURE 3. Mold in which mites were arranged for sectioning.

FIGURE 4. Dipping tube for nuclear track emulsion.

FIGURE 5. Saggital section of female T. neocaldonicus showing spermatheca.

Outlined area enlarged in Figure 6.

FIGURE 6. Enlarged spermatheca from Figure 5.
Magnification: 2500x.

FIGURE 7. Spermatheca full of sperm. Magnification 2500x.

FIGURE 8. Cross-section near the rear showing the spermatheca with duct to the outside.

FIGURE 9. Anterior of saggital section of female
which was fed on ^3H -thymidine.

Note heavy labeling of cuticle.

FIGURE 10. Saggital section of male reared on ^3H -thymidine.

FIGURE 11. Spermatheca, with concentration of grains above it, from a female mated 2 hours before fixation.

FIGURE 12. Autoradiograph of female mated 2 days before fixation.

Note patterns of grains in the two large eggs.

FIGURE 13. Spermathecal area with a concentration of grains extending away from it.

FIGURE 14. Portion of the cuticle of female reared on ^3H -thymidine.

Note grains above cuticle and setae.

FIGURE 15. Portion of mite that received labeling transovarially.

Note that no grains appear above cuticle or setae.

VITA

James Winfred Smith was born in Greenwood, Mississippi, January 27, 1943. He graduated from Leland High School, Leland, Mississippi, in 1961. From 1961-1963 he attended Delta State College and in 1965 received his Bachelor of Science degree in Entomology from Mississippi State University. During the summers of 1961-1965 he was a field aid at the Stoneville Branch Experiment Station, Stoneville, Mississippi. In the summer of 1966 he attended the Institute of Acarology at The Ohio State University as a graduate assistant in Entomology and in August 1967 he received the degree of Master of Science in Entomology at Louisiana State University. He is now a candidate for the degree of Doctor of Philosophy.

In 1966 he married the former Greer Jones and they now have one daughter, Sheryl Elizabeth, born in September 1968.

EXAMINATION AND THESIS REPORT

Candidate: James Winfred Smith

Major Field: Entomology

Title of Thesis: An Autoradiographic Search for the Site of Fertilization in Spider Mites

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination: July 21, 1970