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SOME STUDIES ON GALUMNA VIRGINIENSIS AND MONIEZIA EXPANSA (ACARINA, ORIBATOIDEA; CESTODA: ANOPLOCEPHALIDAE

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INTRODUCTION

Stunkard discovered (1937) that the oribatid mite, *Galumna virginiensis*, serves as the intermediate host for the sheep tapeworm, *Moniezia expansa*. Since that time oribatid mites have been found to serve as vectors for 11 other species of anoplocephalid cestodes. These mites occupy almost every kind of habitat, being especially abundant in pastures where they live on grass blades and roots. They are able to spend the winter in the nymph state, which are found in the greatest abundance in the spring.

Stunkard (1944) was able to rear the mites in the laboratory from the egg to the adult stage. At a temperature of 25° C., and a relative humidity of 82 percent the egg takes 8 to 14 days to hatch into a 6-legged larva. Sixty-five to ninety-four days may elapse before the larva develops into an adult mite. Stunkard stated that the entire life span of these mites is about a year, because all of the adults collected by him in March died during the summer of the same year.

Kates and Runkel (1948) were able to produce infections in 34 percent of the *Galumna virginiensis* present on a small pasture by heavily seeding it with sheep dung containing great numbers of *M. expansa* eggs. These mites are found in this region of Kentucky. Because a survey of the literature showed that no detailed study of the population had been made during the winter months, it seemed desirable to make such a study.

Stunkard (1937) gave no detailed description of the developing larval stages of *M. expansa*. Consequently, it appeared worthwhile to perform some experiments to gain this information, which is presented here.

PART I

MATERIALS AND METHODS

Winter fluctuations in the surface population of *G. virginiensis* were studied by us on a small plot which had been used as a sheep pasture for several years. Since 1948, this plot has been used exclusively for investigation of sheep helminths. In the fall of 1948, the pasture was heavily seeded with sheep feces containing eggs of different species of worms. The following spring in 1949 four weanling lambs were placed on it for less than a month. The pasture then lay fallow until June, when four yearling male lambs were placed on it for a month. Two of these lambs acquired tapeworms indicating that the pasture

has retained the infection which was established the preceding fall. The plot contained 336 square yards of surface area and sloped slightly from the northeast toward the southwest.

Mites were collected from four square feet of this pasture every 48 hours from December 20, 1949, until January 21, 1950, when collections were made every fourth day. Beginning February 10, 1950, collections were made every eighth day and this interval was used until the end of the investigation, March 18, 1950.

The mites were taken from the top inch of sod in a manner described by Jacot (1936). Four separate samples, each one foot square by one inch deep, were taken each time. After the samples were removed they were placed in separate boxes, sod side up, brought to the laboratory and placed in collecting funnels. A collecting bottle partly filled with water was attached to the lower end of the funnel to trap the mites as they dropped from the sod. A one hundred-twenty watt reflector type lamp was placed two feet above each sample to light, warm, and dry the sod sample and left shining on it for 48 hours, at which time the bottles were removed, the mites recovered and counted. In order to be sure that every mite in the jar was collected, the entire collection was filtered through filter paper which was then dried and examined. This procedure made it possible to detect mites with facility.

Weather data were taken when the sod was collected. Temperatures were measured with a Fahrenheit thermometer at the following points: 6 inches above the ground, 1 inch below the surface and 2 inches below the surface. The same thermometer was used for each operation, being left in each position for 3 minutes before the reading was taken. Other weather data were obtained from the local weather bureau. This included a maximum, minimum and average daily temperature, daily precipitation and the degree of cloudiness for each day of the observation period. It was noted whether the soil was dry, muddy, frozen or covered with snow as each sample was taken.

Discussion

Results of the collections are presented in table 1. An average of 83 mites, *G. virginiensis,* was found per square foot of sod at the beginning of the period. After a gradual increase, a peak was reached on January 9, 1950, when 148 mites per square foot were collected. The surface population density declined rapidly until January 21, 1950, when an average of 20 mites per square foot was collected. From January 21, 1950, until March 18, 1950, the population differences fluctuated very little.

Extreme variations in the surface population occurred between one sample and the next as can be seen in table 1, but when the whole observation period is considered, no great difference occurred among the four samples. This variation in numbers of *G. virginiensis,* occurring between one square foot and the next adjacent one has been observed by Kates *et al.* (1948). Krull (1939) stated that the population of oribatids in general varied immensely between sample areas. Such extreme variations have not yet been explained satisfactorily.

Krull stated that oribatid mites feed primarily on hyphae and spores of fungi, cellular material from blades of dead grass and anoplocephaline tapeworm eggs. Kates and Runkel found *G. virginiensis* to be more abundant on sheep pastures than on other pastures. We found a similar situation during the fall of 1949, when very few *G. virginiensis* were found on dairy pastures compared with the number collected from a sheep pasture during the same period. Kates and Runkel showed that the preference *G. virginiensis* has for sheep

TABLE 1. *Mite collections per square foot.*

Date	Sample 1	Sample 2	Sample 3	Sample 4	Average Number Mites/ sq. ft.	Soil Condition
12-20-49	78	72	90	92	83	Moist
12-22-49	21	73	33	44	42	Muddy
12-24-49	73	65	105	35	69	Frozen ½″ deep
12-26 49	91	64	56	95	76	Muddy
12-28-49	80	29	23	26	39	Moist
1-5-50	215	37	56	84	98	Muddy
1-7-50	15	109	148	90	90	Frozen in spots
1-9-50	88	208	..	..	148	Moist
1-11-50	75	59	66	..	66	Moist
1-13-50	46	59	83	..	62	Muddy
1-15-50	58	94	43	27	55	Moist
1-17-50	99	30	7	7	35	Moist
1-19-50	55	..	10	18	30	Top frozen
1-21-50	13	6	27	35	20	Moist
1-25-50	37	15	27	37	29	Dry
1-29-50	21	35	13	11	19	Muddy
1-30-50	44	13	85	62	51	Muddy
2-2-50	17	12	17	14	15	Very muddy
2-6-50	29	..	49	26	34	Moist
2-12-50	32	18	..	32	27	Moist
2-18-50	8	25	56	33	30.5	Moist
2-26-50	11	22	30	..	21	Frozen
3-2-50	25	7	52	39	30	Top frozen(snow)
3-10-50	62	33	33	26	38	Dry
3-18-50	15	28	11	17	18	Muddy
Total...	1,308	1,113	1,120	850	.....	.....
Average/ Sq. ft.	52.3	48.4	48.7	40.5	.....	.....

pastures may be due, in part, to the food habits of the mite. This food habit, likewise, could account for extreme differences of numbers which occur between different adjacent square feet of turf. Organic constitution of the soil could be a factor which would be difficult to observe. The ovine habit of fecal deposition generally allows greater amounts of feces to be deposited in one spot than in a random manner. As these deposits of nitrogenous organic material leach out,

an abundance of plant growth will take place at those points. These areas will in all likelihood include numeros fungi. It seems reasonable to suggest that mites would migrate to these fungus-rich areas for feeding. It would appear that those areas of sod which contain the

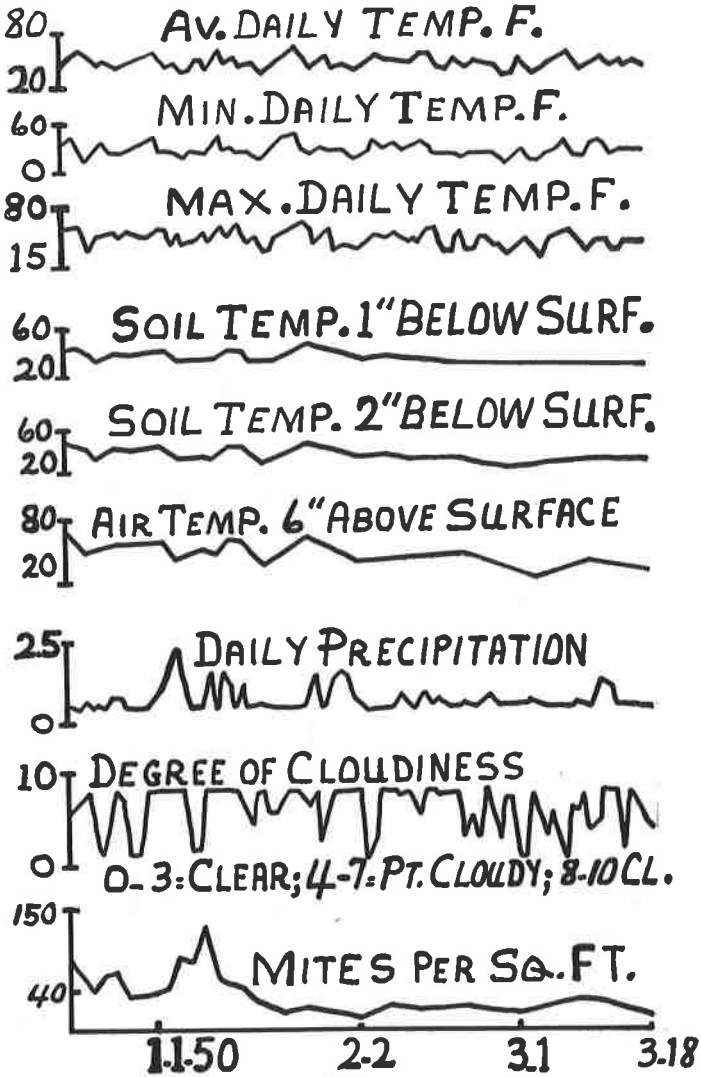

Plate I. Prevailing weather conditions correlated with mite collections, between December 20, 1949, and March 18, 1950.

highest number of *G. virginiensis* may have been areas containing disintegrating fecal material.

An average of 45.8 individual *G. virginiensis* per square foot was

found in the 89 samples collected in our survey. A Kentucky pasture which harbored the same number of *G. virginiensis* per square foot as found in the above sample would contain a surface population of approximately two million individuals of this species per acre. Kates and Runkel (1948) estimated that there were around six million *G. virginiensis* on each acre of the sheep pasture at the U. S. D. A. station at Beltsville, Maryland. Consequently, it would appear that our estimate of two million mites per acre is conservative.

Plate 1 illustrates mite collections and the prevailing weather conditions existing at the time. No correlation could be found between the number of *G. virginiensis* found in any collection and any of the weather data obtained. The highest collection of 148 individuals per square foot was made on a fairly warm, cloudy day with an average temperature of 52° F., which followed two clear colder days. This peak came two days after a rainy period, when almost five inches of rain fell in three days. Krull (1939) stated that an increase in the number of mites, found on grass blades, occurs in the early spring. Although the temperature of this period was much lower than normal for the spring, the precipitation was very similar to spring rainfall. It is suggested that perhaps this period of above normal weather produced a high surface migration which normally would not have occurred until some months later in the year.

PART II

Materials and Methods

All of the mites used in our experiments were collected in a manner previously described, from a dairy pasture. No sheep had been on this ground for several years; consequently, none of the mites could have been naturally infected with *M. expansa*. Mites were collected during the months of August, September, October, November, and December, 1949.

Since no one has reported a suitable method of storing oribatid mites to be used for life history investigations; we devised the following method for keeping mites in a living condition. This method proved to be satisfactory and mites were kept alive in the laboratory for more than six months. Larval mites were found in the culture jars which indicates that the environment simulated the natural one nearly enough so that reproduction occurred. Mites were kept in glass jars which measured 7.5 by 4.0 cm. and had plastic caps. About one half inch of crushed, sterilized sphagnum was placed in the bottom of the jar and into this was stuck a strip of filter paper long enough to reach the top of the jar. More crushed sphagnum was added on both sides of the filter paper until the jar was two-thirds full. The sphagnum presented the mite with a medium similar to that in which it lives naturally. The filter paper acted as a wick carrying moisture in and out of the culture. Sixty drops of water were placed on this wick once each week, if needed. Care was taken not to get the culture too wet, but it was kept moist enough to maintain a high relative humidity. Because the tiny mites become trapped and die in droplets of water condensed on the sides of the jars, a single hole 2.0 cm.

in diameter was drilled in the plastic bottle cap to prevent condensation. A piece of tight fitting porous paper which served as an interliner, was inserted beneath the cap. The hole in the cap covered with porous paper allowed enough evaporation to take place so that little condensation occurred in the jar. These jars were stored in an incubator which was kept at a temperature of 24±2° C.

Tapeworms were procured from a local packing plant. The terminal proglottids were removed from them and washed in running tap water until free of debris. These proglottids were pressed through a fine mesh screen with a pestle. Water was poured through the

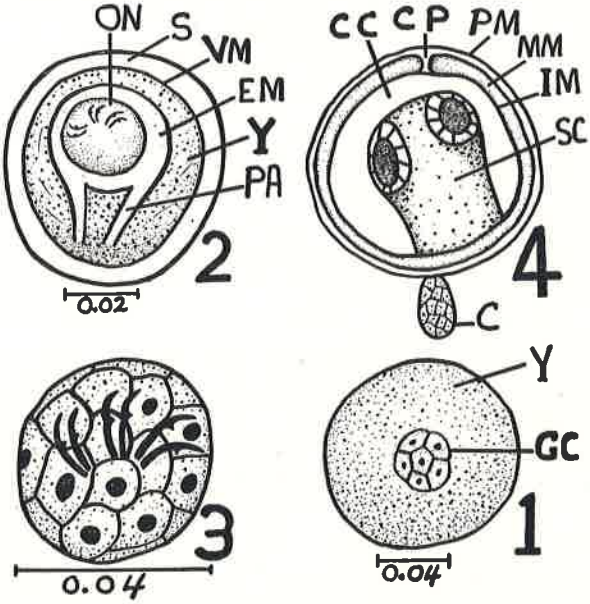

Plate II. Fig. 1. Early larva of *Moniezia expansa*. Fig. 2. Egg of *M. expansa*. Fig. 3. Onchosphere of *M. expansa*. Fig. 4. Fifty-five day old cysticercoid of *M. expansa*. All drawings were made with the aid of a camera lucida. Measurements are all in millimeters. The following abbreviations are used: *CC*, cystic cavity; *C*, cercomere; *CP*, cyst pore; *EM*, embryophore; *GC*, germinal cells; *IM*, inner membrane; *MM*, middle membrane; *ON*, onchosphere; *S*, shell; *PA*, pyriform apparatus; *PM*, peripherial membrane; *SC*, scolex; *VM*, vitelline membrane; *Y*, yolk material.

screen after the proglottids were crushed. The resulting mixture of water and eggs was concentrated by centrifuging and stored in a refrigerator at 4° C.

Sixty drops of highly concentrated mixture of tapeworm eggs were introduced into each of the mite cultures at the beginning of the experiment and no more eggs were added. Ten separate experiments were done.

Conventional methods of fixing and staining larval stages proved impractical. Dissection of the mites to release the cestode larvae was

done by use of fine dissection needles and a binocular microscope. When larvae were found, they were prepared as aqueous mounts and drawings were made with a camera lucida.

Discussion

The results we obtained compare favorably with those obtained by Stunkard (1937-38). But, several significant points are presented here for the first time, we believe.

When the egg is eaten by the mite, the onchosphere is released and makes its way into the body cavity. Within the body cavity the germinal cells begin proliferation and within a short time the onchosphere has lost much of its original form. One onchosphere, recovered four days after the host was exposed to tapeworm eggs, could no longer move its hooks. These hooks also lay in a very different position than in an active onchosphere, a position which suggests atrophy of their muscle-cell attachments. The hooks are apparently lost shortly after their parenchymatous muscle-cell attachments atrophy.

After 14 days at 25° C., the original onchosphere has grown from a diameter of 0.02 mm. to 0.75 mm. and no longer bears any resemblance to its original form. Larvae at this stage of development have very delicate body walls which rupture at the slightest provocation. At an age of 25 days another larvae measured 0.06 mm. by 0.08 mm. and the cells in one end of the specimen presented an appearance distinctly different from those at the opposite end of the body. We observed four larvae at this age, which had begun to resemble cysticercoids. The anterior end, or cysticercoid is now enveloped by a strong membrane and a constriction has begun to form which will separate the cysticercoid from the cercomere. Cystic granules, referred to by Stunkard (1938) as calcareous granules, have appeared, but they are small at this stage. Our tests made by using Alizarin red as an indicator for calcium were negative. We also obtained negative tests for fatty compounds and starch in these granules. We suggest that they may be composed of insoluble ureates. We obtained a positive test for lipoid or fatty substances in the cystic fluid of these parasites by using Sudan IV as an indicator. Cells in the cercomere end of the larvae have not attained the appearance of cells found in the cercomere of fully developed larvae. The pro-cercomere is not surrounded by any distinguishable membrane. The anterior cyst cavity has begun to form; but no sign of the developing scolex could be found. By the age of 42 days, the cyst has attained its familiar form, but is still without a scolex. Cystic granules have become more numerous and larger and by this time germinal cells located at the base of the cyst begin multiplying to form a scolex. By the age of 55 days, the scolex is completely formed and the cysticercoid has become infective.

Four hundred-twenty individuals of *G. virginiensis* which had been exposed to eggs of *M. expansa* were dissected. Forty, or 9.5 percent, of these mites were found to be infected with cestode larvae. Fifty-six larval cestodes of various stages were removed from these 40 mites, an average of 1.4 larvae per mite.

To determine whether or not cysticercoids have reached the in-

fective stage, we fed them to sheep. The lambs used were born in the spring of 1949 and reared in wire pens three feet above the barn floor. Fecal examination indicated the animals to be free of tapeworms. The cysticercoids were administered directly into the rumen of the sheep by means of a stomach tube. This was of heavy rubber, 1.5 cm. in diameter of 75.0 cm. long, with a funnel on one end. To facilitate passage of the tube and prevent injury of the lamb, a speculum was used. This was inserted into the sheep's mouth, then the tube which had been lubricated with a light mineral oil was introduced into the speculum and gently forced down the esophagus to the rumen. Then the cysticercoids were washed down the tube with water. Presence of tapeworm proglottids in the feces a few weeks later indicated that the lambs had become infected. Larvae less than 55 days of age were found to be non-infective, but 55-day-old larvae produced infections in the lambs.

The effects of pure infections of *M. expansa* on the host have been variously described by different authors. Shorb (1939) reported on the effects of the pure infections of *M. expansa* in four lambs and two uninfected lambs for a period of 10 weeks. He concluded that his experiments indicated no loss of weight, unthriftiness, anemia, or manifestation of gastro-intestinal disturbances other than the passing of formed, but softened fecal pellets, rather than the usual solid pellets. The Annual Report made by the Chief of the Bureau of Animal Industry (1948) stated that no pathology was observed in seven six-weeks-old lambs which were infected with this parasite. Kates and Goldberg (1949) found no clinical evidence of infections in 16 lambs, and there was no significant weight differences between infected and uninfected lambs. Freeborn and Stewart (1937) stated that this parasite apparently affected lambs very little, but they may lose a "negligible amount of weight." They stated that infections with this tapeworm makes the lamb less valuable to the purchaser ". . . because they become coarse and less economical for slaughter." Haberman and Carlson (1947) were able to check and control scouring in lambs when they removed the tapeworms by administering lead arsenate. Brown *et al.* (1950) reported from observations made on Kentucky lambs, that fatalities due to tapeworm infections frequently occur in flocks before external symptoms appear. They believed that many cases of scouring which are attributed erroneously to nematode infections, are in reality caused by *M. expansa.* They ascribed symptoms of stiffness, ". . . animal may even be down in hind quarters," and diarrhea to tapeworm infections.

In an experiment done on two lambs with a pure infection of *M. expansa,* Hansen *et al.* (1950), found significant differences between the rate of weight gained by these lambs as compared with two uninfected lambs. They concluded that unthriftiness was exhibited since the infected lambs did not gain weight as fast as the control lambs. They also found a slight trend towards anemia.

The economic significance of this parasite seems to have been stated best by Hansen *et al.* (1950): ". . . the retardation in growth of lambs infected with *M. expansa,* resulting in a delay in arriving

at marketable weight and considerable loss in pounds of meat in large flocks of lambs, is of greater concern than the apparent few mortalities due to tapeworms." We agree with Hansen and Brown. Our infected lambs developed diarrhea, the controls did not.

Freeman (1949), while investigating the life history of *Monoecocestus* sp., anoplocephalid cestodes of the porcupine, discovered that temperature affected the rate of larval development. At 25° C., he found cysticercoids fully developed in 45 days; at 20° C., 52 days were required for full development and 82 days were required for the same development when kept at 15° C. Because *M. expansa* is closely related to *Monoecocestus,* this suggests that there may be similar development in it and the effect of temperature on larval development of *M. expansa* might prove to be of value when applied as a control measure for this tapeworm. Consequently, we propose a plan of using four pastures for lamb production and tapeworm control.

The ewes should be wormed with a taeniacide just before the lambing season. After lambing, tentatively in February, they should be placed in pasture number 1, which should have been kept free of sheep since the preceding April. At the same temperature, it appears safe to assume that larvae of *M. expansa* probably develop at a rate somewhat similar to those of *Monoecocestus.* In this region of Kentucky, the soil temperature seldom averages 25° C. before May. Therefore, assuming that tapeworm eggs were introduced on pasture number 1 in early February by the ewes, it would be the last of March or early April before cysticercoids developed to an infective stage in the intermediate host, *G. virginiensis.* About May 1 the lambs should be moved to pasture number 2, two months after they were placed on pasture number 1, and a month later they should be moved to pasture number 3. The lambs should be marketed some time within the next six weeks in order to realize the most profit. Of course, these pastures should remain free of sheep for the remainder of the year, but they could be grazed by other animals. Breeding ewes should be kept in pasture number four.

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whose display illustrated the trees of Tennessee. In addition to cash prizes these students were awarded an all expense-paid trip to Oak Ridge to compete in the National Science Fair, held May 7-9, 1953.

Mike Stanford, age 16, Martin High School, Martin, Tennessee, is recipient of a $1000 first prize award from the Lion Oil Scholarship Fund Essay Contest. His essay, "Why I Intend to Remain in the South," was adjudged the best among hundreds submitted from the Tennessee-Kentucky area. The gist of his essay was: "I intend to remain in the South and study forestry . . . and help make the South the greatest timber region in the world."

A new chemistry-physiology building costing 1,373,354 is under construction at the University of Tennessee Medical Units in Memphis. This five story building is the first of three additions to be erected under a $4,800,000 expansion program of the University's medical units authorized by the 1951 Legislature.

Also at the University of Tennessee Medical Units, Memphis, Dr. William Hale, senior scientist and head of the Department of Bacteriology and Virology, Brookhaven National Laboratories, Long Island, N. Y., has joined the staff as professor of bacteriology. Dr. Frank L. Roberts, assistant dean of the College of Medicine, has been promoted to associate dean. Other promotions are: Dr. L. R. Fitzgerald and Dr. S. A. Cohn, each to assistant professor of anatomy; Dr. Roger A. Koeppe to assistant professor of chemistry; Dr. W. S. Gilmer to assistant professor of bacteriology, and Dr. Daniel A. Brody to an instructorship in the division of medicine.

Research grants to members of the staff of the University of Tennessee Medical Units awarded recently are: To Dr. Jas. D. Hardy, $8,287 from the U. S. Public Health Service to study body's reaction to certain types of chest surgery, to Dr. J. G. Hughes, $3000 from the United Cerebral Palsy Association to study brain waves of cerebral palsy children while asleep, to Dr. R. R. Overman, director of clinical physiology, grants totaling $43,674 from the Atomic Energy Commission, the American Cancer Society, the U. S. Public Health Service and from Sharp and Dohme, for study of whole body irradiation, transfer of cortisone and other adrenal products, and effect of various diuretic agents on water and electrolyte metabolism, to Dr. Donald B. Zilversmith, $5,292 from the AEC to continue his researches on coating particles of radioactive gold.

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