MITES ASSOCIATED WITH THE PASSALUS BEETLE
II. BIOLOGICAL STUDIES OF *COSMOLAELAPS PASSALI* HUNTER AND MOLLIN (ACARINA : LAELAPTIDAE) [1], [2]

BY

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Cosmolaelaps passali Hunter and Mollin is a laelaptid mite that has been taken only in association with the horned passalus beetle, *Popilius disjunctus* (Illiger). Seasonal occurrence and life cycle studies of this mite were reported in an earlier paper (HUNTER and MOLLIN). The present paper, one of a series on the mesostigmatic mites associated with the horned passalus beetle, is concerned with biological studies of *C. passali*.

METHODS AND MATERIALS.

Mites were reared in circular plastic culture dishes three inches in diameter and two inches deep. Several small ventilation holes were burned in the lid of each dish. The bottom of the dish was covered with previously sterilized frass and wood material from a passalus beetle tunnel or from beetle cultures. In tests in which beetles were kept in the dish, small amounts of rotting, water soaked wood were added to the dishes every other day as food for the beetles and to maintain a high relative humidity. All beetles were completely cleaned of mites before use. When it was necessary to collect the mites at the termination of a test, mites were collected from the frass material by use of a Berlese funnel and from the beetle by examination under a microscope. Unless otherwise stated, all tests and rearings were carried out at a temperature of 29°C.

Experiments were set up to study the effect of temperature upon the number

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of mites found on a beetle. Ten adult mites picked at random from a stock culture were marked with yellow paint following the method of HUNTER (1960). The same procedure was followed with red, black, and silver paints making a total of 40 marked mites. Four randomly chosen beetles were marked with paint on the elytra. The mites and beetles were kept in a plastic shoe box 12 × 6 × 4 inches in size. The beetles were fed water soaked wood as indicated above. Daily records were taken on the number of different colored mites on each beetle while the box was at room temperature (25 ± 2°C.). Then the box was held at 10 ± 1°C. for 30 minutes and the number of mites on the beetles again counted. The box was then returned to room temperature and held at this temperature until the count was taken on the following day. Records were taken daily for a two week period. No beetle or mite mortality occurred during this test period.

In tests to determine if the number of mites found on a beetle could be correlated with the movement of the beetle, a beetle was tied down in each corner of a shoe box containing a small amount of frass material. Forty mites were released in the center of the box (approximately three inches from the beetles), and after ½, 1, 2, and 24 hours the number of mites attached to each beetle was recorded. The mites were then removed from the beetles, and both mites and beetles freed in the box and the test repeated. Tests were carried out at temperatures of 25° and 10° C. Two replicates of each test were run simultaneously at each temperature.

The effect of temperature on reproduction, the rate and type of reproduction, and the degree of association with the passalus beetle required for mite reproduction were investigated. The effect of temperature was investigated by confining six mites, taken from field collected beetles, and one beetle from a laboratory culture, in a circular culture dish. Dishes were held at temperatures of 10°, 21°, 25°, 29°, or 32°C. Temperature variation did not exceed ± 1°C. except for the 25°C. temperature which at times varied as much as ±2°C. Eight dishes were held at each temperature for one month and then checked for mite reproduction.

The rate of reproduction and sex ratio were checked by confining two males and one newly matured female with a beetle in each of 16 culture dishes. The dishes were held four to six weeks in a temperature cabinet at which time the numbers and developmental stages of the mites in each dish were recorded. Parthenogenetic reproduction was investigated by isolating nymphs singly, placing each in a culture dish with a beetle. After maturity the females were moved to a new dish with a new beetle every five days for five weeks. The old dishes with the original beetle in each dish, were held until any mites produced had matured. Six female mites were used in this experiment.

Tests to determine if *C. passali* would reproduce when isolated from a beetle, but provided with beetle fecal material, were carried out. A hole was cut in the lid of a large plastic dish (six inches in diameter and four inches deep), and a culture dish provided with a $^1/_4$ inch mesh wire screen bottom was inserted in the opening of the lid. A beetle and wood were put on the top of the screen ; six adult mites, taken from field collected beetles when the outside temperature had been near

freezing for over three weeks, were put in the large dish. This allowed fecal and frass materials from the beetle to fall through the screen to the mites below, but did not allow the mites direct contact with the beetle. Six replicates of this test were run for one month at which time the bottom dishes were checked for mite reproduction.

The effect upon mite reproduction of the length of time mites were with a beetle was investigated by holding mites in a series of culture dishes with a beetle for 1, 2, 3, or 4 days. After the alloted number of days the beetles were removed and the dishes held for two weeks then checked for reproduction. Thirty-eight dishes with an average of six mites per dish were used in these tests.

Three separate tests were carried out to determine if mites would reproduce when held with starved beetles. In each test 10 mites were confined with a beetle in a culture dish having a charcoal plaster of Paris substrate. Water was added to the substrate regularly to maintain moisture for the mites. The starved beetles were replaced every six days with new beetles which had been starved two days before use in the culture dish. In one test mites were taken from field collected beetles at a time when outside temperatures were below the reproductive range of the mite. In the remaining two tests mites were taken from laboratory cultures.

A series of experiments were carried out to obtain information on the response to light, temperature, and olfactory stimuli of *C. passali*, and especially to determine the importance of legs I as sensory receptors. Adult mites, taken at random from stock culture beetles, were used in all tests. Due to the collection method, most mites used were probably females (see HUNTER & MOLLIN). In these tests mites with both front legs amputated, mites with only one front leg amputated, and control mites with the front legs intact were used. Legs were amputated by placing the mites in a petri dish set in a larger dish of cracked ice ; when the mites were sufficiently cooled that movement ceased, the dishes were set under a microscope and leg I amputated at the genu or tibia with a small scalpal. These mites were then held with beetles until used in tests.

Response to diffused light was investigated by using a petri dish painted black on the bottom, sides, and ½ of the top cover, and covered inside on the bottom with a dampened filter paper. Diffused light was obtained by shining the light from a microscope lamp through a jar of water held on a ring stand 12 inches above the petri dish. Mites were placed in the dish and the number of mites in the lighted portion of the dish counted after 1, 2, and 3 hours. Four groups of control mites — 25 in the first two groups and 50 in the last two groups — were tested. The response of mites with one front leg removed was determined in tests using 12, 45, and 35 mites per test. Three tests of 25 mites each were carried out using mites with both front legs amputated.

Temperature preference was determined by using a temperature gradient sheet made of copper, and consisting of a 20 × 20 inch surface sheet to which a 2 × 2 inch trough — to hold ice as a cooling agent — was soldered so that one inch of the trough extended above and one inch below the surface sheet. A small hole in the

bottom of the trough allowed for water drainage so that new ice could be added as needed during tests. A hot water reservoir 4 × 4 × 8 inches was soldered into a 4 × 4 inch hole in the middle of the surface sheet. Four small tubes fitted with hoses and clamps extended from the bottom of this reservoir. Hot tap water was run into the reservoir as a heat source, and the rate of flow from the tap and escape from the drain tubes was adjusted so that the reservoir held six inches of water at all times. This apparatus gave a temperature gradient from 7° to 40°C. (In later experiments with other animals a light bulb was mounted in the hot water reservoir and used as a heat source.)

In temperature preference tests mites were initially placed near the middle of the gradient surface and allowed to wander freely. During each test the highest and lowest temperatures reached by a mite before turning back were determined by use of a thermocouple and potentiometer. The temperature where the most mites had congregated after 15 minutes was recorded as the preferred temperature.

Olfactory response of these mites was investigated using a glass tube containing paradichlorobenzene crystals at one end of the tube. Mites were released in the tube and their position noted after 10 minutes.

Results.

Factors Affecting the Number of Mites on Beetles.

The 30 minutes time interval at 10°C. was found to be adequate for allowing the maximum number of mites to go onto the beetles. This was based on an experiment in which the box was held at 10°C. and counts taken at intervals throughout a 24 hour period. In this test as many mites (31) were found on the beetles at the end of 30 minutes as were found at readings taken throughout the first eight hours. From the 10*th* to the 24*th* hour there was a slight decrease in the average number of mites on the beetles with 25 mites recorded at the final reading.

The effect of temperature on the number of mites found on each of the four beetles used is given in Table 1. Significantly more mites ($P = 0.01$) attached to the beetles at 10° than at 25°C. A total of 41.2 % of the available mites were found on the beetles at the higher temperature and 82.5 % at the lower temperature. For the two week period the average number of mites on the beetles per day was 16.5 for the higher and 33.0 for the lower temperature. The daily counts show that, except for the first day, the attachment of more mites at the lower temperature was a very consistent response. Daily records of marked mites (see Mollin, 1962) showed a larger fluctuation in the number of mites on a beetle at the higher than at the lower temperature in the day to day counts. At the higher temperatures the mites moved from beetle to beetle or from frass material to beetle while at the lower temperature very little movement occurred.

TABLE 1. — Total number of *C. passali* per day for 14 days on 4 beetles at 25 and 10 ± 2°C.

Beetle	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Totals
25°C.															
I	10	5	8	1	7	4	8	2	7	2	2	7	2	4	69
II	6	2	4	1	5	3	2	2	8	6	2	5	2	3	51
III	10	2	4	4	2	6	3	5	1	5	1	7	3	1	54
IV	10	8	4	2	5	2	1	2	10	4	5	1	2	1	57
Totals	36	17	20	8	19	15	14	11	26	17	10	20	9	9	231
10°C. after 30 minutes															
I	9	8	9	13	10	11	10	5	8	7	9	10	7	9	128
II	6	14	6	4	9	4	3	5	9	15	9	7	11	11	113
III	14	3	6	14	5	2	16	18	14	6	11	12	9	0	130
IV	4	13	15	2	7	8	6	4	7	5	5	2	6	7	91
Totals	33	38	36	33	31	28	35	32	38	33	34	31	33	27	462

No significant difference was found in the total number of mites per beetle at the 25°C. temperature indicating that no one beetle was more attractive to the mites. At the 10°C. temperature significantly fewer mites were found on beetle IV than on any of the other three beetles. At the higher temperature this same beetle had the second highest total number of mites attached.

The results of tests to determine the effect of movement of the beetle upon the number of mites on a beetle are given in Table 2. Five percent fewer mites were found on the tied down than on the freed beetles at the higher temperature, but this difference was not statistically significant. At the lower temperature over 17 times as many mites were on the free moving beetles as on the tied down beetles.

TABLE 2. — Total number of *C. passali* attached to freed compared to tied down beetles at 25° and 10° ± 1°C. Two replicates, using 40 mites each, were run under each condition.

Beetles	Temperature °C.	Time in hours				Total number counted on beetles
		½	1	2	24	
Free	25	15	24	29	38	106
Free	10	50	64	64	63	241
Tied Down	25	12	17	19	47	95
Tied Down	10	0	2	6	6	14

This highly significant difference showed that movement of the beetle has a definite effect upon the number of mites going onto the beetle at this temperature. The fact that the mites did not go onto the beetles as fast in the case of tied down beetles gives some indication that the mites were not directly attracted to the beetles, but probably wandered into the vicinity of the beetle and then located the beetle. This would seem to indicate that under these conditions the mites did not detect the beetles from the center of the box — approximately three inches.

Reproduction Studies.

Reproduction studies at various temperatures (Table 3) showed a very definite range within which reproduction occurred in *C. passali*. Reproduction did not occur at 10° or 32°C. and except for one case, replicate No. 5, did not occur at the 21° temperature. In replicate No.5, the door to the temperature room was accidently left open for a few hours and the temperature rose above 21°C. The fact that reproduction did not occur at 21°C. in any other replicate would indicate that the mites do not reproduce at this temperature. These experiments were carried out over a period of several weeks starting in the late winter. The lowest fluctuations of the 25°C. temperature (laboratory room temperature) would have occurred at the start of these experiments. No reproduction occurred in the first two trials at this temperature, but in later replicates when the outside temperature was higher and the room temperature would tend to be above rather than below 25°, reproduction did occur at this temperature. This would tend to support the general idea that reproduction in *C. passali* does not readily occur at temperatures below 25°C.

TABLE 3. — Reproduction of *C. passali* at various temperatures when confined with a passalus bettle. Six mites and one beetle were used at each temperature in each replicate. + indicates reproduction and — indicates no reproduction.

No.	Temperature °C.				
	10	21	25	28	32
1	—	—	—	+	—
2	—	—	—	+	—
3	—	—	+	+	—
4	—	—	+	+	—
5	—	+ [1]	+	+	—
6	—	—	+	+	—
7	—	—	+	+	—
8	—	—	+	+	—

1. Door of temperature room accidently left open and temperature rose above 21°C.

From the experiment on the rate of reproduction, 182 offspring — 48 males, 54 females, 79 nymphs, and one larva — were taken from the 23 dishes at the end of the test interval. An average of 7.9 offspring, with a range from 4 to 15 offspring, were recovered from the dishes. The large variation in the number of offspring per dish was primarily due to the longer test interval of some dishes possibly allowing the F_1 females to reproduce. In a few dishes where the progeny were removed before the F_1 females could reproduce the females produced an average of one offspring every 5.6 days. Based on the number of mature offspring, a chi-square test indicated that the sex ratio was not significantly different from a 1 : 1 male-female ratio. Adult mites taken in Berlese samples of litter from under decaying legs where passalus beetles were collected also approached a 1 : 1 sex ratio. Male mites were only rarely found attached to beetles in the laboratory, and field collected beetles had almost exclusively female mites attached (see HUNTER and MOLLIN).

In the parthenogenetic tests all isolated females produced offspring. Since previous studies of the duration of the immature stages showed that the mites required an average of 17 days to mature (see HUNTER and MOLLIN), the rate of change of the adult female mites to new dishes followed in these tests would preclude the mating of male offspring with their mother. Therefore, these females must have reproduced parthenogenetically. The total number of mature offspring produced by the six non-fertilized females was 30 males and no females, and averaged one offspring every 7 days — a slightly slower rate than noted above for mated females.

No offspring were produced by the mites isolated from beetles, but provided access to fresh beetle frass and fecal material. After one month, only the six original mites were found in each dish ; when the mites from each dish were put into culture dishes where they had direct contact with a beetle, reproduction occurred in all dishes within two weeks. Further evidence that *C. passali* females must be in direct contact with beetles before reproduction occurs was found when 73 mites taken from field collected beetles in January and February were held in dishes without beetles at 29°C. and provided fresh fecal material daily. No reproduction occurred by the end of the second week and at that time approximately ½ of the mites were divided into three separate dishes, each with a beetle. Two weeks later reproduction was noted in all dishes.

As a result of the findings above, the experiment was set up to determine if the length of time the mites were with a beetle was important in reproduction. Mites used in these tests were taken from beetles collected at a time when outside temperatures, based on the temperature reproduction experiments, were too low for reproduction of the mites to occur. The results of confining the mites with a beetle for various periods of time indicated that the length of time the mites was allowed to remain with the beetle has a definite effect upon reproduction. When the mites were left with the beetle for only one day, no reproduction occurred. One larva was produced from the mites left with a beetle for two days, i.e., one offspring/78

adults. Mites left with a beetle for three days reproduced at the rate of one offspring/3 adults while those with a beetle for four days averaged one offspring/ 2.8 adults.

In tests in which mites were confined with starved beetles no reproduction occurred in the test using field collected mites. Some reproduction occurred during the first two days in tests using mites from laboratory culture. In these two cases the reproduction which occurred soon after initiation of the test was believed due to the mites being in a reproductive state prior to the start of the test. In no test did reproduction continue beyond the first few days.

Sensory Response.

In the tests with normal mites the total percentage of mites in the lighted area of the dish after one hour was 20 %, after two hours 15 %, and after three hours 8 %. This suggested that the mites were at least moderately negatively photokinetic. The direct overhead light may have made it difficult for the mites to orient to the light source. The more pronounced response after the longer periods of time may have been due to random movement of the mites aiding in the initial location of the darkened area of the dish, but the mites undoubtedly remained in the darkened area by choice. The response of mites with one front leg amputated (Table 4) showed that these mites responded in almost the same manner and as quickly as normal mites. In contrast mites with both front legs amputated showed no strong preference to either light or dark areas of the dish.

TABLE 4. — The total percent, based on three tests, of *C. passali* adults remaining in light when one or both front legs were amputated. Readings were taken after 1, 2 and 3 hours. A total of 92 mites with one leg amputated, 75 with both legs amputated were used.

Both front legs amputated		*One front leg amputated*	
Hours in light	% of mites in light	Hours in light	% of mites in light
1	56.5	1	24.0
2	58.7	2	14.6
3	56.5	3	12.0

The results of tests with normal mites for temperature preference are shown in Table 5. The average preferred temperature of the mites was 27.9°C. whereas the average highest and lowest temperatures reached by mites before turning back were 31.6° and 22.1°C. respectively. Mites which had both front legs amputated did not show any temperature preference. When placed at the more extreme temperatures on the surface of the gradient, these mites would move around in a circle until they became inactive due to the heat or cold, or until their wandering movements took them to less extreme temperatures.

TABLE 5. — Response of *C. passali* adults to temperature gradient of 7°C. to 40°C. Twenty-five mites used in each trial. Preferred temperature was taken as temperature at which most mites settled.

Trials	Preferred temperature	Highest temperature reached	Lowest temperature reached
—	—	—	—
1	28.0	31.0	24.0
2	28.0	32.0	20.0
3	29.0	31.5	24.5
4	26.5	32.0	20.0
Average of 4 trials	27.9	31.6	22.1

The response of mites to paradichlorobenzene crystals was tested in a glass tube using normal mites and mites with one or both front legs amputated. The normal mites congregated at the end of the tube farthest from the crystals. Mites with only one front leg amputated reacted in the same manner as normal mites, but mites with both front legs amputated showed no response to the crystals, wandering at random throughout the tube and in some cases crawling over the crystals.

General observations indicated that mites with both front legs missing did not attach to beetles in the culture dishes. When counts were made, it was found that only two out of 200 mites with both front legs amputated had attached to beetles at room temperature (25°C). These two mites were removed from the beetles, and the mites and beetles held for 30 minutes at a temperature of 10°C. No mites attached to the beetles at this temperature. Of 182 mites with only one front leg amputated and exposed to the same temperatures, 68 % attached to the beetles at 25°C. and 82.4 % attached at 10°C. These percentages of attachment were as large as those found in earlier experiments with control mites at these two temperatures.

C. passali is normally attracted to a very specific area of the beetle in front of legs I (see HUNTER and MOLLIN). In a series of tests it was found that the place of attachment on the beetle could be changed by covering the normal area of attachment with finger nail polish or paint. Under these conditions the mites would attach to other areas of the beetle, most attaching to the setae back of the first pairs of legs ; however, some attached in the area of legs III or moved onto the head of the beetle. When the nail polish or paint wore off, the mites would return to the area in front of legs I and reattach. When non-painted beetles were added to a culture dish containing only painted beetles most of the mites would eventually be found attached to the non-painted beetles. When only one side of a beetle was painted, mites would readily attach to the non-painted side indicating that the

paint had little if any reppellent effect. When beetles washed with distilled water or water and a detergent were put in mite cultures, the mites wandered over the beetles but did not attach for about three hours. When given a choice between a recently washed and a non-washed beetle, the mites would attach to the non-washed beetle.

Discussion.

C. passali appears to have a very close association with its host beetle. Undoubtly olfaction is an important stimuli in attracting mites to a beetle, but both temperature and movement of the beetle showed an effect upon the number of mites going onto a beetle. At the two temperatures checked (25° and 10°C.) about 50 % more mites attached to the beetles at the lower temperature. When given a choice in the temperature gradient tests, the mites would not go to temperatures below 20°C. More mites going onto beetles at the lower temperature may be due to the fact that as the environmental temperature dropped, the body temperature of the beetle lagged sufficiently that many mites attached to the beetle because of a temperature differential. More mites were found on field collected beetles taken during the colder months of the year (see Hunter and Mollin) indicating that possibly some factor of this type occurs in nature.

The experiments carried out in this study indicate that the mites probably do not detect the beetle from a distance of three inches, and movement of the beetle in an area greatly increases the opportunity for a mite-beetle contact. Once in the vicinity of a beetle the mites probably find the beetle by a chemical attraction. A chemical attraction is strongly indicated by the fact that mites are not attracted to freshly washed beetles and when given a choice will go to a specific area of the beetle to attach.

The experiments reported here indicate that rather specific conditions are necessary for reproduction in *C. passali*. Although both mated and non-mated females reproduced, reproduction occurs only when the mites are in direct contact with a beetle suggesting that the mites obtain some material or stimulus from the beetle. A mechanical or physiological stimulus can not be ruled out. The fact fact that mites will not reproduce with a starved beetle and must be with the beetle for at least two days before reproduction takes place indicated that the mites were possibly obtaining a food material from the beetle. The absence of reproduction of mites with starved beetles may indicate that this food material is in some way associated with the feeding of the beetle, perhaps a salivary secretion.

The experimental evidence for *C. passali* indicated that the primary photo — thermo — and chemo-receptors are located on the terminal segments of legs I, probably the tarsi. Camin (1953) showed that the photoreceptors of *Ophinonyssus natricis* (Gervais) were located on the pulvilli of legs I, and Evans *et al* (1961) have illustrated a sensory area on tarsus I for a *Zercon* mite. Very likely the first pair of legs act as sensory receptors in most mesostigmatic mites.

SUMMARY.

Biological studies were carried out using adult *Cosmolaelaps passali* Hunter and Mollin. This mite shows a very close association with the horned-passalus beetle, *Popilius disjunctus* (Illiger). Significantly more mites attached to beetles at 10° than at 25°C. A significantly large number of mites attached to beetles that were allowed to move freely in a container compared to beetles restricted to a limited area of the container.

Reproduction of this mite occurred only when the female was in direct contact with a beetle. Mites confined with a beetle one, two, three, and four days produced offspring in direct relation to the number of days with a beetle, no offspring being produced by mites with a beetle for only one day. Mated females produced both male and female progeny; unmated females produced only male progeny. Reproduction did not occur at 32°C. or below 21°C ; maximum reproduction occurred at 28°C. in these tests.

The primary photo — thermo — and chemo-receptors of this mite appear to be located on legs I. Mites with legs I amputated showed no response to light, temperature, paradichlorobenzene crystals, and did not go on to the host beetle. Mites with only one front leg amputated responded in the some manner as control mites — i.e. mites with both front legs intact. Control mites were negatively photokinetic and responded negatively to paradichlorbenzene crystals. The average preferred temperature for control mites was 27.9°C.

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