OVIPOSITION INHIBITION IN *SINELLA CURVISETA* BROOK

(COLLEMBOLA : ENTOMOBRYIDAE)

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

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INTRODUCTION

Diverse environmental factors influence oviposition in Collembola. Of these the various physical factors have been most investigated. The effects of temperature are noted by Davidson (1934), Lindenmann (1950), Marshall and Kevan (1962), and Hale (1965). The optimum temperature for oviposition is about 14°C in Sminthurus viridis (L.) (Davidson, 1934) and about 24°C in Folsomia candida Willem (Marshall and Kevan, 1962). Low temperatures probably inhibit egg deposition in winter in Orchesella spp. (Lindenmann, 1950) and in a series of 15 species representing several genera (Hale, 1965). The effect of humidity has been investigated by Davidson (1934). He found that normal clutches are deposited by females of Sminthurus viridis when soil moisture is above 12 percent. The role of light is noted by Sharma and Kevan (1963a). They indicate that Folsomia similis Bagnall oviposits only in the dark. Soil composition is the subject of experiments by Davidson (1934), Wallace (1967), and Ashraf (1969). They report that fine textured soils are preferred as oviposition sites in Sminthurus viridis (Davidson, 1934; Wallace, 1967) and Onychiurus bhattii Yosii (Ashraf, 1969). The effect of soil pH is explored by Ripper (1930), Maclagan (1932), Davidson (1934), and Ashraf (1969). The optimum pH is about 6.5 in Sminthurus viridis (Maclagan, 1932; Davidson, 1934), about 7.2 in Onychiurus bhatti (Ashraf, 1969), and ranges from 5.8 to 8.0 in Hypogastrura manubrialis

Tullberg (Ripper, 1930).

Several interesting biotic factors regulate oviposition success in Collembola. The effect of crowding on fecundity is described by Green (1964) in parthenogenetically reproducing Folsomia candida. He suggests that reduced fecundity at high densities in this species is due to "stress" caused by jostling. A markedly different influence facilitates oviposition in Onychiurus procampatus Gisin (Hale, 1964). Large females of this species (that consistently differ from females of other species) require the presence of males of another species, O. tricampatus Gisin, to deposit eggs that develop. Small females occur and reproduce with males of O. procampatus.

Personal observation of cultures of Sinella curviseta Brook in this study suggested that an environmental factor negatively affects oviposition. There is a high rate of egg deposition in young cultures, but no oviposition occurs after approximately 10 days in cultures that include many young adults. This absence seemed to indicate that an unknown substance had accumulated that inhibited oviposition. Since this inhibition appeared to represent an interesting example of population regulation, a study of it was undertaken. This paper reports the definition and investigation of aspects of that inhibition.

METHODS

General Methods

Experiments were designed initially to establish that inhibition occurred and then to clarify its general features. With this background, aspects of the biology of inhibition and the chemistry of the inhibitor were investigated. All the experiments are described and interpreted in sequence in the section, "Experiments, Results, and Analyses."

Populations of Sinella curviseta were obtained from a culture maintained by the Acarology Laboratory at The Ohio State University. These populations were descendent from a culture established at the University of Kansas as food for trombiculid mites (Lipovsky, 1951). The common name, springtail, will refer to individuals and populations of this species. Cultures of Sinella coeca (Schött) were derived from cultures maintained at Grinnell College in Grinnell, Iowa.

Mass cultures were reared in small plastic trays with tightly fitting lids; experiments utilized wide mouth, straight sided, screw cap jars in two sizes, 5.5 cm in diameter designated standard, and 4.0 cm in diameter termed small; individuals and pairs were maintained in 1-dram glass vials with plastic caps. Substrates were formed in each of these of a 9:1 mixture of plaster of paris and powdered charcoal as described by Wharton (1946) and Goto (1961). This was

mixed with water, poured in the rearing chamber to a depth of about 1 cm and allowed to set. Water was added to insure a high humidity (ca.95% R.H.) near the substrate surface (Huber, 1958).

Active baker's yeast was provided as food. It was replaced approximately every third day to prevent excessive filamentous fungal growth.

All cultures were maintained at a temperature of 26°C ± 0.5°C in continuous light. (Constant light was essential for other experiments conducted simultaneously.)

Eggs and animals were manipulated using a small aspirator and a moist brush.

Terms

Certain terms require clarification. A used environment is one that has been inhabited for from 8 to 12 days or more, a period sufficient to inhibit oviposition; a clean environment is a previously unused rearing chamber. Layers are animal populations in or recently removed from an ovipositing culture; nonlayers are animals in an inhibiting environment.

Assay

To test the effect of a substance on oviposition a simple procedure was followed. Solids were placed on the substrate surface in clean moistened rearing jars; liquids were utilized to wet the substrate in dried ones. Nonlayers from a single mass culture were added

to treated and control environments. Generally, 5 replicates were tested for each treatment. Fifty individuals formed the test populations in standard containers and the equivalent density, that is, 26 animals, were introduced into small environments. Yeast was supplied as food.

The eggs deposited by test populations were counted 24 and 48 hours after the introduction of the adults. Six days after the onset, the environments were checked for the presence of hatchlings. (At 26°C about 5 days are required for springtail eggs to hatch.) These data from treatment and control populations are presented in the tables.

Cold Bath Trap Collection

Volatile substances were removed and collected from rearing chambers by the air current generated by a Silent Giant™ aquarium air pump. This air current was saturated with water vapor; directed through used environments, a dessicant (a container of Drierite™), and a glass wool filter; and introduced into a cold bath trap. The Pyrex™ vapor trap was partially submerged in a Thermos™ dewar flask filled with dry ice in ethanol. The line connecting the parts of this system was formed of Tygon™ tubing.

Collections were made continuously for 1- and 2-week intervals. During a run the dry ice was renewed twice daily and the environments were replaced about every third day. In the course of the one-week collections the dessicant was in place approximately half the time.

The 1-week collections yielded roughly 10-20 ml of aqueous solution; the 2-week ones, roughly 20-40 ml. The condensate collected by this method was stored in a refrigerator at 4°C.

Chromatography and Spectrometry

In the course of chemical analyses a Hewlett-Packard 402 high efficiency gas chromatograph was utilized. It was employed in association with a hydrogen flame detector with a sensitivity of 4×10^{-12} amperes for full scale output and a 1 millivolt strip chart recorder (Model 7127A). A glass column was packed with 30% polyethylene glycol on Celite 545 (60/80 mesh) as described by Doelle (1967). The operating conditions for this system were: flash heater, 115°C; column temperatures, 70°, 100°, and 110°C; Helium pressure, 2.1 psi; attenuations 4 and 8; and ranges, 10 and 10^2 on the gas chromatograph and temperature, 70°C; Hydrogen pressure, 4.0 psi; and air pressure, 4.0 psi on the hydrogen flame detector. Samples of 2 and 3 µl were injected.

For chemical identification an AEI MS-902 mass spectrometer was employed. It was operated with input from a preparative gas chromatograph. The chromatograph included a porapak Q column and was operated with a column temperature of 110°C at a flow rate of about 20 ml/min of Helium. Attenuations were 32 and 16; jacket pressure was 0.1 mm of mercury; sample size was 2 µl.

EXPERIMENTS, RESULTS, AND ANALYSES

Elucidation of the Problem

Initially an experiment was conducted to confirm the occurrence of the cessation of oviposition. Fifteen cultures of 50 equal-aged animals were established in standard environments. Five of these were maintained with egg removal at 12-hour intervals; five, with hatchling removal at 12-hour intervals; and five, without alteration. Under each of these regimes, populations possessed a rate of oviposition that was initially high and gradually declined and stopped. In all cases, approximately 12 days after its onset, oviposition ceased. Since the cessation occurred at about the same time in each test series, the presence of hatchlings, or higher density was not triggering the inhibition.

Each of the 15 populations exhibited several peaks of egg laying activity. Since the animals were of the same age (having developed from eggs deposited within a 24-hour period) their oviposition was synchronized and the peaks corresponded to the deposition of clutches by females. The first clutch deposited by newly matured females in mass cultures numbered on the average 19 eggs; the second, 21 eggs; the third, 5 eggs; the fourth, 1 egg. Thereafter an egg was deposited infrequently and most of these failed to develop.

The first clutch deposited by the newly matured female in an isolated bisexual pair under optimal conditions averaged 23 eggs; the second, 44 eggs; and third and subsequent ones, 59 eggs each. The marked differences in clutch sizes between females in aging mass cultures and females in clean vials with males provided a quantitative measure of inhibition together with the effect of the larger number of associates present in mass cultures.

The general location of the stimulus that initiates oviposition inhibition was then explored. Laying and nonlaying cultures were maintained and transferred to clean and used environments in all combinations. Both laying and nonlaying populations either maintained in, or transferred to clean environments oviposited; both of these in used environments refrained from oviposition. It can be concluded that the used environments included a component that inhibited springtail oviposition.

In this work equal-aged cultures in standard environments were allowed to mature, half (15 cultures of 50 animals each) were observed and manipulated during the oviposition interval, and half were maintained and utilized after the onset of inhibition. Five cultures of each type were held without alteration; five were transferred to clean environments; and five were transferred to used ones. In these manipulations with nonlayers the multi-aged populations that developed from equal-aged cultures were subsampled to 50 animals each for transfer to used conditions. The numbers of eggs obtained following manipulation appear in Table 1. It is impressive that at markedly

Table 1. Egg populations (above) and hatchling presence (P) or absence (A) (below) following transfer of layers and nonlayers to clean and used environments.

Replicates	Layers to Clean 1	2	3	4	5	Layers to Used 1	2	3	4	5
Egg numbers: 24 hours	104	38	72	157	103	0	3	1	2	0
Hatchlings: 6 days	P	P	P	P	P	A	A	A	A	A

Replicates	Nonlayers to Clean* 1	2	3	4	5	Nonlayers to Used 1	2	3	4	5
Egg numbers: 24 hours	695	805	597	331	281	0	0	0	0	0
Hatchlings: 6 days	P	P	P	P	P	A	A	A	A	A

* The replicates in this series consisted of the populations that developed from cultures of 50 adults. All other test populations here numbered 50 adults.

different densities, that is, high densities in clean environments and low densities in used ones, inhibition was limited to used chambers.

As is apparent from the tables, there is considerable variation between the populations in the number of eggs deposited. This is at least partially explained by the character of the female reproductive cycle. Females of this species produce numerous clutches of eggs. Each clutch is deposited during a precise period near the onset of a stadium. These oviposition periods occupy a maximum of approximately 7.4 percent of the time in the lives of adult females. Clutches average about 60 eggs each. The large potential for oviposition combined with the random nature of the coincidence of oviposition periods and the test period accounts for the variation in egg numbers reported in various experiments.

To begin to characterize the environmental component with the inhibitory effect, several kinds of environmental transfers were performed. Using a cork borer, cores of used substrate were obtained and introduced into clean standard environments. The egg numbers deposited in these environments and those from controls containing clean cores are presented in Table 2. It is apparent that inhibition occurred in populations in environments containing used cores and not in those with clean ones. The inhibitor was transferred in the treated series in the substrate, and therefore, must become at least temporarily attached to it.

A second kind of environmental transfer utilized water extracts

Table 2. Egg populations (above) and hatchling presence (P) or absence (A) (below) with cores of clean substrate and used substrate.

	Clean Cores					Used Cores				
Replicates	1	2	3	4	5	1	2	3	4	5
Egg numbers:										
24 hours	91	71	41	87	84	0	7	10	24	8
48 hours	102	139	233	128	155	1	2	4	33	5
Hatchlings:										
6 days	P	P	P	P	P	A	A	A	A	A

of used environments. Water collected 24 hours after its addition to used environments was applied as a treatment to dried clean substrates. The number of eggs obtained from animal populations introduced into standard environments treated in this way and those obtained from populations on substrates wetted with water extracts of clean chambers and distilled water are given in Table 3. The comparison of treated and control series produces differences even more striking than thoseobtained in the core transfer experiment. It can be concluded that the water extract of used substrates contained the oviposition inhibitor.

Thirdly, air currents were examined as a means of transfer of inhibitor from one chamber to another. When standard environments were connected with an aquarium air pump circulating moist air from one to another, nonlayers in a used environment resumed oviposition. The air current removed the inhibiting compound from used environments. However, the inhibitor cannot be introduced into a clean environment via an air current, even when used environments were connected in parallel to a single clean one and the experiment continued for 48 hours. (Presumably, the concentration of inhibitor substance was too low to produce an effect.)

The volatility of inhibitor, rather than the occurrence of oviposition in response to air movement, is supported by the following observation. When used environments were thoroughly dried and then moistened with distilled water, the populations that were introduced oviposited.

Table 3. Egg populations (above) and hatchling presence (P) or absence (A) (below) following treatment with distilled water, water extracts of clean environments, and water extracts of used ones.

	Distilled Water					Extracts of Clean			
Replicates	1	2	3	4	5	1	2	3	4
Egg numbers:									
24 hours	50	174	160	77	71	54	66	120	68
48 hours	70	211	188	205	159	170	152	125	220
Hatchlings:									
6 days	P	P	P	P	P	P	P	P	P

	Extracts of Used				
Replicates	1	2	3	4	5
Egg numbers:					
24 hours	0	0	0	0	0
48 hours	0	0	0	0	0
Hatchlings:					
6 days	A	A	A	A	A

An experiment was next performed to obtain the volatile components from inhibited cultures. The volatile substances were collected using an apparatus that directed an air current from used environments through a cold bath trap. The condensate collected in this way was applied to dried, clean standard environments to assay its biological activity. Similar runs, including clean, moistened environments in the line, provided condensates that served as controls. The results presented in Table 4 indicated that the oviposition inhibitor was present in collections from used environments and absent in those from clean ones. No egg deposition occurred in environments treated with condensates collected for 1-week periods (i.e., the dilute ones). The population in a small environment treated with condensate collected over a 2-week period with continuous dessication during the collection was killed. These deaths were due to the greater concentration of solutes present in this condensate.

Having determined that inhibition occurs and that it is due to an environmental factor capable of manipulation and collection, several avenues were opened for investigation.

Aspects of the Biology of Inhibition

Sex Affected by the Inhibitor

A cessation of reproduction can be due to a limitation of the sexual activity of males or females or both. To determine the sex or sexes of animals responsive to inhibitor, an experiment was performed in which groups of 25 females were added to different kinds

Table 4. Egg populations (above) and hatchling presence (P) or absence (A) (below) following treatment with distilled water, and vacuum trap condensate from clean and used environments.

Replicates	Used Condensate 1	2	3	4	Distilled Water 1	2	3	4
Egg numbers:								
24 hours		0	4			73	3	
48 hours	0	0	5	6	200+	95	13	151
Hatchlings:								
6 days	A	A	A	A	P	P	P	P

Replicates	Clean Condensate 1	2	Distilled Water 1	2
Egg numbers:				
24 hours	225	431	108	210
48 hours	275	600+	176	372
Hatchlings:				
6 days	P	P	P	P

of environments newly vacated by males. Sperm-free females were added to (a) a used environment following the removal of males and females, (b) a clean environment that had housed spermatophore depositing males for the preceding 2 hours, and as a control, (c) a clean previously uninhabited environment. The 25 females in each of these groups after 2 hours exposure to the respective environments were isolated in individual vials. These vials were observed at intervals for several days and the occurrence of clutches of eggs that developed and hatched was recorded.

Successful clutches were deposited only by females exposed to environments that previously contained males. Of those held in a clean environment (b), 10 of 25 (40%) successfully reproduced; and of those exposed to the used one (a), 8 of 25 (32%) deposited successful clutches. The difference between these groups is not significant ($\chi^2=0.34$; $P>0.05$) and is probably due to the random nature of the encounter with spermatophores and variations in reproductive cycles between the test animals.

Several conclusions can be drawn from these results as to the response of each of the sexes to this inhibitor. Since other experimental work of mine fails to support the occurrence of parthenogenesis in this species, it can be concluded that in both environments males have deposited spermatophores. The absence of a significant difference in the production of successful clutches by females demonstrated that male sexual activity was not significantly affected by the inhibitor.

Females in this experiment picked up spermatophores in both clean and used environments as indicated by their subsequent egg deposition. This capacity must be coupled with at least a limited sperm storage capability to enable these females after a delay to deposit eggs. In several cases oviposition occurred within 30 minutes after transfer to a clean vial. This rapidity suggests that egg deposition, not egg production, was affected. This inhibitor apparently affects specifically the deposition of eggs by females.

Egg Cannibalism

The absence of eggs believed due to oviposition inhibition might be the result of the combination of oviposition and egg cannibalism. Fluctuations in egg cannibalism could then lead to the presence or absence of "inhibition". To evaluate the role of egg cannibalism an experiment was performed in which eggs of known type (from deposition to about 24 hours of age eggs are spherical with a smooth surface; older developing eggs are slightly larger, somewhat elongate, and possess a rough surface) were introduced into unisexual cultures of males and nonovipositing females in standard environments. Sixty eggs of each type were added; the egg populations were counted at 2-hour intervals for about 8 hours; and eggs that had been eaten and smooth eggs that had matured were replaced. This routine was followed for five populations of males and two populations of females in clean environments and repeated for five groups of males and four groups of females acclimated to used environments. The results are presented in Table 5.

Table 5. Egg cannibalism in populations of 50 males and 50 females in clean and used environments.

CLEAN

Replicates	Males 1	2	3	4	5	Females 1	2	$\bar{x}$
Total eggs eaten/50	6	9	9	2	16	12	19	10.4
Exposure period (hrs)	8.7	7.5	6.8	6.5	7.2	9.0	7.8	7.6
$\bar{x}$ eggs eaten/ 50/hr	0.69	1.2	1.3	0.31	2.2	1.3	2.4	1.3

USED

Replicates	Males 1	2	3	4	5	Females 1	2	3	4	$\bar{x}$
Total eggs eaten/50	7	9	18	5	16	25	29	14	9	14.7
Exposure period (hrs)	10.5	9.5	8.0	7.7	6.5	10	9	8.2	6.8	8.5
$\bar{x}$ eggs eaten/ 50/hr	0.64	0.95	2.2	0.65	2.5	2.5	3.2	1.7	1.3	1.7

There is no evidence from these data in support of a differential in egg cannibalism between animals in clean environments and those in used ones. A randomization test (Siegel, 1958) indicated that egg consumption did not vary significantly ($P>0.05$) between the environments. This finding excludes egg cannibalism as the source of inhibition.

Several other interesting points emerged from the results of the egg cannibalism experiment. The data suggested that females tend to consume more eggs than males. This apparent difference was not supported by statistical test (randomization test; $P>0.05$).

It is striking that of 205 eggs consumed, 202 were of one type, smooth, and only 3 were older, rough eggs. Since care was exercised in egg manipulation, egg damage due to handling was minimal. Although smooth eggs lacking protective outer membranes are more likely to be damaged in handling and severely damaged eggs are probably more heavily subject to predation, the effect of damage seems totally inadequate as an explanation for the difference in cannibalism. Sinella curviseta selects the eggs it ingests.

The two egg types differ in their percentages of reproductive success, that is, maturation and hatching. When bisexual pairs of adults are maintained in isolation, the clutches that they produce include some faulty eggs. Eggs that failed to mature formed approximately 2 percent of each of 46 clutches (about 2700 eggs) examined. These remained smooth failing to develop the rough surface characteristic of eggs that hatch. In contrast, all rough eggs

observed in the present work subsequently hatched.

It would seem to be of adaptive advantage to eat an egg that may or may not develop, rather than an egg certain to mature. By selecting smooth eggs this springtail chooses those less likely to develop and hatch.

In the cannibalism experiment each of the 350 animals in a clean environment consumed an average of 0.026 eggs per hour. Considering that about 10 hours is required to deposit a clutch, and about 24 hours is necessary for egg development to produce rough eggs, adults are typically exposed to smooth eggs for about 34 hours. During this interval an average animal of either sex ingests 0.88 eggs. If it is assumed that two adults are associated with each clutch and that they remain in close proximity to the eggs for this interval, the pair would be expected to consume 1.76 eggs. Since an average clutch is composed of about 60 eggs, approximately 1.2 faulty eggs are present in the environment of these animals.

It is possible that animals are capable of selecting for ingestion among the smooth eggs those that are faulty and will not develop. Due to the difficulty of obtaining faulty eggs and handling them without damage, I was unable to evaulate this possibility. Since the number of eggs consumed and the number of faulty eggs present are similar, this possibility, though not confirmed, is not eliminated.

Green (1964) measured egg cannibalism in female Folsomia candida. He reported an average consumption in that species of 1.5 eggs per day. This is similar to an average of 0.89 in female Sinella curviseta.

Extent of Inhibition

To explore the occurrence of this oviposition inhibition in other species, an experiment was performed with Sinella coeca (Schött). Populations of this species were challenged with water extracts from used Sinella curviseta cultures. In the controls, animals were introduced into standard chambers after the substrate was moistened with distilled water. The data from this experiment appear as Table 6. In Sinella coeca oviposition was inhibited by a factor from used environments under the same conditions that produced inhibition in Sinella curviseta. Having been demonstrated in two species, this phenomenon may be of relatively common occurrence in laboratory populations.

Source of Inhibitor

Springtail cultures, in addition to animals, contain living yeasts (as collembolan food) and produce an array of fungi and bacteria. These were partitioned into two groups: animal (including the subjects' intestinal flora and fauna) and non-animal, to investigate the source of inhibitor.

In an experiment 10 standard environments were allowed to become used in the absence of animals. The series was wetted, yeast added and replaced every third day according to the usual routine, and, in addition, each was opened for about 5 minutes daily to allow airborne fungi and bacteria to contaminate the food. After 18 days, animal populations were introduced with fresh food into these. A

Table 6. Sinella coeca egg populations (above) and hatchling presence (P) or absence (A) (below) following treatment with distilled water and water extracts of used environments.

	Distilled Water					Extract of Used				
Replicates	1	2	3	4	5	1	2	3	4	5
Egg numbers:										
24 hours	61	21	41	32	7	0	0	0	0	0
48 hours	68	45	38	52	50	0	0	0	0	0
Hatchlings:										
6 days	P	P	P	P	P	A	A	A	A	A

clean series of 10 environments of equal age served as controls.

The results that appear in Table 7 indicate that inhibition has occurred. It appears that Sinella curviseta responded by oviposition inhibition to a plant product.

Of the nonyeast fungi the genera that occurred most commonly and abundantly in the rearing chambers were Fusarium and Streptomyces. These were isolated, grown in pure culture, and utilized in experimental tests. Initially, fungal cells in liquid media were homogenized and the resulting mixtures were applied to dried, clean Sinella environments. Subsequently, several other treatments were applied. None of these produced oviposition inhibition in animal populations.

The strain of Saccharomyces cerevisae present in collembolan food was isolated, maintained in pure culture, and subjected to experimentation. Of the treatments of liquid media applied, some contained sufficient alcohol to kill animal populations, but these in diluted series failed to yield inhibition. Although inhibition was not produced in these experiments, neither yeast nor the above fungi are eliminated as the source of inhibitor.

Evidence obtained in chemical analyses suggests that yeast is the source of inhibitor.

Aspects of the Chemistry of Inhibitor

A water extract obtained from used environments was utilized to analyze the crude properties of inhibitor. Water extracts that had been subjected to simple treatments were added to dried clean standard

Table 7. Egg populations (above) and hatchling presence (P) or absence (A) (below) in environments aged in the absence of animals and clean ones.

	Clean Environments									
Replicates	1	2	3	4	5	6	7	8	9	10
Egg numbers:										
24 hours	0	146	0	103	50	80	50	0	88	56
48 hours	0	148	117	175	54	116	109	203	293	94
Hatchlings:										
6 days	A*	P	P	P	P	P	P	P	P	P

	Aged Environments									
Replicates	1	2	3	4	5	6	7	8	9	10
Egg numbers:										
24 hours	1	0	38	0	4	0	32	10	9	0
48 hours	0	0	22	0	1	0	10	5	16	0
Hatchlings:										
6 days	A	A	A	A	A	A	A	A	A	A

* Numerous eggs were present that subsequently hatched.

environments. The effects of the treatment were evaluated by comparison of oviposition in treated series with the performances in environments wetted with distilled water and others wetted with untreated water extracts of used environments.

The data from these in Table 8 suggest one property of inhibitor. These demonstrate that this substance is relatively stable as (1) it can be stored with refrigeration for in excess of 5 months, and (2) it can be heated in a boiling water bath without loss of biological activity.

The combination of relative stability and volatility demonstrated for inhibitor suggested that it was simple in form and could be analyzed by chromatography. Less complex organic compounds can be separated in a gas-liquid chromatograph according to the method described by Doelle (1967). Such a system was utilized to analyze the volatile components collected from springtail environments in the cold bath trap for which inhibition had been demonstrated.

When the most concentrated of the condensates was injected, the chromatogram shown in Figure 1 was obtained with two peaks and a deviation from the baseline. This analysis indicated that three discrete organic compounds were present in the condensate from used environments.

When the control cold trap collection from clean, moistened environments that failed to affect oviposition was injected, the chromatogram in Figure 2 resulted. It includes one peak with the

Table 8. Egg populations (above) and hatchling presence (P) or absence (A) (below) following treatment with aged extract, heated extract, and distilled water.

	Distilled Water					Aged Extracts				
Replicates	1	2	3	4	5	1	2	3	4	5
Egg numbers:										
24 hours	68	78	62	15	45	0	0	0	0	0
48 hours	53	71	123	34	88	0	0	0	0	0
Hatchlings:										
6 days	A*	P	P	P	A*	A	A	A	A	A

	Heated Extracts				
Replicates	1	2	3	4	5
Egg numbers:					
24 hours	0	0	0	0	0
48 hours	0	0	0	0	0
Hatchlings:					
6 days	A	A	A	A	A

* Hatchlings were present on day 7.

Figure 1. Gas chromatogram of condensate collected in the cold bath trap from used environments. Sample size, 3µl; column temperature, 110°C; attenuation, 4; range 10.

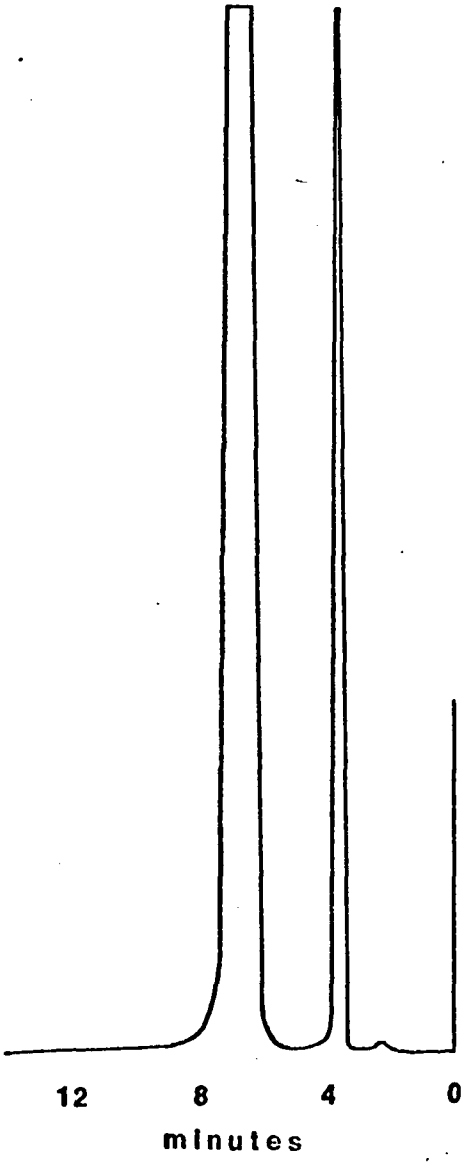
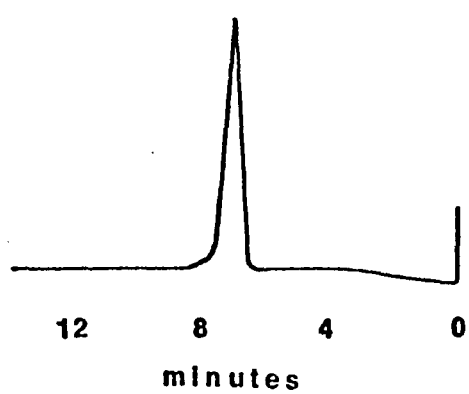

12
8
4
0
minutes

Figure 2. Gas chromatogram of condensate collected in the cold bath trap from clean environments. Sample size; 3 µl; column temperature, 100°C; attenuation, 8; range, 10^2.


12
8
4
0
minutes

same retention time as the most concentrated component above.

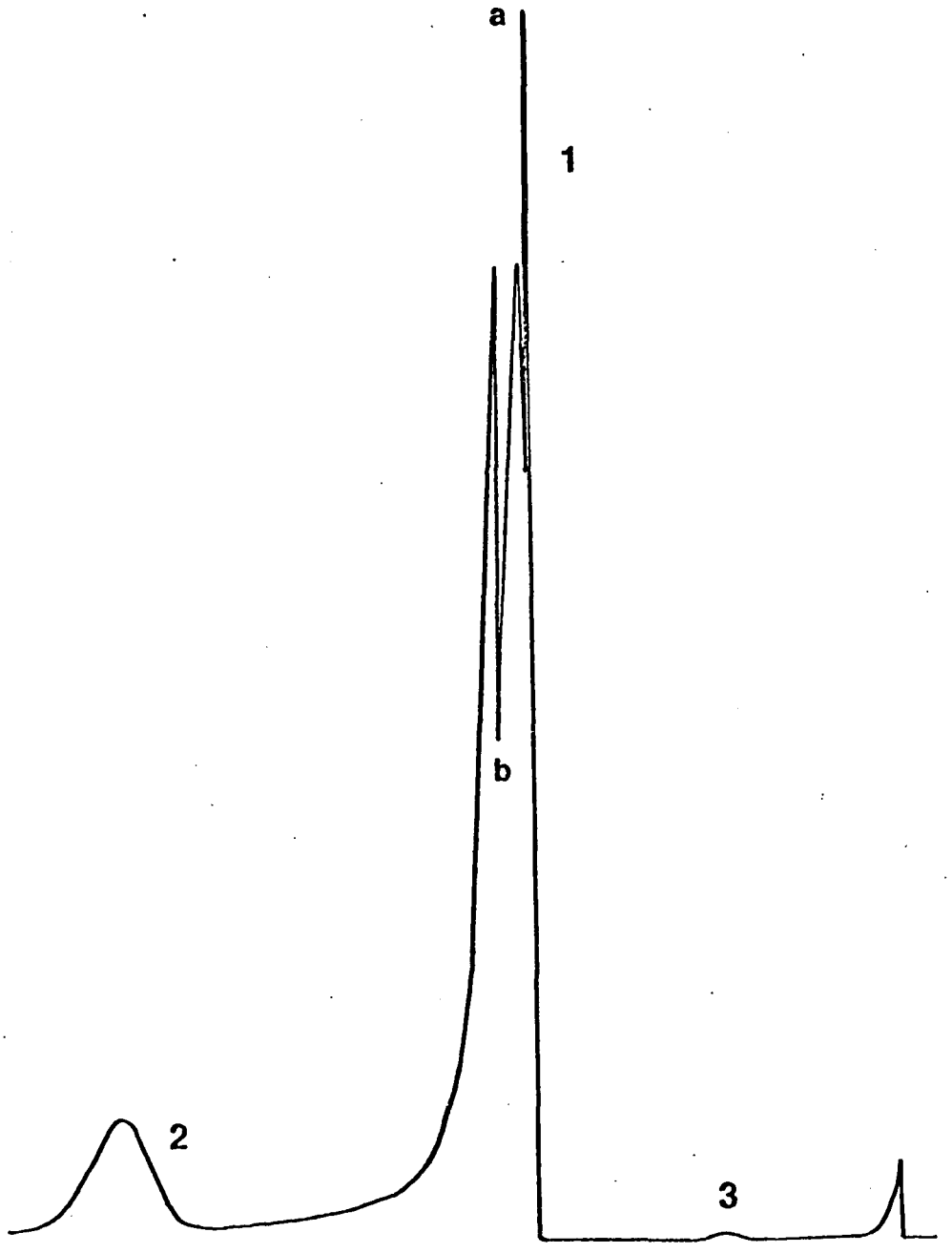
The identification of the organic compounds in the most concentrated active condensate was accomplished using mass spectrometry. The chromatogram obtained from the preparative chromatograph connected to this unit appears as Figure 3. This analysis indicated that the major peak (1) was produced by ethyl alcohol; the lesser one (2), by acetone; and the deflection (3), probably by methyl alcohol.

With identified products, inferences can be made concerning their sources. It seems certain that the ethyl alcohol was produced by the metabolic activity of yeast. It is reasonable to conclude that the other organic components, in light of their nature, are also probably derived from yeasts.

The concentrations of these substances was approximated by comparison of the peak heights of compounds in condensate with the heights obtained for standard solutions. The most concentrated cold trap collection from inhibited cultures contained about 1.97 percent ethanol and about 0.14 percent acetone. The less concentrated condensate of this kind that produced inhibition included 0.012 percent ethanol and 0.005 percent acetone. The control condensate from clean chambers contained approximately 0.14 percent ethanol.

Ethyl alcohol in the controls collected from clean environments could have originated in either or both of two sources: laboratory room air and ethyl alcohol in the dewar flask. To

Figure 3. Gas chromatogram obtained in mass spectral analysis. 1, ethyl alcohol; 2, acetone; 3, methyl alcohol. a, attenuation changed to 32; b. attenuation changed to 16. Sample size, 2 µl; column temperature, 110°C; attenuations, 32 and 16.


a
1
b
2
3

investigate the latter, the cold trap apparatus was operated with n-propanol substituted for ethanol. Chromatography of the condensate collected under these conditions from clean environments indicated that n-propanol and ethanol were present in this condensate. Even though the n-propanol upon analysis was found to contain a small amount of ethanol, it seems probable that both of the sources suggested above contributed to the 0.14 percent in the original control.

Experiments were now conducted to specify from among the compounds present, the inhibitor. Ethanol and acetone were applied in the concentrations reported for the dilute and concentrated collections to clean dried environments; methanol (which was too dilute for its concentration to be determined accurately) was added in 0.14 percent solution. Populations exposed to 1.97 percent ethanol were killed; populations in environments including all other specified concentrations of ethanol, acetone, and methanol oviposited. Treatments with solutions of ethanol ranging from 1.97 percent, which was lethal, to 0.14 percent, which had no effect, confirmed the earlier results with yeast cells in liquid media, namely, if females can live in the ethanol containing environment, they oviposit. The combination of acetone and ethanol was then applied as treatment. When the percentages reported for the dilute condensate that inhibits oviposition were added, egg deposition ensued. This evidence indicates that none of these compounds produces inhibition.

The question then arises: How can a mixture produce an effect

that its organic components alone or in combination fail to produce? Several explanations are offered that possibly resolve this conflict. It may be that the quantity of inhibitor present in the condensate is so small that it is not detected by the detector (with a sensitivity of 4×10^{-12} amperes for full scale output) associated with the gas chromatograph. Since this detector is sensitive to carbon-containing compounds, only those are recorded. It is possible that a noncarbon-containing substance, such as ammonia, is present and produces inhibition. Finally, it may be that two substances produce inhibition and that this investigation combines properties of the two in an inappropriate way.

DISCUSSION

Oviposition Inhibition in Other Arthropods

Although Sharma and Kevan (1963b, p. 71) comment that for Pseudosinella petterseni Börner, "the continuous presence of yeast . . and its fermentation [might] inhibit further oviposition," this is the first documented case of oviposition inhibition in Collembola. A similar negative relationship between oviposition and yeast has been described by David and Herrewege (1969) in Drosophila melanogaster Meigen. They conducted experiments in which flies were allowed to select oviposition sites. Females repeatedly chose a medium lacking yeast for egg deposition. It seems probable that the substance that affects Sinella also repels Drosophila. The effect of ethyl alcohol on oviposition has been explored in investigations by Matsutani (1958) on Drosophila. He found a reduction in fecundity in flies reared in contact with several concentrations of ethanol.

Consequences of Oviposition Inhibition

Yeasts occur commonly near the surface of soils. They are especially abundant in orchards and soils rich in humus, ranging up to 245,000 per gram of soil (Lund, 1958). Assuming that yeasts are the source of inhibitor, it seems improbable that metabolic products of yeasts would reach sufficient concentrations in these soils to inhibit oviposition in Collembola. If such inhibition occurred, it

would perhaps explain the patchy distribution of various species of Collembola within habitats (for example, Poole, 1962; Hale, 1966), and the changes in location observed in aggregations in time (Wallace, 1957, 1967).

In the laboratory, this phenomenon markedly influences the growth and age structure of populations of Sinella curviseta. The products of yeast metabolism may affect, perhaps similarly, other collembolan species, Drosophila, and any laboratory reared arthropods supplied yeast as food. It is important that such artifacts be recognized to prevent confusion and misinterpretation of experimental results.

LITERATURE CITED

Ashraf, M. 1969. Studies on the biology of Collembola. Rev. Ecol. Biol. Sol 6:337-347.

David, M.J., and J.V. Herrewege. 1969. Action réplusive de la levure vivante sur l'oviposition de Drosophila melanogaster Meig. Comptu Rendu 268:1778-1780.

Davidson, J. 1934. The "lucerne flea" Smynthurus viridis L. (Collembola) in Australia. Congr. Sci. Ind. Res. Organ. Bull. 79:1-66.

Doelle, H.W. 1967. Gas chromatographic separation of micro quantities of acetone, C_1-C_7 alcohols, and diacetyl. J. Gas Chromatogr. 5:582-584.

Goto, H.E. 1961. Simple techniques for the rearing of Collembola and a note on the use of a fungistatic substance in the culture. Entomol. Mon. Mag. 96:138-140.

Green, C.D. 1964. The effect of crowding upon the fecundity of Folsomia candida (Willem) var. distincta (Bagnall) (Collembola). Entomol. Exp. Appl. 7:62-70.

Hale, W.G. 1964. Experimental studies on the taxonomic status of the Onychiurus armatus species group (Collembola) Rev. Ecol. Biol. Sol 1:501-510.

_____. 1965. Observations on the breeding biology of Collembola. Pedobiologia 5:146-152, 161-177.

_____. 1966. A population study of moorland Collembola. Pedobiologia 6:65-99.

Huber, I. 1958. Color as an index to the relative humidity of plaster of paris culture jars. Proc. Entomol. Soc. Wash. 60:289-291.

Lindenmann, W. 1950. Untersuchungen zur postembryonalen Entwicklung Schweizerischer Orchesellen. Rev. suisse Zool. 57:353-428.

Lipovsky, L.J. 1951. Collembola as food for chiggers. J. Parasitol. 37:1-2.

Lund, A. 1958. Ecology of yeasts. p. 63-92. In A.E. Cook [ed.] The chemistry and biology of yeasts. Academic Press, New York.

Maclagan, D.S. 1932. An ecological study of the "lucerne flea" (Smynthurus viridis, Linn.). I. Bull Entomol. Res. 23:101-145.

Marshall, V.G., and D.K.M. Kevan. 1962. Preliminary observations on the biology of Folsomia candida Willem, 1902 (Collembola, Isotomidae). Can. Entomol. 94:565-586.

Matsutani, K. 1958. Studies on the resistance of insects to chemicals. II. Effects of ethyl alcohol on the resistance and oviposition of adult flies of Drosophila melanogaster [in Japanese, English summary]. Physiol. and Ecol. 8:28-31.

Poole, T.B. 1962. The effect of some environmental factors on the pattern of distribution of soil Collembola in a coniferous woodland. Pedobiologia 2:169-182.

Ripper, W. 1930. Champignon-Springschwänge, Biologie und Bekampfung von Hypogastrura manubrialis Tullb. Z. angewandte Entomol. 15:546-584.

Sharma, G.D., and D.K. McE. Kevan. 1963a. Observations on Folsomia similis (Collembola:Isotomidae) in Eastern Canada. Pedobiologia 3:48-61.

_____. 1963b. Observations on Pseudosinella petterseni and Pseudosinella alba. Pedobiologia 3:62-74.

Siegel, S. 1958. Nonparametric statistics for the behavioral sciences. McGraw-Hill Book Co., New York. 312 p.

Wallace, M.M.H. 1957. Field evidence of density-governing reaction in Sminthurus viridis (L.). Nature 180:388-390.

_____. 1967. The ecology of Sminthurus viridis (L.) (Collembola). I. Processes influencing numbers in pastures in Western Australia. Australian J. Zool. 15:1173-1206.

Wharton, G.W. 1946. Observations on Ascoschöngastia indica (Hirst, 1915) (Acarinida:Trombiculidae). Ecol. Monogr. 16:151-184.