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MONOFACTORY INHERITANCE OF AUTOGENY IN *Aedes atropalpus*¹

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INTRODUCTION. Ovarian development in mosquitoes can be classified into two types. Females which require a blood meal for the production of mature eggs are called anautogenous, whereas those lacking this requirement at least for the first egg batch are called autogenous.

Although biochemical and physiological aspects of autogeny have been extensively investigated in several species (Beckel, 1954; Chen, 1967; Kardos, 1959; Lea, 1964), the mode of inheritance of this character has been carefully analyzed only in the *Culex pipiens* (L.) complex. Multifactorial modes of inheritance of autogeny have been postulated for this mosquito group (Spielman, 1957; Laven, 1967). In an effort to ascertain if a similar, complex genetic control mechanism was present in another mosquito species, the present study was undertaken. Two rather closely interrelated biological activities were examined in the rockpool mosquito, *Aedes atropalpus* (Coq.). First, both types of egg development were analyzed and the mode of inheritance of this character was determined with crossing experiments. Second, blood-feeding activities of autogenous and anautogenous strains were characterized and the genetic basis of blood-feeding was investigated.

MATERIALS AND METHODS. Laboratory colonies were initiated from field collections made at 12 sites in 7 states (Table 1). In all cases except one, only larvae and pupae were collected. The exception involved eggs collected from an oviposition trap. Large bulbed pipettes were used in extracting the aquatic stages from rockpools.

Aquatic stages were reared in enamel pans with a water temperature range of $26 \pm 3^\circ \text{C}$. Larvae were fed on liver powder (Nutritional Biochemicals Co.). Adults were maintained at $24 \pm 1^\circ \text{C}$. and a relative humidity of 70-80 percent. Sugar cubes and slices of canned apples were provided as food for adults.

Initial observations involved measurement of autogeny by counting eggs that had been deposited by females that had been denied access to blood. However, some females developed eggs but retained them in the abdomen. Therefore, the criterion for autogeny used in the present work was based on the degree of follicular development, as measured in dissected females.

Ovaries were dissected from anesthetized females which had the abdomen in a drop of saline. The abdomen was grasped with two forceps, one holding the first segment and the other holding the seventh. The latter segment was slowly pulled until the intact ovaries appeared.

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The preparation was covered with a coverslip and the slide was examined under the compound microscope. The length of largest follicle was measured with an ocular micrometer and the amount of yolk was estimated.

The TEXAS (TEX) strain was anautogenous, whereas the other 11 strains were autogenous. Most of the crosses reported here were between TEXAS and

BASS ROCKS (BR); however, supplementary crosses used two other strains as the autogenous parent. All crossing experiments were performed in mass mating cages $1\frac{1}{2}' \times 1' \times 1'$. Most crosses had about 100 individuals of each sex.

Blood-feeding activity was examined in two strains which displayed the two types of egg development. Females were offered a blood meal in the form of an

TABLE 1.—Field collecting sites of strains of *A. atropalpus* used in autogeny and blood-feeding studies.

Strain name	Location *	Field collecting site		
		Collector (s)	Date	Remarks
BASS ROCKS	E. Gloucester Essex Co. Mass.	G. O'Meara	June 1967	Coastal rockpool
CHESTERFIELD	W. Chesterfield Hampshire Co. Mass.	G. O'Meara	June 1967	Inland rockpool
GARRETT	Swallow Falls (SP) Garrett Co. Maryland	G. B. Craig, Jr.	June 1967	Inland rockpool
GOOSEBERRY	Gooseberry Falls (SP) Lake Co. Minn.	G. B. Craig, Jr. & J. Truman	August 1967	Inland rockpool
GUNPOWDER	Gunpowder Falls Harford Co. Maryland	G. B. Craig, Jr.	June 1967	Inland rockpool
JAY COOK	Jay Cook (SP) Carlton Co. Minn.	G. B. Craig, Jr. & J. Truman	August 1967	Inland rockpool
JIM FALLS	Jim Falls Chippewa Co. Wisconsin	G. B. Craig, Jr. & J. Truman	August 1967	Inland rockpool
LAB	Bulls Bridge Litchfield Co. Conn.	J. Anderson	July 1965	Inland rockpool
LOWELL	Lowell Middlesex Co. Mass.	G. O'Meara	June 1967	Inland rockpool
MONTGOMERY	Great Falls Montgomery Co. Maryland	G. B. Craig, Jr.	June 1967	Inland rockpool
ROCKPORT	Rockport Knox Co. Maine	G. O'Meara	June 1967	Coastal rockpool
TEXAS	Austin Travis Co. Texas	B. Hoffmann	October 1967	Oviposition trap for <i>A. aegypti</i>

* SP=State Park.

anesthetized mouse for 30 minutes daily from the 4th through the 11th day after emergence. Blood-fed females were removed daily and their ovaries were examined within one hour after taking the blood meal. For the characterization of blood-feeding activity approximately 500 females from both the BASS ROCKS and TEXAS strains were tested. In addition, 500 TEXAS x BASS ROCKS F_1 hybrid females were also tested. Each sample was divided into 6 gallon cages, 3 containing an equal number of males and 3 containing only females. Both apple and sugar cubes served as a source of food. Progeny of backcrosses and F_2 progeny were tested in a similar manner. In all cases where crosses are reported, the females are indicated first.

RESULTS. Of the 12 strains studied, 11 laid fertile eggs without access to a blood meal. These strains have been maintained on a bloodless diet for at least 15 generations. Only females of the TEXAS strain uniformly required blood. Among several thousand TEXAS females examined, none developed eggs without a blood meal.

Fig. 1 shows the rate of follicular growth for BASS ROCKS, TEXAS and F_1 hybrid (TEX x BR) females. Additional experiments have shown that ovarian diapause is also absent in the reciprocal F_1 hybrid (BR x TEX), as well as in females of the GARRETT and LAB strains. Furthermore, F_1 females resulting from crossing GARRETT (GAR) or LAB with TEXAS in either direction,

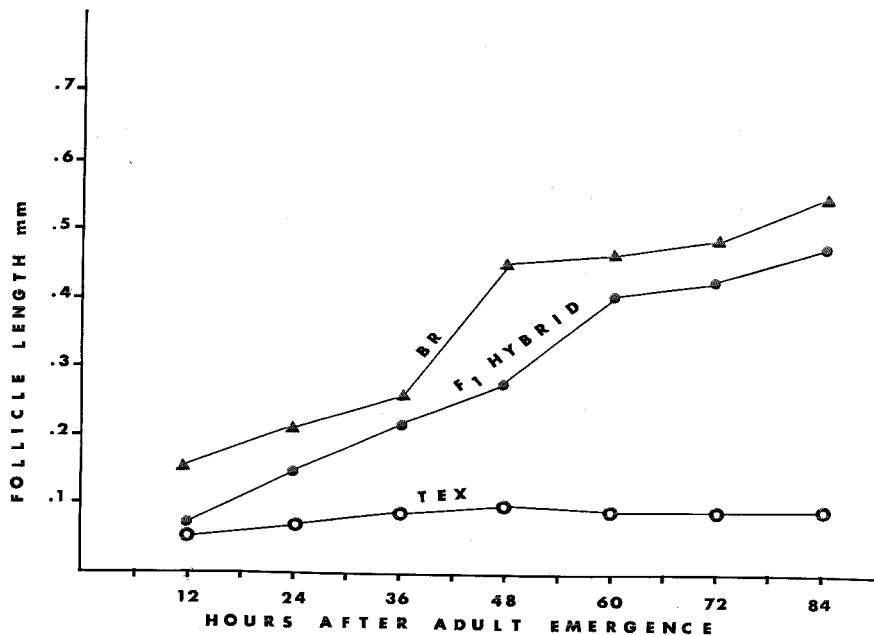


FIG. 1.—Follicular development in females of the BASS ROCKS (BR) and TEXAS (TEX) strains and of the F_1 hybrid, TEXAS x BASS ROCKS. For each time interval follicles of at least 10 females of each type were measured. The greatest 95% confidence limit is .02 mm.

had an ovarian developmental pattern similar to that resulting from crosses between TEXAS and BASS ROCKS. First, nearly all F_1 individuals lacked a diapause stage. Second, the follicular growth rate of all F_1 's was slightly but significantly slower than that of the autogenous parental strain.

After analyzing these follicular growth patterns, a criterion for autogeny was established. If follicular length in a non-blood-fed female was equal to or greater than 0.3 mm when examined 60 hours after emergence, then such a female was considered to be autogenous. Using this criterion, egg development was characterized in four strains of *A. atropalpus*. BASS ROCKS, GARRETT (GAR) and LAB were almost completely autogenous, as were all F_1 hybrids of these strains with the TEXAS strain. Yet TEXAS females were completely anautogenous (Table 2). Among several thousand mosquitoes dissected in the course of this study, there was no difficulty in distinguishing autogenous from anautogenous ovarian development. Ovarian mosaics and follicular development intermediate between the diapause stage and the lowest level of recognized autogenous development were rarely observed.

Table 3 contains the results of reciprocal backcrosses between the TEXAS and BASS ROCKS strains. Since hybrids be-

TABLE 2.—Occurrence of autogeny in parental strains and F_1 hybrids of *Aedes atropalpus* used in crossing experiments.

Cross		Progeny	
♀	♂	No. examined	% autogenous
GAR	GAR	217	98
BR	BR	257	98
LAB	LAB	230	97
TEX	TEX	301	0
.....			
GAR	TEX	287	98
TEX	GAR	143	97
BR	TEX	148	98
TEX	BR	266	97
LAB	TEX	125	97
TEX	LAB	174	97

tween these two strains were almost uniformly autogenous, a relatively simple genetic hypothesis was postulated. According to this hypothesis autogeny is controlled by a single, dominant, autosomal gene, with anautogeny recessive. The validity of this hypothesis was tested by comparing the observed with the expected results of each cross by means of chi-square tests. The results fitted expectation in all eight backcrosses. The segregation of autogeny in F_2 progeny of two reciprocal crosses is shown in Table 4. Both BASS ROCKS and GARRETT were used as the autogenous strain. Again the results fit the hypothesis of a single gene.

TABLE 3.—Occurrence of autogeny in backcrosses of F_1 hybrids to autogenous (BASS ROCKS) and anautogenous (TEXAS) parental strains.

Cross		Progeny		X^2 *	P †
♀	♂	No. examined	% autogenous		
TEX	F_1 (TEX x BR)	395	53.2	2.75	n.s.
TEX	F_1 (BR x TEX)	544	47.1	.89	n.s.
F_1 (TEX x BR)	TEX	222	46.4	.65	n.s.
F_1 (BR x TEX)	TEX	117	47.0	.13	n.s.
.....					
BR	F_1 (TEX x BR)	366	98.6	.72	n.s.
BR	F_1 (BR x TEX)	254	98.4	.20	n.s.
F_1 (TEX x BR)	BR	210	97.6	.19	n.s.
F_1 (BR x TEX)	BR	101	97.0	.50	n.s.

* Based on the hypothesis that autogeny is controlled by a single, dominant, autosomal gene.

† n.s.=not significant at .05 level of probability.

TABLE 4.—Occurrence of autogeny in F_2 progeny of crosses between autogenous (GARRETT or BASS ROCKS) and anaautogenous (TEXAS) strains.

Cross		Progeny			
♀	♂	No. examined	% autogenous	X^2 *	P †
F_1 (GAR x TEX)	F_1 (GAR x TEX)	482	73	.05	n.s.
F_1 (TEX x GAR)	F_1 (TEX x GAR)	521	72	.83	n.s.
F_1 (BR x TEX)	F_1 (BR x TEX)	425	71	1.25	n.s.
F_1 (TEX x BR)	F_1 (TEX x BR)	510	71	1.17	n.s.

* Based on the hypothesis that autogeny is controlled by a single, dominant, autosomal gene.

† n.s.=not significant at .05 level of probability.

The above experiments with BASS ROCKS and GARRETT were conducted by one of us (G.F.O.). A second set of experiments was conducted in another building by G.B.C. using the LAB strain as the autogenous parent (Table 5). Reciprocal F_1 s and all eight backcrosses with LAB and TEXAS gave segregation essentially identical to that obtained with BASS ROCKS and GARRETT (Tables 3 and 4). Thus it would appear that autogeny in the LAB strain is also controlled by a single dominant gene. Finally, crosses and backcrosses among the autogenous strains (LAB, BASS ROCKS, and GARRETT) gave progeny that were entirely autogenous, thus indicating allelism among these strains.

Blood-feeding activity, expressed as the cumulative percentage of females taking an initial blood meal, was measured daily starting on the 4th day after emergence. Fig. 2 illustrates blood-feeding activities

of BASS ROCKS, TEXAS and F_1 (TEXAS x BASS ROCKS) females. Although the F_1 s were autogenous, their blood-feeding activity resembled that of the TEXAS strain. After 7 daily offerings of a source of blood, less than 5 percent of the BASS ROCKS females had blood-fed, whereas more than 90 percent of the TEXAS and F_1 females had blood-fed.

Ovaries of all blood-fed females were examined within one hour after the blood meal (Table 6). As expected, nearly all of the F_1 and BASS ROCKS females examined contained mature follicles, while follicles of the Texas females were uniformly at the diapause stage.

Results of 3 backcrosses of F_1 to BASS ROCKS are shown in Table 6. Essentially all backcross progeny were autogenous, again conforming with the single gene hypothesis. However, approximately half (58, 56, and 47 percent) of these

TABLE 5.—Occurrence of autogenous females in backcross and F_2 progeny of crosses between the LAB and TEXAS strains.

Cross		Progeny	
♀	♂	No. examined	% autogenous
F_1 (LAB x TEX)	LAB		
F_1 (TEX x LAB)	LAB	38	100
LAB	F_1 (LAB x TEX)	93	100
LAB	F_1 (TEX x LAB)	41	100
F_1 (LAB x TEX)	TEX	47	100
F_1 (TEX x LAB)	TEX	59	49
TEX	F_1 (LAB x TEX)	98	46
TEX	F_1 (TEX x LAB)	82	51
F_1 (TEX x LAB)	F_1 (TEX x LAB)	60	52
F_1 (LAB x TEX)	F_1 (LAB x TEX)	221	72
		104	70

TABLE 6.—Blood-feeding activity of autogenous and anautogenous progeny of crosses involving BASS ROCKS and TEXAS.

Cross		Progeny				
		Total no. examined	Autogenous		Anautogenous	
♀	♂		No. examined	% blood-fed *	No. examined	% blood-fed *
TEX	TEX	561	0	0	561	91
BR	BR	455	448	3	7	100
TEX	BR	460	450	94	10	90
F ₁ (TEX x BR)	BR	102	100	58	2	100
BR	F ₁ (TEX x BR)	73	73	56	0	0
BR	F ₁ (BR x TEX)	154	152	47	2	100
F ₁ (TEX x BR)	F ₁ (TEX x BR)	510	362	84	148	97

* Cumulative % blood-fed after 7 blood offerings.

progeny took a blood meal. This might indicate action of a single gene, with the recessive condition inhibiting blood-feeding. Among the F₂ progeny listed in Table 6, about 75 percent were autogenous and 84 percent of these individuals took a blood meal, whereas nearly all (97 percent) of the anautogenous individuals took a blood meal.

DISCUSSION. Evidence obtained from a variety of crossing experiments between autogenous and anautogenous strains indicates that autogeny in *A. atropalpus* is controlled by a single dominant, autosomal gene. Chi-square analysis indicated no significant departure from the expected results. Reciprocal crosses to the F₁, all eight possible backcrosses and four crosses to the F₂ showed segregation in accordance with expectation. BASS ROCKS and GARRETT, the two autogenous strains which were utilized in crossing experiments, originated from widely separated and distinctly different habitats. Yet each of these strains gave similar results in crossing experiments with TEXAS. Moreover, experiments with the LAB strain conducted by a different investigator in another laboratory building also gave results fitting the hypothesis.

A monofactorial mode of inheritance was proposed by Roubaud (1930) for autogeny in *C. pipiens*. He postulated that autogeny was the result of a single recessive gene in the homozygous condition. When he crossed an autogenous

with an anautogenous strain, autogeny disappeared in the F₁ and reappeared in the F₂ generation. Similar results were obtained by Callot (1947) and Weyer (1935). However, Spielman (1957) discounted a monofactorial hypothesis for this species. After statistically analyzing the results of an extensive series of crosses and backcrosses, he suggested that autogeny was controlled by factors present on all 3 linkage groups. In the same study he found the mutant yellow fat color, which is regulated by an autosomal recessive factor, was not linked to autogeny. Therefore, inheritance based on factors present on only 2 chromosomes was also postulated. Khan (1963) performed crossing experiments where each of the 3 linkage groups were marked with a visible mutant; he concluded that linkage group 2 had relatively little influence on the expression of autogeny. This supports the work of Spielman since the factor for yellow fat color probably belongs to linkage group 2. In an effort to account for the apparently conflicting results of crossing experiments using different strains, Laven (1967) has proposed a working hypothesis involving a two-factor inheritance with one factor having a dominant-recessive correlation, while the other is represented by a series of multiple alleles. Thus a complex control mechanism has replaced the rather simple one proposed by earlier workers.

Not only does the genetic mechanism

for autogeny in *C. pipiens* appear to differ from that in *A. atropalpus* but the relative sensitivity of each genetic system to environmental factors differs greatly. In *C. pipiens* earlier workers found that autogeny was either not expressed in F_1 hybrids between autogenous and anautogenous strains or was expressed only slightly. In reciprocal F_1 hybrids, Spielman (1957), working with American strains, observed a percentage autogeny of 50.6 and 73.9 respectively. A still higher proportion of autogeny (83.2 percent) was observed by Krishnamurthy and Laven (1961) in F_1 hybrids obtained from crossing an Indian anautogenous strain with a European autogenous strain. Laven (1967) implied that such differences have a genetic basis. However, Krishnamurthy and Laven (1961) showed that if overcrowded conditions existed in larval cultures, the amount of autogeny in F_1 hybrids dropped below 50 percent.

Environmental and nutritional factors can drastically affect the expression of autogeny in other *Culex* mosquitoes. Kardos (1959) demonstrated that larval nutritional level could affect the development of autogeny in *Culex tarsalis* Coq. Also in *C. ursalis*, the length of the photoperiod can markedly influence ovarian development (Harwood, 1966). Because the expression of autogeny is so sensitive to environmental factors, such factors are frequently difficult to separate from genetical factors. On the other hand, autogeny in *Aedes atropalpus* is both stable and uniform and is relatively unaffected by environmental variations. In contrast with *Culex* mosquitoes, *A. atropalpus* exhibited nearly complete penetrance, stable expression, and complete dominance for this character.

Since autogeny is not sex-linked (linkage group 1), this character may now be used as a marker for one of the two autosomes of *A. atropalpus*.

The TEXAS strain differs markedly from the BASS ROCKS strain in blood-feeding activity (Fig. 2). In the laboratory Davis (1940) and Trembley (1945 and 1947) used mouse, guinea pig, spar-

row, man, white rat, and chicken; with all of these hosts, *A. atropalpus* seldom fed or was an irregular feeder. Literature reports on field behavior are conflicting; several reports, especially those concerning southern populations, describe females as avid and vicious biters on man. Other reports, especially those from the north, indicate that blood-feeding on man is uncommon. During our field collecting of northern populations biting was rarely observed, even where adult populations were at a high density. Thus our field observations, together with results of experiments reported in the present work (Fig. 2), indicate that populations of *A. atropalpus* do differ in their blood-feeding activity. Furthermore, results shown in Table 6 suggest that this activity is controlled by a single gene with avidity for blood dominant. Also this gene appears to be closely or completely linked to the gene for autogeny. However additional experimentation is need before the genetic relationship between the two factors can be determined.

The raw material for genetic analysis, namely alternative forms of given traits, appear to be abundant in this species, since in the course of this study several morphological variations were noted. This fact together with the ease in colonizing and simplicity of rearing make this species an excellent subject for genetic investigation.

SUMMARY. The mode of inheritance of autogeny was analyzed in several strains of *Aedes atropalpus*. Eleven field populations from Maine, Massachusetts, Connecticut, Maryland, Wisconsin and Minnesota, were autogenous whereas a population from Texas was anautogenous. Reciprocal crosses between various autogenous strains and the TEXAS strain gave F_1 females that were uniformly autogenous, albeit the rate of ovarian development was slightly slower than that in females from autogenous strains. Backcrosses to parental strains and F_2 progeny showed that autogeny is controlled by a single, dominant, autosomal gene.

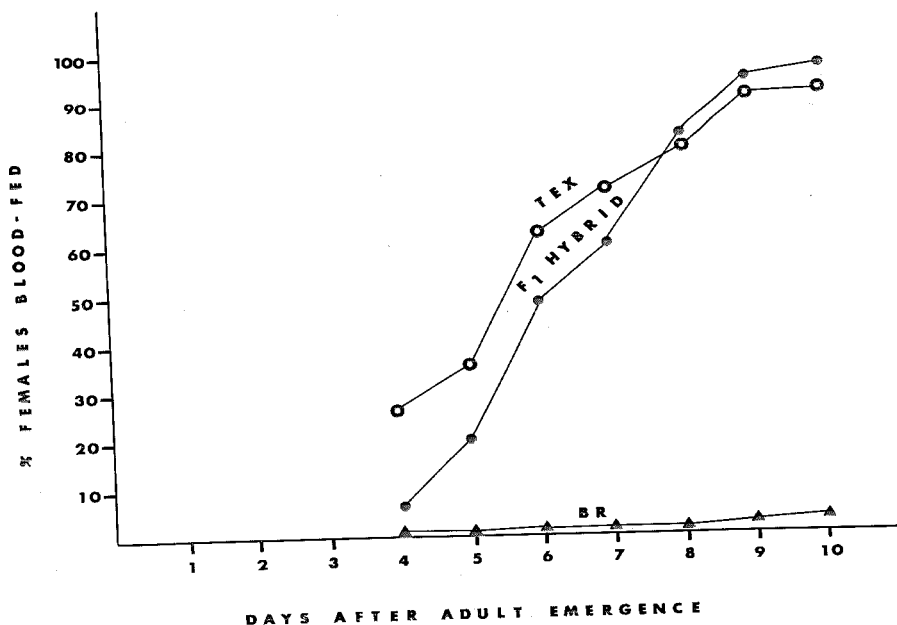


FIG. 2.—Blood-feeding activity of females of the BASS ROCKS (BR) and TEXAS (TEX) strains and of the F₁ hybrid, TEXAS x BASS ROCKS. Activity measured as the cumulative percentage blood-fed.

Number tested: BR=455

TEX=541

F₁=484

Anautogenous females blood-feed readily 4 days after emergence whereas autogenous females seldom take a blood meal. F₁ hybrids blood-feed readily even though they are uniformly autogenous. Results of backcrosses and F₂'s suggest monofactorial inheritance with very close linkage between blood-feeding and autogeny.

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A DEVICE FOR THE DRIP APPLICATION OF INSECTICIDE CONCENTRATES

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INTRODUCTION. The drip method of applying insecticides to flowing and impounded waters for mosquito control has been used intermittently for the past 20 years. Numerous methods have been used to introduce chemical insecticides in both the concentrate and dilute form. With the development of organophosphate compounds with improved stability, renewed attention has been given to the use of the drip technique employing insecticide concentrates.

Knowles and Fisk (1945) employed a pump to transfer water into an air-tight bottle connected to a sealed reservoir containing the insecticide solution. With an increase in air pressure in the reservoir, the larvicide was discharged via a tube. Wiscup *et al.*, (1946) described a drip can regulated by a metering valve from an oil stove. Geib and Smith (1949) employed the siphon principle to discharge a dilute

DDT solution from a 50-gallon drum reservoir, via a glass metering tip. Lancaster, (1964, 1965) used the same principle to apply emulsifiable concentrates through hypodermic needles for mosquito control in rice fields. Gahan *et al.*, (1955) designed an applicator for dispensing dilute insecticides which had a constant head column fed by gravity flow. The height of the liquid column was regulated by the liquid level in the well; when the level decreased, air flowed into the sealed reservoir breaking the vacuum and filling the column enough to cover the lateral opening. When the air supply was cut off, a vacuum formed again and the flow ceased.

Recently Mulla and Darwazeh (1968), and Sjogren and Mulla (1968), employed a constant head applicator which used calibrated discs to vary the discharge rate desired. In using this apparatus to dispense emulsifiable concentrates, problems were encountered by the senior author due to the inability to precisely adjust the discharge rate to that desired and the tendency of the orifice to clog when using commercially available formulations.

The device described in this paper was designed for simplicity, portability, accuracy of discharge and ease in the regula-

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