EFFECTS OF LOW BIURET UREA ON NATURAL POPULATIONS OF *HIPPELATES COLLUSOR* (TOWNSEND) AND *LEPTOCONOPS KERTESZI* (KIEFFER)[1]

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ABSTRACT. Reductions of naturally breeding field populations of *Hippelates collusor* and *Leptoconops kerteszi* were obtained with granular and spray applications of urea ($CO(NH_2)_2$) to the soil. Control ranged from 10 to 96 percent, depending on the dosage and method of application (disced or surface application). Possible modes of action and the use of urea, as a substance harmless to natural enemies, in the integrated control of pestiferous soil arthropods are discussed.

The observation that larvae of the eye gnat, *Hippelates collusor* (Townsend), dispersed naturally away from high larval densities in the soil without internecine struggle, suggested that some secretion of the larvae may provide a stimulus to migration (Legner, 1966). Research on other insects showed that bacteriostatic secretions are produced by some dipterous larvae (Pavillard and Wright, 1957; Landi, 1960). These might have some effect on the dispersion of other individuals. The possibility that *Hippelates* larvae may elaborate biologically active substances was suggested by the observation that fungus growth was inhibited in yeast cultures containing gnat larvae (Legner, 1966; Spielman, 1962). Further studies showed that *H. collusor* larvae consistently responded to increasing inoculation densities in the soil, where greater distances of migration produced a proportionally higher larval mortality (Legner and Olton, 1969).

Leads to the possible nature of dispersion inciting substances produced by dipterous larvae were found in work by Robinson (1935), Robinson and Norwood (1934), and Wigglesworth (1956). Uric acid is apparently the substance produced in greatest quantity, although other compounds such as allantoin are also found.

Preliminary laboratory tests with allantoin and synthetic urea showed that only the latter compound caused a mortality of *Hippelates collusor* larvae when applied to the medium in which the larvae were developing. The tests excluded any dispersing action of urea on the larvae, but mortality was high enough to suggest field testing. Studies were eventually extended to include *Leptoconops kerteszi* (Kieffer), a biting gnat that breeds in river bottom and moist sandy soils in southern California.

Earlier tests by B. R. Bartlett (personal communication), Division of Biological Control, University of California, Riverside, showed that urea, a common fertilizer, possessed no lethal properties for a wide array of beneficial organisms. This report presents results of field experiments in which urea and a control material (ammonium nitrate) were applied to breeding habitats of eye gnats and the *Leptoconops* biting gnats.

Earlier, it had been demonstrated that their principal natural enemies in the re-

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spective habitats could survive such treatment. This was determined by comparing the longevity of 5 to 10 individuals, caged with the control materials, with the longevity of others in vials containing urea granules and vials that had plaster-of-paris bases and were wetted with urea concentrates. The *Hippelates* parasitoids thus tested were *Spalangia drosophilae* Ashmead, *Phaenopria occidentalis* Fouts, and *Hexacola* sp. *Hippelates* predators tested were mixed cultures of the staphylinids, *Apocellis analis* LeConte, *Lobrathium lituarium* LeConte, *Neobisnius paederoides* LeConte, *Philonthus alumnis* Erichson, *Philonthus hepaticus* Erichson, and *Platystethus spiculus* Erichson; the carabid, *Agonoderus maculatus* LeConte; and the dermapteran, *Euborellia annulipes* (Lucas). Predators of *Leptoconops* tested were the carabid, *Bembidion* sp., and the staphylinids *Carpelimus* spp., *Myllena* sp., *Neobisnius paederoides* LeC., and *Philonthus grandicollis* Horn. There were no detectable differences in longevity in cultures exposed continuously to urea and the controls up to 30 days. The determination of the above species as key natural enemies of the respective hosts was made by Legner (unpublished data).

METHODS AND MATERIALS. Experiments were conducted in breeding habitats of *Hippelates collusor* in the Coachella Valley in 1966 and 1968, and in the habitat of *Leptoconops kerteszi* near the Santa Ana River in 1969.

The selected dosages of low biuret urea [4] were based on practical fertilizer applications on citrus used in the Coachella Valley (600–1,300 lbs./acre), and in rates equivalent to the estimated current cost of insecticide applications in the Santa Ana River. The synthetically produced urea chosen produced a maximum of only 0.15 percent biuret, a compound that is toxic to some kinds of vegetation. Another desirable quality of urea that made it especially suitable for this study was that it leaves little or no residue. Urea is converted to ammonic nitrogen, carbon dioxide, and nitric nitrogen and does not cause appreciable increases in soil salinity even when used in large quantities over long periods of time.[5]

Three experiments were conducted with field-breeding populations of *H. collusor*, and one experiment with *L. kerteszi.*

Hippelates collusor. EXPERIMENT I. A four-year old citrus orchard near Mecca which had a history of high *Hippelates collusor* density was used for the experiment. The orchard, which had an alfalfa cover crop, was cultivated three times to a depth of 8 inches, one day after irrigation on April 21, 1966. Four days later on April 25, it was divided into 16 separate units, each unit containing five citrus trees on each side and 528 square feet of cultivated alfalfa. This unit constituted one replicate. Granules (size # 5–8) of low biuret, 46 percent urea [$(CO(NH_2)_2)$] were used in the treatments with each dosage (354, 708, and 1,417 lbs./acre active material) being spread over the plots by hand. Control plots were untreated. The experimental design was completely random with each treatment being replicated four times.

Immediately after application, both treatments and controls were wet with 31 gallons of water per replicate to begin the granule dissolving process. Two hours later, eight emergence traps (Mulla, 1961 and Fig. 1) were placed at random in each replicate and the soil banked around each trap, to prevent the escape of emerging individuals from the trap area and to exclude further wetting during irrigation. *H. collusor* adults which emerged were collected weekly for 6 weeks, and their average oven-dry weight measured. Analyses of variance were performed for each collection date and significant treatment differences were tested.

EXPERIMENT II. The soil in the same orchard was cultivated 3 times to a depth of 12 inches on June 9, 1966. Granular applications of urea (354, 708, and 2,834

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[4] Norsk Hydro, Oslo, Norway. Viking Ship Urea 46%, The Fertilizer of Modern Agriculture.

[5] Shell Chemical Corporation Technical Bulletin No. 12A-R. Shell Urea.

FIG. 1.—Emergence units for the collection of *Hippelates* and *Leptoconops* adults from the soil. Photo shows units in place during *Hippelates* experiment I.

lbs./acre active material) were made on the same day followed by a fourth cultivation which mixed the granules into the breeding habitat in the soil to a depth of about 8 inches. The orchard was flood-irrigated 4 hours after the treatments were applied. Four days later on June 13, emergence traps were placed in each plot and adults collected for 3 consecutive weeks thereafter.

EXPERIMENT III. A 30-year-old date grove near Thermal, with a cover of Sudan and Bermuda grasses, was irrigated on May 29, 1968. Four days later the grove was cultivated three times to a depth of 8 inches. Granular treatments were applied by hand in a randomized complete block design to 528 square-foot plots (2,064 lbs./acre NH_4NO_3, and 354 and 2,834 lbs./acre urea, active material) followed by a fourth cultivation to mix granules into the soil. Emergence traps were fixed 10 days after treatment on June 16 and 5 weekly adult emergences recorded.

The traps were removed on August 1 after emergence was complete. The grove was disced three times to a depth of 8 inches on September 9, and the traps reset at random in the plots 10 days later on September 19, without additional treatments. Six weekly adult collections were gathered.

Flood irrigations were made during experiments I–III on the average of every 16 days; however, none of this water entered the emergence traps.

Leptoconops kerteszi. Three locations in the Santa Ana River bottom were selected as potential study sites in July 1969. Initial adult yields from the respective areas indicated that a site located 1 mile above the Prado Dam near the city of Norco had a substantial biting gnat density with a minimum sample variation. Experimental plots were arranged contiguously in the area with each plot measuring 45′ x 15′ (675 square feet). Plots were replicated four times and treatments applied at random as granules and dissolved granule sprays directly to the surface of the soil on August 8, 1969 (118.8, 237.6, 475.2 lbs./acre urea, active material). The same traps that were used in the *Hippelates* experiments were set at random in the plots at the rate of 6 per plot, and emerged adults were collected weekly for 4 weeks.

On September 20, the 6 traps in each replicate were moved to new random positions within the same plot, and weekly collections were resumed for another 4 weeks. A final movement of the traps was made on October 21 and 4 weekly collections were made.

Emerged adults were sexed and data recorded separately according to the type of application (granules or sprays) and treatment. A factorial analysis of the data was made and the significance of treatments, application types, sex and interactions tested.

Statistical Analyses. Each set of emergence data was assessed by analysis of variance on the basis of either the completely random or randomized complete block design with subreplicates, as indicated. Because of the existence of zero values in the data, the validity of the analyses was ensured by employing the $\sqrt{X+1/2}$ transformation, where X is equal to the number of adult gnats of one sex in each subreplicate. However, because of practical disadvantages in the use of transformed data in discussion, means in the text are expressed in terms of the original variates.

Results. *Hippelates collusor.* Weekly emergence records of *H. collusor* adults from soil treated with surface applications of urea without cultivation (Experiment 1) are shown in Table 1. There was variable emergence in the different treated areas, but the totals of all collections showed a slight significant (5 percent level) control effect for the medium and high dosages only (10.40 percent and 14.57 percent). Gnat emergence increased where low dosages were applied (Table 2). There were no significant differences in the oven-dry weights of adults collected during the initial 3 weeks of emergence.

Results of the second experiment, in which granule treatments were mixed into the soil by one cultivation after application are shown in Table 2. Here significant reductions in emergence exceeding 50 percent were recorded at all dosage levels and at all 3 weekly collections. There were no significant weight differences.

Similar results were shown in the third experiment, where treatments were also mixed into the soil by a cultivation (Table 3). However, recultivation following the first period of emergence resulted in an increase of emergence from the treated areas. This could have been the result of the increased growth of vegetation produced in the treatments by the fertilizer effect of urea.

Leptoconops kerteszi. Table 4 shows significant reduction (over 51 percent) in emergence from all dosages of urea applied to the surface of the soil without cultivation. The fact that *L. kerteszi* larvae and pupae develop primarily in the upper inch of soil, may be responsible for significant effects at such low dosages compared to the experiments with *Hippelates.* One initial treatment was sufficient to reduce significantly the *Leptoconops* emergence for at least three field generations (Table 4) from August 14 through November 11. There were no significant interactions nor total emergence differences between spray and granular applications. Females predominated in all replicates, and no significant treatment size differences were detected.

Discussion. The level of control achieved with both species may, under certain conditions, be sufficient to warrant widespread application. Degree of annoyance to humans is difficult to measure and will vary according to meterological conditions. Often a 50 percent reduction, as shown in most of these tests, will suppress the pest population sufficiently to reach a tolerable level.

The long range effect of continuously reducing gnat population densities by 50

TABLE 1.—Emergence of *Hippelates collusor* adults from soil treated with granular applications of UREA applied on the surface at Mecca, California, April, 1966.

Collection Date	Mean number and oven-dry weight of adults emerged/meter-square [1]										
	354 lb.[2]			708 lb.			1417 lb.			Control	
	x̄	mean wt.[4]	% reduction[3]	x̄	mean wt.	% reduction	x̄	mean wt.	% reduction	x̄	mean wt.
5/4/66	20.81	1.15	36.93+	17.81	1.20	26.31+	5.63	0.90	57.14	13.28	1.20
5/11/66	95.56	1.17	56.29+	44.18	1.10	5.29+	31.50	1.20	24.50	41.59	1.20
5/18/16	73.56	1.02	13.48	77.19	1.30	9.25	91.16	1.40	6.58	85.16	1.10
5/25/66	18.75	....	28.26	9.06	....	65.27	12.50	....	52.22	26.16	....
6/1/66	1.63	....	46.51+	1.22	....	33.33+	1.25	....	33.33+	0.84	....
to											
6/8/66	1.41	....	60.52+	0.69	....	16.66+	1.13	....	50.00+	0.56	....
Total	211.72	1.11	20.84+%*	150.15	1.20	10.40%*	143.17	1.17	14.57%*	167.59	1.17

[1] 8 subreplicates in 4 blocks.
[2] Active Urea per acre.
[3] Percent reduction over control (+ indicates greater than control).
[4] Oven-dry weights are 1×10^{-4} g.
* = significant at 95% level.

TABLE 2.—Emergence of *Hippelates collusor* adults from soil treated with granular applications of UREA disced-in 6 inches at Mecca, California, June, 1966.

Collection Date	Mean number and oven-dry weight of adults emerged/meter-square[1]										
	354 lb.[2]			708 lb.			2834 lb.			Control	
	$\bar{x}$	mean wt.[4]	% reduction[3]	$\bar{x}$	mean wt.	% reduction	$\bar{x}$	mean wt.	% reduction	$\bar{x}$	mean wt.
6/22/66	4.06	1.40	67.27	3.84	1.30	69.09	1.91	1.10	84.54	12.37	1.20
6/29/66	48.75	....	55.40	46.31	....	57.66	24.19	....	77.87	109.19	....
to 7/5/66	15.28	....	63.24	18.22	....	56.21	16.94	....	59.27	41.53	....
Total	68.09	1.40	58.25%**	68.38	1.30	58.07%**	43.03	1.10	73.61%**	163.09	1.20

[1] 8 subreplicates in 4 blocks.
[2] Active Urea per acre.
[3] Percent reduction over control.
[4] Oven-dry weights are 1×10^{-4} g.
* = significant at 95% level.
** = significant at 98% level.

TABLE 3.—Emergence of *Hippelates collusor* adults from soil treated with granular applications of ammonium nitrate and UREA, disced-in 6 inches at Thermal, California, June, 1968.

Collection Date	Mean number adults emerged per meter-square[1]						
	NH_4NO_3 2064 lb.[2]		Urea 354 lb		Urea 2834 lb.		Control
	x̄	% reduction[3]	x̄	% reduction	x̄	% reduction	x̄
First Trap Set							
6/24/68	0.03	88.66	0.13	73.33	0.03	93.33	0.47
7/2/68	9.69	59.31	8.91	62.59	0.66	97.24	23.81
7/9/68	2.25	63.63	3.56	42.42	0.28	95.45	6.19
to							
7/18/68	0	100.00	0.03	66.67	0	100.00	0.09
7/24/68	0	100.00	0.09	50.00	0.03	83.33	0.19
Total	12.00	60.97%**	12.72	58.63%**	1.00	96.74%**	30.75
Second Trap Set (Soil disced)							
9/27/68	9.22	48.47+	5.94	20.00+	5.09	6.74+	4.75
10/4/68	20.44	4.66	22.94	6.53+	33.50	36.00+	21.44
10/11/68	12.53	52.36+	6.78	11.98+	9.16	34.81+	5.97
10/18/68	5.09	72.39+	1.53	8.16+	1.47	4.25+	1.41
10/25/68	1.19	47.36+	0.94	33.33+	0.59	5.00	0.63
to							
11/4/68	1.28	56.09+	1.03	45.45+	0.63	10.00+	0.56
Total	49.75	30.13+%*	39.16	11.23+%	50.44	31.08+%*	34.76

[1] 8 subreplicates in 4 blocks.
[2] Active material per acre.
[3] Percent reduction over control (+ indicates greater than control).

TABLE 4.—Emergence of *Leptoconops kerteszi* adults from Santa Ana River bottom soil treated with granular and spray applications of UREA combined, applied on the surface on August 8, 1969.

Collection Date	Trap Set	Mean number adults emerged/meter-square[1]						
		118.8 lb.[2]		237.6 lb.		475.2 lb.		Control
		x̄	% reduction	x̄	% reduction	x̄	% reduction	x̄
8/14/69	I	3.375	0	3.958	14.74+	1.875	44.44	3.375
8/26/69	"	3.750	10.00	2.208	47.00	0.875	79.00	4.167
9/4/69	"	1.083	64.38	0.333	89.04	0.583	80.82	3.042
to								
9/9/69	"	0.208	70.59	0.125	82.35	0.042	94.12	0.708
Total	"	8.417	25.46%*	6.625	41.33%**	3.375	70.11%**	11.292
9/20/69	II	1.917	8.69+	0.875	50.00	3.083	43.24+	1.750
9/26/69	"	2.458	66.09	4.792	33.91	2.625	63.79	7.250
10/4/69	"	3.250	70.34	3.208	70.72	3.125	71.48	10.958
to								
10/11/69	"	1.167	65.43	1.083	67.90	0.667	80.25	3.375
Total	"	8.792	62.32%**	9.958	57.32%**	9.500	59.28%**	23.333
10/21/69	III	3.167	48.99	2.667	57.04	3.125	49.66	6.208
10/28/69	"	4.708	58.60	2.042	82.05	2.958	73.99	11.375
11/4/69	"	4.708	51.29	6.417	33.62	7.208	25.43	9.667
to								
11/11/69	"	2.792	50.73	0.750	86.76	1.375	75.73	5.666
Total	"	15.375	53.29%**	11.875	63.92%**	14.667	55.44%**	32.917
Grand total (8/14–11/11)		32.584	51.75%**	28.458	57.86%**	27.542	59.22%**	67.542

[1] 6 subreplicates in 4 blocks.
[2] Active Urea per acre.
[3] Percent reduction over control (+ indicates greater than control).

percent or more, could, theoretically, result in a lowering of the breeding threshold; especially in the presence of uninterrupted activity by natural enemies, whose efficiency appears to increase at lower host densities (E. F. Legner, unpublished data).

Several possible modes of action are suggested for urea in these experiments, but further research is needed to definitely establish any one. Concurrent laboratory tests have largely eliminated the factor of any repellent action of urea on the larvae of either species. Starvation is probably not involved as indicated by the uniformity of oven-dry weights of survivors. It was observed that dissolved urea recrystallizes in a myriad of jagged crystals. This could interfere with larval respiration, and possibly abrade the larval integument. Also, the mode of action might involve the favoring of a pathogenic organism in the soil. The abundance of *Saprolegnia* fungi pathogenic to gnat larvae in some soil cultures treated with urea favors this hypothesis. However, there is no experimental evidence that would eliminate the action of the material as a stomach poison.

Further study to define the active component which results in the insecticidal action of urea could lead to a more practical application of substances that reduce the natural breeding potential of pestiferous soil arthropods, and which at the same time may not create problems of resistance and destruction of natural enemies.

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