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Distribution, Abundance and a Probability Model for Larval
Culicoides variipennis^{1/} on Dairy Farms in New York State^{2/}
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ABSTRACT

Aquatic habitats on New York state dairy farms were sampled to determine the distribution and abundance of larval Culicoides variipennis (Coquillett), and to develop a probability model for the presence of the species. Larvae were found on 5 to 7 of 8 dairies selected randomly in each of 7 widespread counties; overall, 46 of 56 farms (82%) were positive. Of 626 substrate samples examined, 294 (47%) contained C. variipennis larvae. Larval density was 1-10, 11-49 and >49 larvae per 150 cm³ sample in 72%, 14% and 14% of positive samples, respectively. Larvae occurred in a wide variety of farm habitat 'types' and 'locations', but were particularly common in cattle-modified substrates in pastures and cow yards where the source of water was 'springfed' or 'milkhouse effluent'. Only 1 of 108 samples from 20 dairy manure storage systems contained C. variipennis larvae.

Nine variables associated with larval habitats contributed significantly to a stepwise logistic regression equation that predicted the presence or absence of C. variipennis with an accuracy of 80.07%. The 'degree of animal access', 'pH', 'nitrate concentration', 'water source=milkhouse effluent', 'phosphorous concentration', 'habitat type=stream', 'water source=spring fed', and 'sample period' were positively associated with the presence of larvae; '% organic matter' was negatively associated. These findings are discussed in terms of their relevance to Northeast dairy operations.

Mild summers, extensive forage acreage and a proximity of consumer population centers combine to support extensive dairy farming in the Northeastern United States. Also common to the region is a rich Culicoides fauna (Foote and Pratt 1954), several species of which, including C. variipennis, seek blood from pastured livestock (Schmidtman et al. 1981). Reported here are data concerning the larval development sites of C. variipennis on dairy farms in New York state and a probability model for predicting the presence or absence of the species.

Though not currently recognized to be of economic importance, "eastern" C. variipennis is related to populations of the C. variipennis group that transmit bluetongue virus of domestic ruminants in the Western U.S. (Jones et al. 1981). The immature stages of eastern C. variipennis have been reported from substrates bordering streams, ponds and puddles, particularly those contaminated by animal manure (Jones 1961, Hair et al. 1966, Kardatzke and Rowley 1971, and Battle and Turner 1972). In the Northeast, neither the prevalence of aquatic habitats that support larval development nor the density of larval populations is known (Jamnback 1965), although eastern populations of C. variipennis have been reported to be sparse and less dense than western populations (Wirth and Jones 1956, Jones 1961).

Materials and Methods

Fifty-six dairies, 8 in each of 7 New York state counties with extensive dairy farming (Fig. 1), were selected randomly from township farm lists. Access to farms and permission to sample aquatic habitats for C. variipennis larvae were obtained by NYS Cooperative Extension staff. Four farms in each county were visited between mid-April and mid-May 1980 and 4 more were visited during July 1980. Twenty manure storage systems typical of those constructed in recent years to accommodate increases in dairy herd size (Ainslie and Natzke 1980), were sampled in July 1980.

Permanent and semi-permanent aquatic habitats on each farm were examined by taking 3 sets of 3 (9) 150 cm³ substrate samples from around the perimeter of standing waters or along the margin of streams. Each habitat was classified by 'general type', 'location on farm', 'source of water', 'degree of animal access' and 'degree of manure loading' (see Table 2). Substrate samples were taken by inserting a shallow-scoop trowel at the waterline of sediment accumulations and removing an aliquot of surface mud up to ca. 10 cm X 5 cm and 2 cm thick from below the water; late instar C. variipennis larvae are generally most numerous in this zone (Barnard and Jones 1980). The trowel was briefly drained of free water and the substrate placed in a container marked at a volume of 150 cm³. Several aliquots were taken to make up each 150 cm³ sample. If the habitat was small and uniform, only 1 or 2 sample sets (3 or 6 samples) were taken. Substrate samples were placed individually in plastic bags and held in an insulated chest on ice packs until they were refrigerated at ca. 4.5°C.

In the lab, 2 samples of each sample set, a total of 626 samples, were individually washed through a 60-mesh sieve; the 3rd sample was frozen for chemical analysis. Ceratopogonid larvae remaining in the sieve from each sample were back-flushed into a white enamel pan and transferred into 70% alcohol. Third and 4th instar C. variipennis larvae were identified by the anteriorly-narrowed head capsule, heavily-sclerotized pharyngeal armature and size.

Substrate samples used for analysis of chemical content (n=199) were thawed at 4.5°C, weighed, oven-dried and analyzed for pH, % organic matter, soluble salts, nitrate, phosphorous, potassium, magnesium and calcium. Samples were tested by the Department of Agronomy, New York State College of Agriculture and Life Sciences, Cornell University, in accordance with procedures in Grewelling

and Peech (1965).

The data, 20 variables for each sample set (excluding manure storage system samples), were analyzed by the technique of logistic regression (see Hanushek and Jackson 1977) to identify predictor variables strongly associated with the presence of C. variipennis and to estimate the probability of presence of C. variipennis in a sample from that sample's characteristics. The logistic regression model is

$$\lambda_i = \log[p_i/(1-p_i)] = \sum_{j=1}^k \beta_j x_{ij} \quad \text{for } i=1, 2, \dots, n,$$

where x_{ij} is the value of the j th predictor variable in the i th sample, β_j is the coefficient of the j th predictor, p_i is the probability of presence of C. variipennis in a sample with predictors equal to $x_{i1}, x_{i2}, \dots, x_{ik}$, and λ_i is the log odds or logistic transform of p_i . The β_j 's and p_i 's are unknown, and are estimated from the predictors x_{ij} and the responses Y_i , which are 1 if C. variipennis is present in the i th sample and 0 otherwise. Predictor variables used in the logistic regression model were selected in a stepwise manner, and their coefficients estimated at each step. Logistic regression is more appropriate for this problem than either discriminant analysis or linear regression on the Y 's (see Lee 1980). The computer analysis was performed using the program BMDPLR in the BMPD statistical package, supplemented by the program LOGIST in the SAS statistical package.

Results

Third and 4th instar C. variipennis larvae were recovered from 5 to 7 out of 8 dairies in each of 7 counties; overall, 46 of 56 farms (82%) were positive. Maximum larval density was 1-10 larvae per sample on 24 (43%) farms, 11-49 larvae per sample on 10 (18%) farms, and >49 larvae per sample on 12 (21%) farms. Of 626 substrate samples examined, 294 (47%) were positive;

212 samples (72%) contained 1-10 larvae per sample, 42 (14%) had 11-49 larvae per sample and 40 (14%) had >49 larvae per sample. The mean numbers of C. variipennis larvae recovered from aquatic habitats categorized by their "general type", "location on farm" and "source of water" are presented in Table 1.

The range of values observed for each habitat variable, along with data for several substrate samples, are presented in Table 2. Overall mean substrate chemical values and values for samples in which C. variipennis was either present or absent are listed in Table 3. Of the 20 manure storage systems examined, only 1 was positive for C. variipennis (Table 4); larvae were found at the base of a steeply-graded bank in rain-water diluted floating debris.

The predictors selected for the logistic regression equation give the estimated log odds of the probability of the species' presence in a sample when multiplied by their estimated coefficients and summed. These predictors are defined in Table 5, where they appear with their estimated coefficients, the estimated standard errors of these coefficients, and the standard deviations of the predictors. The estimated probability of presence of C. variipennis in a sample is $\hat{p} = \exp(\hat{\lambda}) / [1 + \exp(\hat{\lambda})] = [1 + \exp(-\hat{\lambda})]^{-1}$. This model has a goodness-of-fit chi-square statistic of 236.7 on 220 degrees of freedom, giving $p = .210$. This statistic tests the hypothesis that the data are consistent with the specified model. A small p-value, e.g., $p < .05$, would indicate that the model does not fit the data adequately.

The predictors can be used to classify each sample by whether the estimated probability p_i of the presence of C. variipennis is above a chosen threshold. Thus, in each sample, C. variipennis was predicted as being either present or absent, depending on whether p_i is above or below the threshold; also, the actual presence or absence was determined. For the threshold values of

.435 and .375, the 306 samples are cross-classified as follows:

<u>.435</u>	<u>Predicted</u>		<u>.375</u>	<u>Predicted</u>	
	Present	Absent		Present	Absent
Present	128	23	Present	136	15
<u>Observed</u>			<u>Observed</u>		
Absent	38	117	Absent	57	98

The threshold value of .435 maximizes the overall correct prediction rate among all possible choices of the threshold value, giving a rate of $(128 + 117)/306 = 80.7\%$. The threshold value of .375 maximizes the overall correct prediction rate among all choices of the threshold value that provide correct classification in at least 90% of those cases where C. variipennis is present. This threshold value has a slightly lower overall correct prediction rate of 76.47%, but has a 90.07% correct prediction rate for those samples in which C. variipennis is present.

Discussion

The data show that C. variipennis larvae are common and abundant in aquatic habitats on New York State dairy farms. In addition, the presence of larvae on a high percentage of widespread farms that varied in soil types and animal management, as well as on dairies in Wisconsin and Virginia (Jones 1961, Hair et al. 1966), suggests strongly that the species also occurs on many other dairies in the northeastern United States.

In terms of habitat acceptability, the presence of larvae was largely determined by variables linked to the modification of aquatic substrates by cattle. For example, the equation selected by the logistic regression indicates that 'degree of animal access', 'nitrate concentration', 'pH', 'phosphorus concentration' and 'water source=milkhouse effluent' were positively associated with the species' presence. These variables

are all attributable to cattle or their or urine and manure, which contain high levels of phosphorus, nitrogen, urea and other nitrogenous products. Further, the latter compounds combine with water during early aerobic decomposition to form ammonium carbonate, which breaks down into ammonium hydroxide and carbon dioxide, resulting in an increase in pH as CO_2 gas escapes (Salter and Schollenberger 1939). This reaction may also account for the basic pH of C. variipennis habitats noted by Hair et al. (1966), Kardatzke and Rowley (1971) and Battle and Turner (1972). Dairy milkhouse effluent also consists largely of manure and hoof dirt mixed in water (Zall 1972), and therefore it is not surprising that milkhouse drainage substrates were exploited by C. variipennis, although the relationship has not been reported previously. Milkhouse effluent flows are heated, sporadic and contain mild detergent and disinfectant, but larvae were both common and abundant in this habitat.

The contribution of 'animal access' as a predictor of C. variipennis larvae also reflects modification of aquatic habitats by cattle. Cattle trample stream and pond margins, destroying vegetation and creating the sediment accumulations (Meehan and Platts 1978) that are characteristic of habitats exploited by larvae. Cattle also represent a dependable blood meal source that is attractive to host-seeking females (Schmidtman et al. 1981). Access to ponds and streams by cattle is therefore adaptive to C. variipennis, benefitting both larval and adult stadia.

The variable 'sample period' is the only significant predictor of C. variipennis larvae not directly associated with cattle. The first sample period, mid-April to mid-May, was selected with the thought that sampling would precede the emergence of overwintered larvae. However, it is now apparent that spring emergence of adult C. variipennis in New York occurs during May (Mullens, unpublished data). Therefore, some habitats that were

positive early in the season may have been negative when sampled late in the first period, leading to the inclusion of 'sample period' in the model equation. Differing larval population phenologies also may underlie the large standard errors for mean numbers of larvae in individual habitat categories (see Table 1); in addition, these values likely reflect a clumped larval distribution (variance : mean significantly >1 , Southwood 1978) and variation between similar habitats that were grouped by gross description.

With all other predictor variables held constant, the logistic regression equation selected indicates that an increase in substrate '%-organic matter' decreases the probability of finding C. variipennis larvae in a sample. This finding appears to contradict the well-accepted positive relationship between manure pollution and larval populations (Jones 1961, Hair et al. 1966, Battle and Turner 1972). However, the dairy farm substrates observed were generally high in '%-organic matter', exceeding the levels reported previously for C. variipennis habitats (Kardatzke and Rowley 1971, Battle and Turner 1972). Further, larvae were seldom present in substrates with very high ($>28\%$) organic content, a condition often associated with heavy manure loading; also, our field notes show that substrates consisting of 'frothy, decaying plant matter' also were generally void of larvae. Thus, high organic content appears to be limiting to C. variipennis larvae, and an increase in '%-organic matter' would be expected to have a negative effect on the probability of the species' presence. The variables 'habitat type=stream' and 'water source=spring fed', both positive predictors, may also reflect the negative effect of high organic matter through adding oxygen and diluting manure, countering the high biological oxygen demand of decomposing organic matter. The recovery of larvae in a single sample of water-diluted debris from a manure storage system is consistent with this interpretation. Also, an inverse relationship between high organic pollu-

tion and numbers of mosquito larvae has been reported in swine and poultry waste lagoons (Rutz et al. 1980).

Thus the modification of aquatic habitats by cattle, a common occurrence on dairy farms, is a primary determinant of habitat acceptability for larval eastern C. variipennis. As illustrated by the probability equation, the relationship between the presence of larvae and aquatic habitats is complex and involves numerous factors, several of which we have identified and interpreted. On the other hand, many other aspects of the relationship, both ecological and physiological, remain unknown. This information is needed for dealing rationally with an insect of potential economic importance which, through its close association with commercial agriculture, is both common and abundant.

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Footnotes

- 1/ Diptera: Ceratopogonidae
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Table 1. Descriptive summary of *C. varipennis* larval development sites on New York State dairy farms, 1980.

Habitat type	No. habitats: % positive	No. samples: % positive	\bar{x} no. larvae /sample (S.E.)	Habitat location	No. habitats: % positive	No. samples: % positive	\bar{x} no. larvae /sample (S.E.)	Water source	No. habitats: % positive	No. samples: % positive	\bar{x} no. larvae /sample (S.E.)
[1] ^{1/} Pond	61:51	260:39	10.1 (2.4)	[1] Pasture	45:53	196:32	6.1 (1.5)	[1] spring-fed [4] continuous stream flow ^{3/}	36:56 9:44	152:33 44:27	7.3 (1.9) 1.8 (0.6)
				[2] Cow-yard	6:23	32:69	33.0 (15.2)	[1] spring-fed [2] milkhouse effluent [3] silo effluent [4] continuous stream flow	2:100 1:100 1:0 2:100	12:100 6:100 2:0 12:33	85.7 (36.4) 3.2 (1.4) 0 0.8 (0.5)
				[4] Other	10:25	32:19	11.0 (6.9)	[1] spring-fed [2] milkhouse effluent	6:17 4:50	16:13 16:25	0.8 (0.6) 23.8 (15.2)
[2] Stream ^{2/}	52:67	226:52	33.8 (7.8)	[1] Pasture	37:73	184:49	22.9 (6.4)	[1] spring-fed [2] milkhouse effluent [4] continuous stream flow	6:100 2:50 29:69	28:89 10:40 146:42	111.4 (30.0) 1.9 (1.5) 3.1 (0.9)
				[2] Cow-yard	11:64	26:67	93.9 (40.4)	[1] spring-fed [2] milkhouse effluent [4] continuous stream flow	2:100 3:67 6:50	8:100 8:50 10:40	179.9 (54.1) 226.0 (178.6) 1.3 (0.5)
				[3] Road-side ditch	3:67	10:70	10.2 (6.1)	[2] milkhouse effluent [4] continuous stream flow	2:100 1:0	8:88 2:0	12:8 (7.4) 0
				[4] Other	1:100	6:100	73.0 (33.5)	[2] milkhouse effluent	1:100	6:100	73.0 (35.5)
[3] Inter-mittent flow	28:37	82:36	5.4 (2.2)	[1] Pasture	7:57	26:58	12.2 (5.7)	[1] spring-fed [2] milkhouse effluent [3] silo effluent	1:0 5:80 1:0	2:0 22:68 2:0	0 15.5 (7.1) 0
				[2] Cow-yard	7:42	24:20	4.9 (4.1)	[1] spring-fed [2] milkhouse effluent [3] silo effluent	1:0 3:67 3:33	2:0 10:40 12:8	0 8.9 (7.5) 0.1 (0.1)
				[3] Road-side ditch	1:0	2:0	0	[2] milkhouse effluent	1:0	2:0	0
				[4] Other	13:30	30:17	0.4 (0.2)	[2] milkhouse effluent [3] silo effluent	9:44 4:0	22:23 8:0	0.5 (0.2) 0
[4] Bog	15:41	58:25	1.4 (0.7)	[1] Pasture	13:46	50:25	1.4 (0.8)	[1] spring-fed	13:46	50:26	1.4 (0.8)
				[2] Cow-yard	1:100	6:33	1.8 (1.5)	[4] continuous stream flow	1:100	6:33	1.8 (1.5)
				[4] Other	1:0	2:0	0	[2] milkhouse effluent	1:0	2:0	0
Totals	156:50	626:39	16.8 (2.9)	[1] Pasture	102:60	456:38	12.3 (2.6)	[1] spring-fed	67:55	270:41	26.0 (4.9)
				[2] Cow-yard	25:56	88:57	44.3 (14.9)	[2] milkhouse effluent	32:59	112:49	24.4 (10.3)
				[3] Road-side ditch	4:50	12:58	8.5 (5.2)	[3] silo effluent	9:11	24:4	<0.1 (<0.1)
				[4] Other	25:31	70:24	11.4 (4.7)	[4] continuous stream flow	48:63	220:36	2.5 (0.5)

1/ Numbers in brackets refer to the data set code.

2/ Even sluggishly moving water is included in the "Habitat type = stream" category.

3/ Includes only streams originating well upstream from habitat sampled.

Table 2. Data set organization and range of habitat variable values, *Culicoides varipennis* larval survey, New York State dairy farms, 1980.

Sampling variables								Continuous variables								Categorical variables				
								Kg/ha												
County	Period	Farm	Habitat number	Sample set	No. samples	No. <i>C. varipennis</i> larvae	No. larvae in sample 1	% Organic matter	pH	Phosphorus	Potassium	Magnesium	Calcium	Nitrate	Soluble salts (K x 10 ⁵)	Habitat type	Habitat location	Habitat water source	Degree animal access	Degree manure loading
1-7 ^{1/}	1-2	1-8	1-7	1-3	1-2	0-1344	0-618	0-77	4.9-8.1	4-8921	43-8699	125-4818	803-62453	0-40	2-2098	1-4 ^{3/}	1-4 ^{4/}	1-4 ^{5/}	0-3 ^{6/}	0-3 ^{7/}
1 ^{2/}	2	6	2	3	2	292	226	10	7.4	134	339	638	8565	4	85	2	2	1	2	2
1	2	8	1	2	2	71	41	19	7.2	723	571	803	9814	9	165	3	1	2	1	1
4	2	5	3	1	2	0	0	31	7.2	71	714	2471	62453	9	302	1	4	1	1	1
4	2	6	3	1	2	62	9	8	7.3	60	357	558	6781	4	94	1	1	1	2	2

1/ Values in horizontal column represent range of values for each variable.

2/ Values taken from original data set, 4 farms, 4 habitats.

3/ 1 = pond; 2 = stream; 3 = Intermittent flow; 4 = bog.

4/ 1 = pasture; 2 = cow yard; 3 = road-side ditch; 4 = other.

5/ 1 = spring fed; 2 = milkhouse effluent; 3 = silo effluent; 4 = continuous stream flow.

6/ 0 = none; 1 = occasional access by one to several cattle; 2 = sporadic access by few to numerous cattle; 3 = continuous access by numerous cattle.

7/ 0 = none; 1 = little to some manure loading; 2 = moderate manure loading; 3 = heavy manure loading.

Table 3 Descriptive summary of substrate chemical values.

% organic matter	pH	Kg/ha: \bar{x} (SD)					Soluble Salts(k x 10 ⁵)
		Phosphorus	Potassium	Magnesium	Calcium	Nitrate	
14 ^{1/} (11)	6.7(0.7)	379(904)	748(910)	831(729)	9332(9547)	10(7)	162(186)
12 ^{2/} (7)	6.9(0.6)	417(1012)	700(1012)	773(507)	9190(7521)	9(6)	124(94)
16 ^{3/} (14)	6.5(0.7)	343(786)	794(1244)	887(891)	9466(1197)	10(7)	199(336)

1/ Values in horizontal column based on all substrate sample sets (n = 306).

2/ Values in horizontal column based on substrate sample sets with C. varilpennis larvae (n = 151).

3/ Values in horizontal column based on substrate sample sets without C. varilpennis larvae (n = 155).

Table 4. Description of dairy manure storage systems surveyed for C. varilipennis larvae, New York State, 1980.

Type of system	No. systems	No. samples	No. samples with <u>C. varilipennis</u>	Chemical content of substrates							
				pH	% organic matter	kg/ha					Soluble salts (Kx10 ⁵)
						p	K	Mg	Ca	No ₃	
Earthen dike; semi-solid manure; bedding added	8	72	0	8.3 ^{1/}	53.9	65901	152697	76626	171232	97	17848
Earthen dike; semi-solid manure; milk house effluent and bedding added	8	72	1	8.3	46.6	62486	132644	58839	170841	81	12815
Earthen dike; liquid manure	1	9	0	7.3	3.5	7810	10230	3933	10010	41	825
'Slurry store'; semi-solid manure	1	3	0	7.8	53.8	81400	405900	95480	200200	110	18480
'Agway type'; semi-solid manure; bedding added	1	3	0	7.3	55.6	57860	299750	54560	105600	55	20790

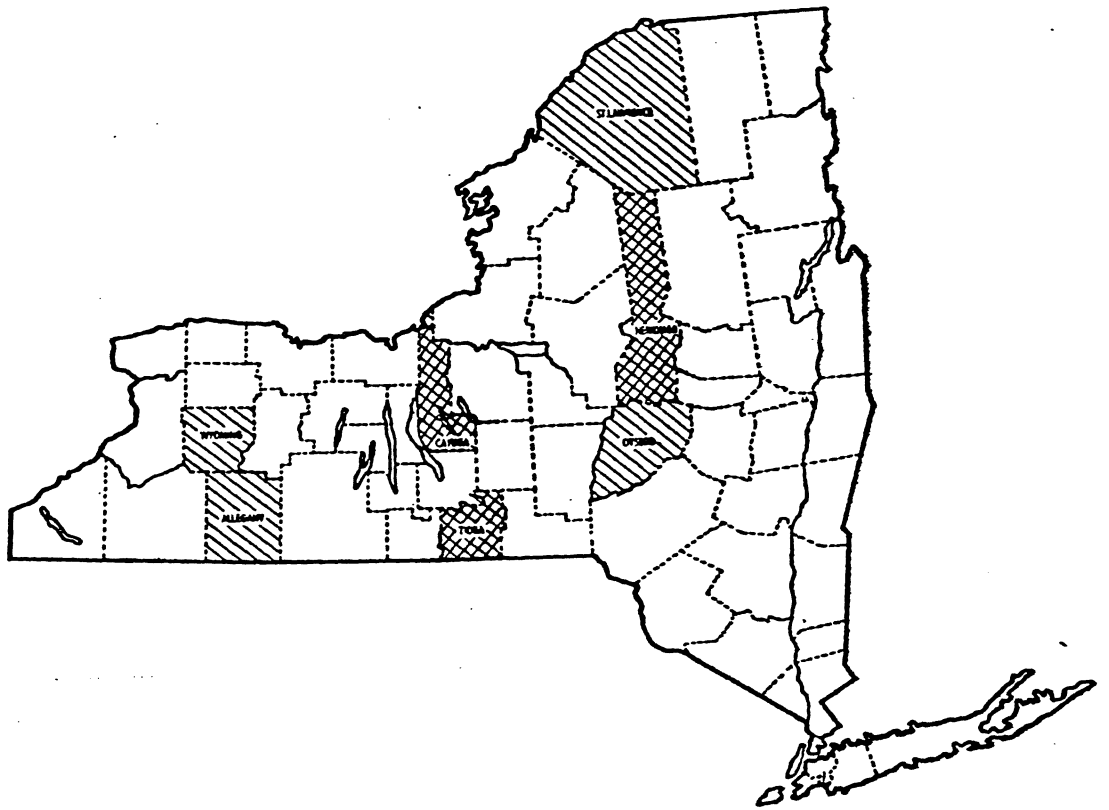
^{1/} Values represent averages for all samples.

Table 5. Logistic regression equation predictors, estimated coefficients, estimated coefficients standard errors, and predictor standard deviations.

Predictor		Estimated Coefficient	Estimated Coefficient Standard Error	Predictor Standard Deviation
DATE	-1 for early sample period +1 for late sample period	0.350	0.156	1.002
HMW2	1 if habitat general type is flowing, 0 otherwise	1.033	0.367	0.478
HSC1	1 if habitat source of water is spring-fed, 0 otherwise	0.963	0.389	0.498
HSC2	1 if habitat source of water is milkhouse, 0 otherwise	1.627	0.537	0.393
DEGANL	degree of animal access, rated 0 (none) to 3 (heavy)	1.584	0.258	0.765
PCTORG	percentage of organic matter	-0.157	0.031	11.266
PH	pH	1.031	0.291	0.675
LP	logarithm (base 10) of P in kg/ha	1.267	0.364	0.770
LN03	logarithm (base 10) of N03 in kg/ha	1.825	0.619	0.297
Constant		-11.676	2.322	--

Figure Caption

Fig. 1. Counties in New York state where dairy farms were examined for larval C. variipennis. Cross-hatching indicates counties where dairy manure storage systems were also examined.



Running head:

Schmidtman et al. Culicoides variipennis, larval populations and probability model for New York State dairy farms.

Index words: Culicoides variipennis; larval development on dairy farms; logistic regression; probability model.