ABSTRACT

BEHAVIOR AND SURVIVAL OF THE ADULT CEREAL LEAF BEETLE, OULEMA MELANOPUS (L.)

By

Richard Alfred Casagrande

Adults of the cereal leaf beetle (CLB) *Oulema melanopus* (L.) survive the winter in highest densities at the edge of woodlots. Other habitats include sparse woods, fence rows, dense woods, and croplands (in order of "preference"). Beetles overwintering at the ground surface generally do not receive lethal low temperature exposures. However the small proportion overwintering above ground does receive chronic exposures to temperatures below 25^{0}F, the upper threshold for cold-induced mortality. A predictive model is developed to relate mortality to the duration and severity of continuous cold exposures. Experiments also reveal the significance of preconditioning and recovery from successive cold exposures in determining the mortality from low temperatures.

Two control features are evaluated which are based on adult cereal leaf beetle behavior. Oviposition is greatly reduced by resistant wheat of the variety Vel. Plantings of mixtures of resistant and susceptible wheat resulted in intermediate reductions in oviposition. Within generation of the beetle seemed unaffected by the field plantings of resistant wheat as did the behavior and survival of the larval parasite *Tetrastichus julis* (Walker).

Richard Alfred Casagrande

Strip spraying utilizes the CLB behavioral trait of taking frequent short flights within grain fields in a control program that entails spraying a field with narrow bands of a persistent insecticide. As a result of their normal movement beetles contact the insecticide. A mathematical model is developed which is based on a 1-dimension diffusion equation and functions for insecticide decay and effect on beetles. This model simulates various strip spray experiments and shows strip spraying to be an efficient and economical means of reducing adult densities--a conclusion that is verified by field experimentation.

Cereal leaf beetle behavior is considered to be a factor in natural population regulation as a shift of the majority of the CLB population from oats to wheat is observed during a phase of declining regional densities.

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By

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PREFACE

The basic theme of this thesis is concerned with the behavior and survival of populations of cereal leaf beetle (CLB) adults. To this end, I conducted field research at the Gull Lake Research Farm between 1972 and 1974. Basic objectives included determining where beetles overwintered, how they moved around in the spring and summer, and the rate of survival throughout the year. Insofar as I shared these objectives with my predecessor on this project, Dr. William G. Ruesink, who used similar techniques in gathering the same types of data, I have incorporated much of his data, taken from his Ph.D. thesis and field notes, and integrated them with my results. This allows a reevaluation of his data in light of subsequent work and provides a more complete data set on which to base current interpretations. Rather than crediting every individual table or estimate which was taken from Ruesink's work, it should suffice to say that all data collected prior to 1972 was taken by Bill. Other researchers are referenced whenever their work is used.

In the course of conducting and analyzing this field work, 3 special areas of interest and research opportunity developed. These projects, described in Appendices A-C, are all outgrowths of the basic study on behavior and survival. Since in all 3 cases, the techniques, analysis, and discussions are very different from the basic field work, these research efforts were each written as separate sections.

The first section discusses field work at Gull Lake concerned with the behavior, survival, and distribution of adult cereal leaf beetles. Early observations on adult movement and susceptibility to insecticides led to the idea that strip spraying might be developed as a control measure against the CLB. This concept is developed in Appendix A, which discusses the field and lab measurements, the theory, the economics, the computer simulations, and the field results of strip spraying for CLB control. Field observations made for this project also contributed to an interpretation of the field densities measured at Gull Lake.

One of the more important and least understood aspects of CLB dynamics is overwintering mortality. A model predicting overwintering mortality could be an important component of an on-line system for CLB management. For this reason, a fairly comprehensive set of lab experiments was conducted to determine and model the response of CLB's to low temperatures. This work, reported in Appendix B, though not complete in all respects, allows a new insight and some new dimensions to the subject of overwintering survival, a topic which has not received anywhere near the attention it deserves in entomological research.

The third appendix discusses an evaluation of the first large scale planting of wheat resistant to the cereal leaf beetle. The orientation in this project is toward determining the impact of the wheat on the beetle and its parasites, and the probable outcome of releasing resistant wheat in a control program. As with each of the other projects, this effort provided additional insight into the behavior of the beetles; but, probably to a greater extent than with the other chapters, the interpretation of these results was influenced by the results of the other Gull Lake field work.

INTRODUCTION

The life cycle of the cereal leaf beetle (CLB) in North America has been reported by Castro _et al_. (1965). The within-generation population dynamics was reported by Helgesen and Haynes (1972), and the interaction with parasites was described by Gage (1974).

In modeling the dynamics of CLB populations, certain new information has been required on the between-generation dynamics. Initial field work allowing preliminary estimates for these parameters was conducted during 1970 and 71, and was reported by Ruesink (1972). This thesis discusses subsequent field and lab work intended to refine estimates of these parameters and includes the results reported by Ruesink (1972) as well as additional results of 1972-74.

The cereal leaf beetle, a recently introduced pest of small grains, overwinters in the adult stage in Michigan. In the spring, adults feed and oviposit on a variety of grasses, but are found in greatest densities in small grains where subsequent larval feeding can seriously damage the crop. The adults have been found overwintering in a number of protected sites, generally at or near ground level. Castro (1964) found overwintering CLB's under the bark of trees, in logs, in folded leaves, in straws on the ground, in corn stubble, in bailed hay, farm structures, beehives, field margins, and in woodlots. In areas of high CLB densities, adults are frequently found overwintering in large numbers in grain stubble, especially where the stubble field borders a woodlot or dense fence row to the north and/or east.

Overwintering mortality was measured near Galien in southwestern Michigan by Castro (1964) at 100% in caged beetles exposed 4 and 12 feet above ground. Beetles held in cages at the ground surface experienced 68% mortality in the winter of 1962-63, and 48% the following year. Denton (1973) measured adult CLB mortality in the same area during the winter of 1971-72 and found that by March 28, 1972, adult mortality in standing wheat stubble averaged 69.9% vs. 49.1% in prostrate stubble. Yun (1967) and Wellso _et al_. (1970) recorded the mortality rate of beetles stored at 38°F in the lab during the winter. Both reports noted about 4% mortality during the first 2 months in storage and a gradual increase in mortality rate after that time, such that by the end of March there was about 82% mortality observed by Yun, and about 75% observed for two consecutive years by Wellso _et al_. Castro (1964) noted apparent predation on beetles in moist overwintering sites as evidenced by a high incidence of body fragments and low density of living beetles in these sites which had high numbers of predaceous insects.

Following emergence from overwintering sites, the movement of spring adults has been assumed by numerous authors to have a rather rigid chronological order: spring grasses, to winter grains, to spring grains. In an experiment which involved spraying all the wheat fields in a township to kill adult CLB's and measuring the impact of this treatment on CLB densities in oat fields, Wells (1967) noted no decline in populations in oats as compared to controls. Despite some complicating factors, his results cast some doubt on the accepted sequence of movement.

Gage (1974) found densities of egg and larval stages of cereal leaf beetles to be related to planting dates of wheat and oats. Late planted wheat and early planted oats had higher densities than the normal plantings of these crops, indicating further complexity in the behavior of the adult beetles.

METHODS

The Study Area

An 1842 acre area in the northeast corner of Kalamazoo County, Michigan, was chosen for this study; the majority of that acreage belongs to Michigan State University Kellogg Biological Station. For the purposes of estimating the total number of cereal leaf beetles in this region, the 1842 acres were divided into several categories, then density estimates were taken from each category. The 1327 acres under cultivation was distributed among about 300 fields ranging in size from .8 acres to 35.8 acres. The remaining 515 acres was subdivided as follows: woods, 262 acres; fence rows, 13 acres; roadsides, 27 acres; and others, 213 acres. The final category contains such things as lakes, roads, buildings, and lawns. None of these were sampled as they were considered unsuitable as habitats for the cereal leaf beetle.

Overwintering Sites

An extensive search was conducted over a 7-year period to find the preferred overwintering sites of the cereal leaf beetle. Three basic methods were used to determine overwintering sites. During the summer and fall of 1969, samples of 3 square feet were dug to a depth of 3 inches and all plants and soil in these samples were run through a cotton gin trash mill. This machine was acquired from the Plant Pest Control division of the U.S. Department of Agriculture where it had been designed and used to survey for pink bollworm larvae in the trash left from ginning cotton.

The machine consists of 2 revolving screen cylinders which sift the material of the sample into 3 parts according to particle size (Curl and White, 1952).

When the soil was loose and dry, this machine efficiently separated the beetles from the soil and most of the debris. Excessive moisture caused mud to clog the screens, so the beetles were not then separated out. For this reason, this technique was not used in the spring.

Especially designed emergence traps (Gage and Haynes, 1975) were used from 1971 to 1975 to sample the number of beetles emerging, and the rate of emergence, from overwintering sites. These pyramid traps covered a square yard of ground surface and caught emerging insects in a pan of ethylene glycol when they reached the top of the screen sides.

Beetles were also found in their overwintering sites by direct observation in 1970 and 71. Old fence posts were torn apart, bark was stripped from wild grape, and leaf litter was sifted in the field. These latter techniques did reveal some beetles, but, in general, the gin mill and emergence cages provided the most information.

Cage Studies of Adult Mortality

In 1970, the mortality rate of adults was studied using 6.6 ft. square (1 milliacre) x 6 ft. high plastic screened cages. These cages were equipped with a zipper door and open bottoms with plastic flaps to be buried so that beetles could be confined to host plants in the field. Two cages were used for spring adults: during May they were in wheat, and in June they were moved to oats. Four cages were used for summer adults: 2 in oats and 2 in corn for the first 3 weeks of July. In every case when a cage was first set up in a new location, it was necessary to remove the resident beetles before the study began. This was accomplished by a visual search using a hand aspirator to collect every beetle seen. When no more could be found, the person left the cage for about ½ hour and then repeated the search. Normally the second search caught about 1/10th as many beetles as the first.

Each week 250 beetles were put into each empty cage. After 6 to 8 days the cages were again emptied using the same search process described above. When the beetles were introduced into an emptied cage at the start of each trial, the jar containing them was opened and placed inside the cage. Those found dead in the jar when the cage was emptied a week later were subtracted from the number introduced before computing mortality.

In 1971-74 a different type of cage was used to evaluate adult CLB mortality in the field. Since beetle densities in the study area were greatly reduced, it was decided that the large cages were too inefficient. Smaller cages, with fewer beetles were used, allowing more replicates, and statistical comparisons of survival rates in different crops. The cages constructed for this purpose were made from plastic screen formed into a cylinder 12 inches high by 3 inches in diameter. These were attached to a small stake and fitted with 1-inch thick foam rubber endpieces which were slit to allow a plant to be inserted through a cage. Ten beetles were placed in each of these cages for a few days, and at the end of the exposure period the dead and alive beetles were counted. These small cages had the advantage of minimizing microclimatic effects and allowing a determination of whether any beetles had escaped.

Population Survey

In 1971 and 1972, each grain field in the study area was sampled to determine the number of adult beetles in that field. Each of the approximately 50 fields were sampled at regular intervals to determine changes in the populations of both spring and summer adults. In 1973 and 74, about 50 non-grain survey sites were added to determine the distribution of beetles throughout the study area. Mortality rates were determined from the survey data by determining the rate of decrease of the regional population each year.

The sampling techniques used in the survey varied with crop height and beetle density. In 1971, grain less than 10 inches tall was sampled using a thrown stick technique, while taller grain was swept with a 15-inch diameter sweepnet. One sample with the stick technique consisted of: 1) throwing a 12-inch garden stake at least 10 feet; 2) moving the stake 2 stake lengths further down the grain row; and 3) counting the beetles in 12 inches of 2 adjacent grain rows. One sample with the sweepnet technique consisted of 10 sweeps, each 5 feet long, keeping the top rim of the net as close as possible to the top of the grain plant.

Sweepnet catch per sweep (C) was converted to number per square foot (D) by the equation given in Ruesink and Haynes (1972):

$$D = C\ (0.20 + 10^{K})$$

where $K = -.06 + .02\ H - .017\ (T + 10S) + .661\ \log_{10}(W+1)$,

H = grain height (inches),

T = temperature (^{o}F),

S = solar radiation (cal/cm^{2}/min), and

W = wind (mph).

Most of the needed weather data were available from a weather station set up within the study area; however, some data came from U.S. Weather Bureau records for Jackson, Michigan.

In 1972, because of reduced CLB densities, the number of sweeps in a sample was increased from 10 to 20 and, in short plants, rather than counting the beetles in 2 linear feet, a 30 linear foot sample was used. In 1973, a 25 sweep sample was used, and in 1974 the sample was increased to 50 sweeps. In both years, the 30 linear foot sample was used. In all 4 years, 4 samples were taken in each field on each sample date.

RESULTS

Direct Observation of Overwintering Sites

On November 6, 1970, an old weathered fence post was torn apart: 18 live and no dead beetles were found in its cracks and crevices. This sample indicated that significant numbers of beetles may overwinter in micro-habitats which are difficult to quantify.

In early April of 1971, additional fence posts, logs, etc., were examined to determine if large numbers of beetles successfully overwintered in such habitats. Of the 162 beetles found in 4 old fence posts, a decaying stump, and under wild grape bark, only 9 were alive. Since these samples were taken before the weather was warm enough for spring emergence to begin, the difference between the observed survival in November and in April represents overwintering mortality. In November 100% of the beetles were alive, while in April only 6% were alive; therefore, overwintering mortality in above ground exposed habitats for 1970 is estimated at 94%.

Gin Mill Samples

The gin mill samples taken during August 11-18, 1969 (Table 1), showed CLB's distributed among all habitats sampled. The beetles were found to be distributed throughout croplands at a mean density (weighted by sample size) of 5,740.4/acre compared to 18,754.9/acre in non-croplands. An analysis of individual samples indicated that beetles were randomly distributed in croplands. A chi-square test indicated no significant departure ($P<.75$) of the observed densities from a poisson distribution.

Table 1. Cereal leaf beetle adults found in ½-square yard samples processed in the gin trash mill in late summer and fall of 1969.

Habitat	Acres	August 11-18				Sept.-Oct.		Oct. 23-Nov. 6	
		CLB's	Samples	CLB's/Acre	Total CLB's (1,000's)	CLB's	Samples	CLB's	Samples
Croplands									
Idle	529	3	7	6,222	3,291	1	18	0	9
Grain Stubble	263	11	21	7,606	2,000	0	30	0	3
Alfalfa	216	1	9	1,613	348	0	26	0	12
Corn	267	2	6	4,840	1,292	1	45	2	18
Non-Croplands									
Woods	262	7	12	8,470	2,219	1	9	0	3
Fence Rows	13	13	6	31,460	409	4	23	0	0
Roadsides	27	37	21	25,583	691	0	15	0	4
Weeds	25	5	9	8,066	202	0	20	0	3

Furthermore, the coefficient of dispersion (C.D. = variance/mean) of 1.149 is close to the 1.0 expected for a random distribution. In the non-croplands however, beetles are not randomly distributed. A C.D. of 4.568 and a highly significant chi-square indicate a significant clumping or aggregation of beetles compared to a poisson distribution.

The subsequent samples taken from late September to early November indicate a large decrease in beetle density from that determined by the August samples. Of the 161 samples taken in croplands during these 2 sample periods, only 4 CLB's were found (.075/yd^2) compared to the 1.186/yd^2 observed in August. A similar decrease was observed in the non-croplands where 5 beetles were found in 77 samples (.195/yd^2) compared to 3.875/yd^2 in August. Clearly, the beetles had moved between the late summer and fall samples. This movement is best interpreted in light of overwintering results and, hence, this aspect is deferred to the discussion.

Emergence Traps

From 1971 to 1973 1 yd^2 emergence traps were set up each spring in the Gull Lake study area to determine where beetles overwintered and the rate of spring emergence. These traps were not placed at random in the environment, but were placed in categorized habitats listed in Table 2. For the purpose of determining overwintering emergence, the woods in the study area was classified as either dense (mature trees with a litter ground cover), or sparse (open with grass ground cover). The perimeter of each woods, including 20 feet into the woods, was classified as woods edge. The results of these 386 samples are included in Table 2. Since CLB densities decreased in each of the 3 years, it is not possible to make direct comparisons between yearly catches. Furthermore, since there are clearly differences in

Table 2. Cereal leaf beetle adults caught emerging from overwintering sites at Gull Lake between 1971 and 1973 using 1-square yard emergence traps.

	Croplands					Non-Croplands				
	Idle	Corn	Alfalfa	Stubble	Total for Croplands	Dense Woods	Sparse Woods	Woods Edge	Fence Rows	Total
1971										
CLB's	3	2	--	1	6	2	68	5	36	
Traps	8	1	--	3	12	2	4	4	8	
Density/Yd^2	.375	2.000	--	.333	.500	1.000	17.000	1.250	4.500	24.250
% of Total					2.062	4.124	70.103	5.155	18.557	
1972										
CLB's	0	0	0	0	0	10	47	66	4	
Traps	10	15	9	13	47	29	44	30	38	
Density/Yd^2	.000	.000	.000	.000	.000	.345	1.068	2.220	.105	3.738
% of Total						9.230	28.571	59.390	2.809	
1973										
CLB's	--	--	--	--	--	4	27	30	5	
Traps	--	--	--	--	--	20	59	66	23	
Density/Yd^2	--	--	--	--	--	.200	.458	.455	.217	1.330
% of Total	--	--	--	--	--	15.038	34.436	34.211	16.316	
Weighted $\overline{X}$					.419	11.307	33.358	40.602	9.137	94.823
Adjusted $\overline{X}$					.442	11.924	35.179	42.819	9.636	100.000

Note: -- = not sampled

overwintering densities among the habitats, and unequal efforts went into sampling habitat types each year, it is not very meaningful to directly compare total CLB catch per year. Comparisons between years were made by determining yearly density/yd^2 of CLB's in each habitat type, summing these densities, and determining the % of this total that came from each habitat type. This % of total distribution presented in Table 2 is independent of sampling effort and regional density and, thus, the 3 years' results are comparable. The average distribution for the 3 years was determined by weighting the yearly distributions by the number of samples per habitat, and this distribution was then adjusted to total 100%. On the basis of this adjusted mean % distribution in Table 2, it appears that beetles successfully overwinter in the highest densities at the edge of woods (42.8%). Sparse woods is a slightly less favorable habitat (35.2%), followed by dense woods (11.9%), fence rows (9.6%), and croplands (.4%).

To determine the generality of this distribution in an area with a different CLB density and different topography, a total of 90 emergence cages were placed near Galien, Michigan, in 1974 and 75. These results (Table 3) were analyzed in the same manner as the Gull Lake samples, giving a reasonably similar distribution of emerging CLB's. The primary difference was a much higher density in fence rows (36.1% vs. 9.6% at Gull Lake). This difference in density reflects a difference in the types of fence rows--those at Gull Lake consisted of mostly shrubs with an occasional large tree, compared to more mature and dense trees in the sampled fence rows at Galien. Since, except for fence rows, the distribution of CLB's at Galien was quite similar to the Gull Lake distribution, a mean distribution was calculated for the 2 sites by weighting the estimates by sample

Table 3. Cereal leaf beetle adults caught emerging from overwintering sites near Galien, Michigan, during 1974 and 1975 using 1-square yard emergence traps.

	Croplands		Non-Croplands				
	Stubble	Total for Croplands	Dense Woods	Sparse Woods	Woods Edge	Fence Rows	Total
1974							
CLB's	1	1	4	10	9	41	
Traps	3	3	5	7	6	8	
Density/Yd^2	.333	.333	.800	1.429	1.500	5.420	9.482
% of Total		3.512	8.437	15.071	15.819	57.161	
1975							
CLB's	--	--	23	77	79	58	
Traps	--	--	12	12	12	12	
Density/Yd^2	--	--	1.917	6.417	6.583	4.833	
% of Total			9.706	32.491	33.331	24.470	19.750
Weighted $\overline{X}$		3.512	9.333	26.073	27.494	37.546	103.958
Adjusted $\overline{X}$		3.378	8.978	25.080	26.447	36.117	100.000

Note: -- = not sampled

size and adjusting the resulting distribution to total 100%. This weighted mean distribution for 5 years' data from 2 sites shows beetles emerging from woods edge in the highest density (40.4% of the total). Sparse woods is slightly less favorable (33.4%), followed by fence rows (15.0%), dense woods (11.1%), and croplands (.6%).

Distribution in Overwintering Sites

The results of the 5 years' individual emergence trap catch are summarized in Table 4. To determine whether the beetles were randomly distributed, a poisson distribution was fit to each of the 5 data sets (Table 5) using the mean number of CLB's per trap to determine the distribution, and the observed and expected distributions were compared with a chi-square test. Each of the 5 years' results turned out to be significantly different from a poisson distribution. Since the coefficient of dispersion was greater than 1 for each year, it is apparent that the beetles are aggregated in their overwintering sites. An inspection of the observed and expected frequencies reveals that in each year there were more zeros and more large numbers than would be expected in random distribution. This is probably a reflection of the differences in density among the different habitat types, environmental heterogeneity within habitat types, and possibly a tendency for beetles to cluster in overwintering sites.

Rate of Emergence From Overwintering

During the 3 years that natural emergence was measured at Gull Lake, the traps were checked at frequent intervals to measure the rate of emergence from overwintering sites. Additionally, in the fall of 1973, 15 sample sites were selected in sparse woods, dense woods, and idle grass fields (5 sites/habitat) and 2 square fiberglass screened envelopes with

Table 4. Densities of cereal leaf beetles caught emerging from overwintering sites at Gull Lake and Galien, Michigan, in each of the 1-square yard emergence traps.

	FREQUENCY OF OCCURRENCE				
	Gull Lake			Galien	
CLB's/Trap	1971	1972	1973	1974	1975
0	12	148	129	14	16
1	6	22	23	5	12
2	5	7	10		4
3	2	3	3	4	3
4	2	2	1	2	6
5		2	2		3
6	1				3
7		1		2	2
8				1	1
9					
10				1	1
11				1	2
Additional Trap Catches (each number occurred only once)					
	16	12			15
	65	15			16
		30			17
					20
					24
					25
					31
Total Traps	30	188	168	30	60
Total CLB's	117	127	66	68	288
$\bar{X}$ CLB's/Trap	3.90	.68	.39	2.27	4.80
s^2	142.64	7.45	.76	10.41	47.25
C.D. $\left(= \frac{s^2}{\bar{X}}\right)$	36.58	10.69	1.97	4.59	9.84

Table 5. Analysis of 5 years of individual emergence trap catches comparing observed frequencies to those expected from a poisson distribution.

	1971			
Dens.	Obs. Freq.		Exp. Freq.	
0	12	23	.61	7.60
1	6		2.37	
2	5		4.62	
3	2		6.00	
4	2		5.85	
5	0		4.57	
6	1	3	2.97	5.98
7	0		1.65	
>7	2		1.36	

χ^2 = 42.46**

	1972			
Dens.	Obs. Freq.		Exp. Freq.	
0	148		95.67	
1	22		64.63	
2	7		21.83	
3	3	11	4.92	5.88
>3	8		.96	

χ^2 = 71.30**

	1973			
Dens.	Obs. Freq.		Exp. Freq.	
0	129		113.42	
1	23		44.56	
2	10	16	8.75	10.02
>2	6		1.27	

χ^2 = 15.80**

	1974			
Dens.	Obs. Freq.		Exp. Freq.	
0	14	19	3.10	10.14
1	5		7.04	
2	0		7.99	
3	4		6.04	
4	2	7	3.43	5.83
>4	5		2.40	

χ^2 = 16.61**

	1975			
Dens.	Obs. Freq.		Exp. Freq.	
0	16	32	.49	8.55
1	12		2.37	
2	4		5.69	
3	3		9.10	
4	6		10.92	
5	3		10.48	
6	3		8.39	
7	2		5.75	
8	1	11	3.45	6.81
9	0		1.84	
>9	10		1.52	

χ^2 = 84.46**

25 adult CLB's in each were placed in each site at the soil surface. The rate of emergence of these beetles after cutting open the envelopes in the spring of 1974 is included in Table 6.

In Fig. 1, the results in Table 6 are plotted on a log-probability scale, omitting the 8% of the beetles that emerged after May 18 in 1971. The relationship between cumulative % emergence on a probit scale and the log of degree-days > 48^{0}F is quite linear. Regression equations fit to this transformed data gave an average r^2 of .968. The degree-day values corresponding to 50% emergence for 1971-74 are 121, 83, 135, and 123 ^{0}D>48, respectively. These occurred on April 21, 28, 17, and 22 in the respective years. From the regression equations fit to the data in Table 6, the rate of emergence was determined for each year. These results, presented in Fig. 2, show that for 3 years the emergence curves were very similar; however, in 1972 emergence peaked earlier and was completed sooner on a degree-day scale than in other years. It is interesting to note that this year was relatively cool and, although the peak was earliest on a degree-day scale, it was the latest of the 4 by calendar date (April 26). Peak emergence at Gull Lake from 1971-74 occurred between 75 and 125 degree-days which corresponded to April 18-26.

Regional Emergence at Gull Lake

To determine the number of cereal leaf beetles emerging from overwintering in the 1842-acre study area, the mean distribution for the 3 years of Gull Lake results was used. Since in each year the number of traps used and beetles caught was relatively small, it was felt that the average of the 3 years was more representative of a single years' distribution than the density actually measured. This is particularly true in 1972 and 73 when no beetles at all were found in croplands.

Table 6. Cumulative emergence of cereal leaf beetle adults from overwintering sites as measured by 1-square yard emergence cages.

1971			1972			1973			1974		
Date	$^{o}D>48$	Cum. Em.	Date	$^{o}D>48$	Cum. Em.	Date	$^{o}D>48$	Cum. Em.	Date	$^{o}D>48$	Cum. Em.
April			April			April			April		
16	78	12	17	47	6	17/18	117	22	2	31	1
18	95	16	19/20	70	27	20	157	44	9	46	1
21	124	67	21/22	71	27	23	194	53	13	76	32
30	134	67	24/25	72	28	27	212	60	15	83	42
May			27	79	77	May			17	88	72
5	149	72	29	95	103	1/ 2	224	63	19	96	79
7	164	88				8/ 9	299	65	21	120	192
10	195	98	May			11	328	66	24	134	205
15	240	106	1/ 2	125	110	15	344	66			
			4/ 5	151	117				26	147	272
18	298	108	6	157	125				28	179	305
22	342	108	9	165	126				30	203	311
25	386	112									
29	412	116	13	205	127				May		
June									2	219	320
1	452	116							4	233	330
5	508	116							6	242	332
8	608	117							9	245	336
									12	261	337
									14	270	341
									17	299	342
									19	322	349

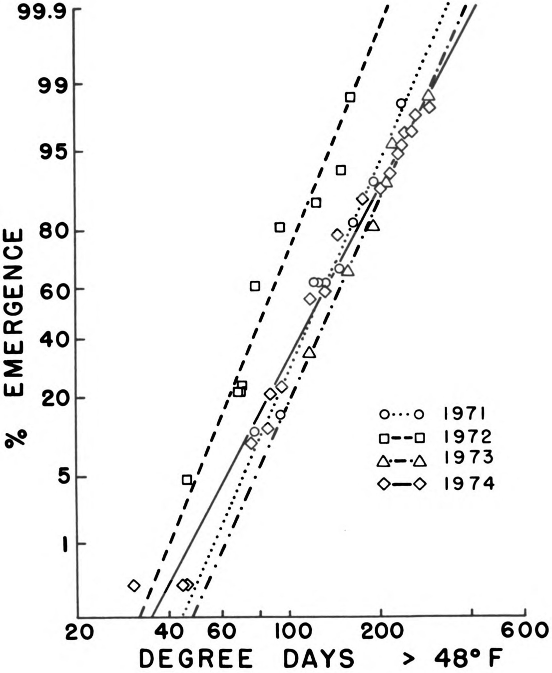

Fig. 1. Cumulative emergence of CLB's as a function of degree-days > 48^{o}F for 4 years' data from Gull Lake, Kalamazoo County, Michigan. (Neg. 752244-12)

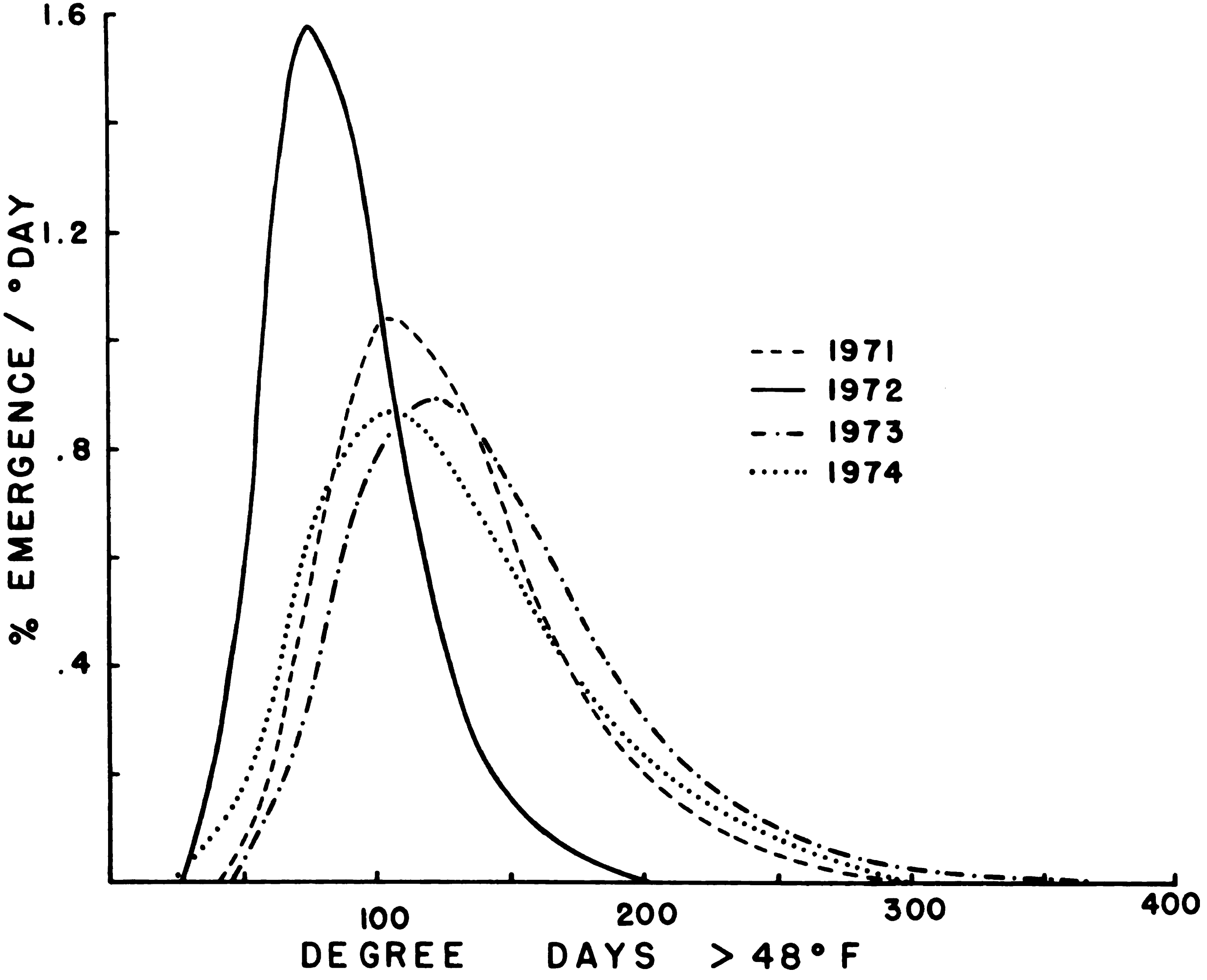

Fig. 2. Emergence rates of CLB's as a function of degree-days > 48^{0}F for 4 years' data from Gull Lake, Kalamazoo County, Michigan. (Neg. 752244-21)

In this analysis, the adjusted 3 year mean distribution for Gull Lake (from Table 2) was used to distribute the total density/yd^2 (from Table 2) among the 5 habitat types and the resulting densities were multiplied by the acreage of each habitat type (Table 7). It should be noted that in this analysis, roadsides which were not sampled were assumed to have the same densities as fence rows. (This assumption was necessary because it proved impossible to prevent vandalism of cages along roadsides. The assumption is reasonable because of the similarity of these 2 habitats at Gull Lake. In addition, it is not too important because of the relatively small acreage and low densities involved.) The results of these calculations in Table 7 show that regional densities of emerging CLB's decreased from almost 9 million in the study area in 1971 to about 1.3 million in 1972 to less than 500,000 in 1973.

Cage Studies of Adult Mortality

Adult mortality rates measured by the 1970 cage study (Table 8) were first computed as % mortality over the entire sample interval (M), which varied from 6 to 8 days. Conversion to % mortality per day (M_d) as a standard base for each interval requires the following equation:

$$M_d = 1 - (1 - M)^{1/n}$$

where n = the number of days in the sample interval. Percent mortality per degree-day was calculated using the same data and the same equation as for the daily rate. These rates are also included in Table 8.

The observed mortality rate began at 7.1% per day, decreased to 3.5% per day 2 weeks later, then increased again until mid-June. By then the field population was so reduced that it was impossible to collect enough beetles to restock the cages. For newly emerged summer adults, the mor-

Table 7. Determining regional spring emergence of cereal leaf beetle adults.

	HABITATS					
	Croplands	Dense Woods	Sparse Woods	Woods Edge	Fence Rows and Roadsides	Total
Acres	1,327	123	123	16	40	
% Distribution ($\overline{X}$)	.442	11.924	35.179	42.819	9.636	100
Calculated Densities (Yd^2)						
1971	.1072	2.8916	8.5309	10.3836	2.3367	24.250
1972	.0165	.4457	1.3150	1.6006	.3602	3.738
1973	.0059	.1586	.4679	.5695	.1282	1.330
Beetles/Habitat						
1971	688,511	1,721,427	5,078,615	804,106	452,385	8,745,044
1972	105,974	265,334	782,846	123,950	69,735	1,347,839
1973	37,894	94,418	287,550	44,102	24,820	488,784

Table 8. Adult mortality as computed from the 1970 cage study.

Date					Mortality (%)		
From	To	Days	dd_{48}	Replicate	Total	Per day	per dd_{48}
May 7	May 15	8	116	W1	38.8	7.1	.50
				W2	49.6		
May 15	May 22	7	100	W1	20.2	5.0	.36
				W2	39.3		
May 22	May 29	7	95	W1	16.8	3.5	.26
				W2	27.1		
May 29	June 4	6	99	01	23.5	6.4	.40
				02	41.2		
June 4	June 11	7	148	01	35.5	8.4	.41
				02	54.6		
June 30	July 7	7	190	01	41.7	10.9	.42
				02	60.7		
				C1	57.8		
				C2	58.8		
July 7	July 14	7	173	01	37.7	9.2	.42
				02	67.2		
				C1	36.8		
				C2	57.3		
July 14	July 21	7	151	01	22.6	7.4	.36
				02	54.7		
				C1	53.7		
				C2	28.9		

tality rate began at 10% per day in the first week of July and decreased to 7.4% per day 2 weeks later. By then the summer feeding period was completed and the beetles were moving into estivation sites.

The 1972-74 studies using the small cages which confined 10 CLB's to a single plant (Table 9) gave results reasonably similar to 1970. In most of these exposures either 10 or 20 replicates of 10 beetles each were used; however, frequently some beetles escaped from a cage and those cages were not included in the results. Percent mortality per day and per degree-day were determined as for 1970.

In general, the results of the 4 years' studies indicate that % mortality per day starts out fairly high in early May, then decreases until mid-June when it again increases. The first 3 years' results are quite similar, but 1974 clearly shows a lower mortality rate. On a degree-day scale, the decreasing and then increasing mortality rates are still apparent.

The variation of the mortality rates on a calendar day and degree-day scale can be compared by calculating the coefficient of variability ($C.V. = 100S/\overline{X}$) of the mortality rates for each season. The results of these calculations on the data in Tables 8 and 9 indicate no relative advantage of one time scale over the other, as in 2 years one scale is better and for the other 2 years the other scale results in less variability. The mean of the 4 years' C.V.'s indicates no difference between the 2 scales (59.6 vs. 60.1 for dates and degree-days respectively).

In 1973, the mortality of adults was compared on the primary host crops in the study area. Beetles were collected by sweeping in a crop, then holding them in the 12-inch cylindrical cages on that same crop.

Table 9. Adult mortality as computed from cage studies in 1972-1974.

Date			Crop	Dead	Alive	% Mort./Day	°D>48	%/°D
1972	May	10-12	Wheat	12	108	5.132	19	.553
		24-26	Wheat	11	89	5.660	22	.528
	June	5- 7	Oats	4	86	2.247	38	.120
1973	May	7-10	Wheat	32	169	5.616	42	.412
		11-15	Wheat	15	175	2.035	16	.513
		21-25	Wheat	17	103	3.747	54	.282
	June	4- 8	Oats	22	158	3.207	86	.151
		11-14	Oats	29	141	6.044	71	.263
		21-25	Oats	27	43	11.469	74	.656
1974	May	2- 9	Wheat	11	69	2.091	26	.567
		9-15	Wheat	1	149	.111	34	.020
		16-22	Wheat	6	154	.635	64	.060
		22-30	Wheat	2	148	.168	101	.013
	June	1- 7	Oats	12	158	1.213	125	.059
		7-14	Oats	45	135	4.030	125	.230
		22-28	Oats	30	90	4.680	89	.323

Mortality rates in different crops were compared simultaneously by using several cages with 10 CLB's per cage in each crop. These results (Table 10) indicate no difference in survival among the several pairs of crops tested, except between wheat and oats (beetles survived longer in oats). This difference between wheat and oats may be real or, in light of the other results, it might be an artifact of sampling: for example, it is possible that some pebbles got into the net when sweeping the 10-inch oats. In each of the 4 years' caged mortality studies, the cages were moved from wheat to oats the first week in June. A comparison of the mortality rate in wheat to that in oats the following week reveals that in 2 years there was a greater rate of mortality per day in wheat than in oats, and in the other 2 years the reverse was true. On the basis of these results, it appears that the mortality rate of spring adults may be the same on all hosts.

Population Survey

The weekly sweepnet survey results (Tables 11 and 12, and Fig. 3) show the densities of adult CLB's in the 1842-acre study area in the springs of 1971-74. In the first 2 years, only the croplands were sampled; however, in 1973 and 74 all representative habitats were sampled. In summarizing the sample results, the density in each of the grain fields was multiplied by the size of the individual field and similar crops were then added together. In fields which were not sampled and in all non-crop areas such as fence rows, pastures, idle land, etc., an average density was determined for the habitat type and multiplied by total acres of suitable grasses within that habitat type (only the grass component of these habitats was deemed suitable for CLB's and for sweepnet sampling). For example, roadsides were estimated to be 70% grasses, fence rows 85%, and idle fields were individually estimated for percent composition of suitable grasses.

Table 10. Comparisons of caged adult mortality rates on different crops in 1973.

Date	Crop	$\bar{X}$ % Mortality	Standard Deviation	Samples	Significance Level
May 5-10	Quack grass	13.000	18.288	10	N.S.
	Wheat	16.000	17.889	20	
May 11-15	Rye	12.000	12.293	10	N.S.
	Wheat	17.000	13.375	10	
May 21-25	Quack grass	7.895	11.343	19	N.S.
	Wheat	14.167	11.645	12	
June 4- 8	Oats	12.222	13.086	18	.05
	Wheat	24.737	20.377	19	

Table 11. Seasonal density of adult cereal leaf beetles measured by a sweepnet survey in 1971 and 1972.

1971			
Date	°D>48	Winter Grain	Spring Grain
--Overwintered Beetles--			
May			
5	149	1,571,000	N.S.
10	195	1,091,000	N.S.
19-20	321	716,000	6,380,000
22	342	620,000	5,601,000
26-27	387	873,000	5,416,000
29	412	339,000	2,873,000
June			
2- 3	466	64,000	814,000
5	535	96,000	1,108,000
9-10	620	66,000	96,000
12	679	17,000	146,000
14	727	80,000	184,000
17-18	801	9,000	61,000
22	935	12,000	26,000
--Summer Beetles--			
June			
25	1,020	42,000	502,000
28	1,115	101,000	780,000
30	1,185	380,000	1,360,000
July			
2	1,232	404,000	940,000
5- 6	1,307	263,000	856,000
8	1,391	0	459,000
10	1,444	2,000	422,000

1972			
Date	°D>48	Winter Grain	Spring Grain
--Overwintered Beetles--			
April			
19	69	0	0
28	88	98,505	0
May			
5	144	582,306	0
11	181	449,668	4,208
18	270	124,418	121,067
25	422	142,660	140,983
June			
2	544	71,136	134,882
14	773	12,142	39,714
22	909	3,601	23,680
--Summer Beetles--			
July			
4	1,110	172,260	476,469
7	1,146	232,997	1,443,086
11	1,243	23,881	1,165,060
13	1,299	35,497	1,877,786
17	1,399	33,204	1,111,166
21	1,517	21,898	161,320
24	1,616	9,468	225,381

Table 1[illegible]. Seasonal density of adult cereal leaf beetles as measured by a sweepnet survey in 1973 and 1974.

Table 12. Seasonal density of adult cereal leaf beetles as measured by a sweepnet survey in 1973 and 1974.

Date		$^{o}D>48$	Wheat	Oats	Rye	Grain Stubble	Alfalfa	Pastures	Idle	Corn Stubble	Roadsides and Fence Rows	Total
1973												
May	9	305	116,386	0	47,718	45,084	73,996	12,909	33,030	46,643	17,486	393,252
	18	355	150,095	1,967	54,848	18,704	142,776	37,939	70,900	2,492	10,132	489,853
	24	420	323,969	31,785	53,019	3,847	24,316	46,904	31,517	4,297	31,673	551,327
June	1	515	71,772	61,970	0	3,518	0	9,773	24,815	0	14,653	186,501
	8	667	26,138	71,928	47,647	1,924	0	11,345	18,280	0	1,285	178,547
	14	820	8,315	21,702	7,704	1,451	0	8,703	0	0	4,171	52,046
	20	972	5,738	26,468	0	0	0	0	0	0	0	32,206
	27	1,123	10,129	25,443	0	2,963	0	0	38,473	0	0	77,008
Samples			26	14	8	14	11	3	8	5	11	100
1974												
May	7	243	1,340,309	800	0	11,127	92,001	0	34,270	--	28,493	1,507,000
	20	337	937,819	18,560	5,922	35,626	70,956	15,492	59,644	--	29,125	1,173,134
	27	433	757,606	92,102	5,603	3,876	29,733	0	5,780	--	74,318	969,018
June	3	542	23,191	22,233	0	486	5,836	0	0	--	2,240	53,986
	10	714	15,851	59,846	0	6,928	0	0	10,572	--	6,962	100,159
	17	800	7,837	195,859	0	0	0	0	6,274	--	4,190	214,160
	24	930	7,898	12,562	0	0	0	0	0	--	5,176	25,636
July	1	1,065	15,306	13,984	0	0	2,706	0	0	--	0	31,996
Samples			32	11	4	12	11	2	11	--	11	94

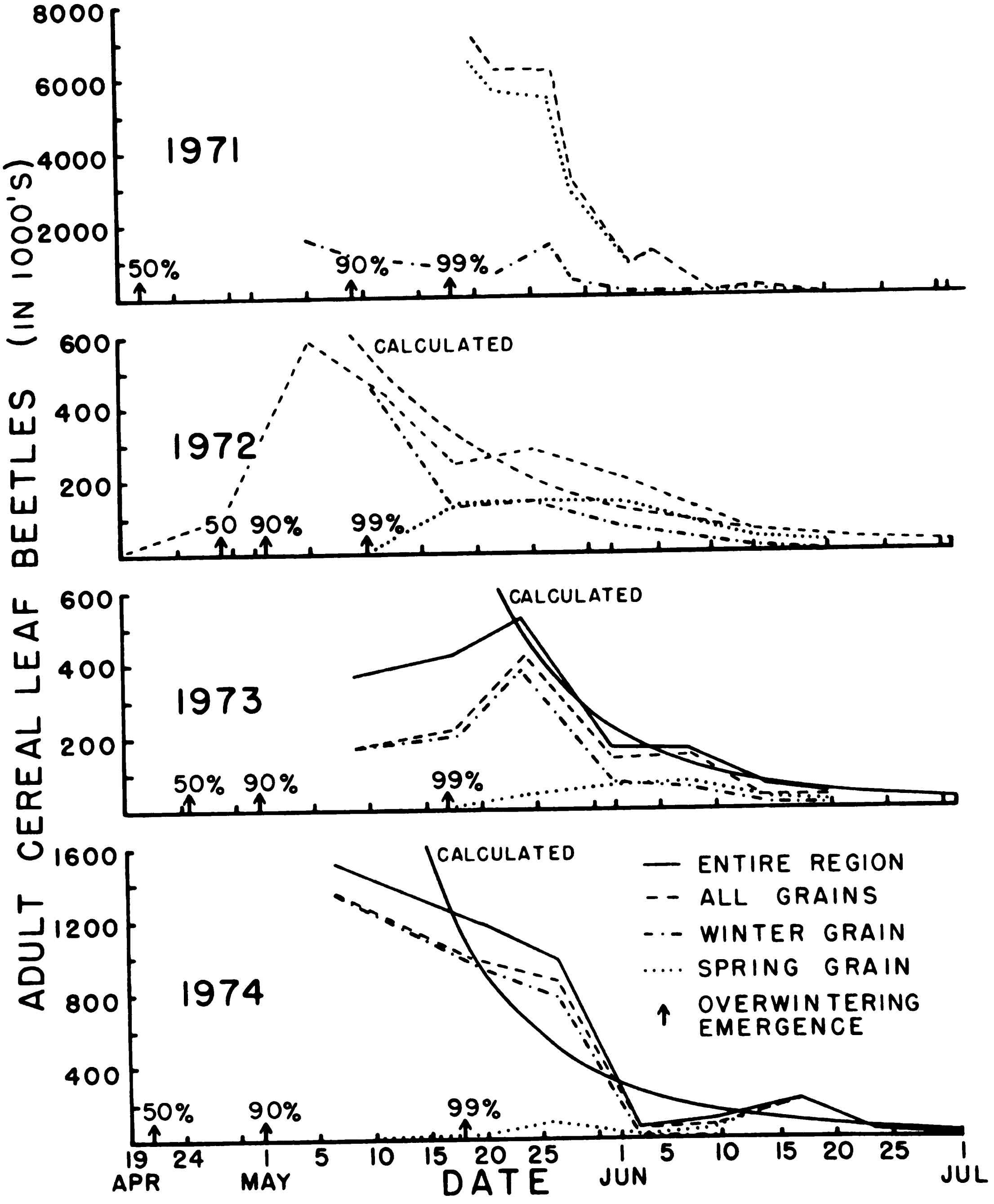

Fig. 3. Adult CLB's in an 1842 acre study area as measured by a sweepnet survey throughout the springs of 1971-1974. Calculated densities are determined by fitting an exponential decay to the declining phase of the regional densities. (Neg. 752244-23)

After emergence from overwintering, CLB densities are observed to decline until the last week of June when the summer generation begins to emerge. The increased densities observed in July result from the new summer beetles which feed for a few weeks before leaving the grain fields for estivation.

Adult Mortality From Survey Data

The results of the 4 years' population surveys for spring adults can be used to compute mortality rates if 2 assumptions are made. First, it is necessary to assume that for 1971 and 72 the rate of decline of populations in all grains combined is the same as the rate of decline of the regional population. An inspection of the 1973 and 74 results in Fig. 3 shows this to be a good assumption. The second assumption is that the migration of beetles into and out of the 1842-acre region balances out and, hence, any decline in density is due to mortality alone. This assumption also seems reasonable considering the size of the region, the diversity of habitats, the densities of beetles, and the relative isolation from other grain-growing regions. In 1972 and 74, adult densities peaked in the fields before emergence from overwintering sites was complete (Fig. 3). Since during this period the field density reflects recruitment from overwintering as well as mortality, the field densities that were measured before 99% emergence from overwintering were not included in mortality estimates. The rate of decline of CLB numbers after the peak densities following 99% emergence is suggestive of an exponential decay of the type $\frac{dN}{dt} = \lambda N$ where λ is a constant decay rate. By performing a linear regression of the natural log of density vs. time ($\ln y = \lambda X + b$) the decay constant was determined for the years 1972-74, and the % mortality per day was calculated as $M_d = 1 - e^{\lambda}$. The curves fit to the data of 1972-74 in Fig. 3 are the product of the regression equations which gave mortality rates of 4.45, 6.83, and 5.93% per day for 1972 to 74, respectively.

Because 11 of the 22 oat fields were sprayed with insecticide in early June of 1971, it was not possible to use the same technique to evaluate mortality for that year. To evaluate mortality in 1971, assumptions were made that all beetles in a field at the time of spraying were killed by the insecticide, and that between-field adult migration was small so that only the beetles in the field at the time of spraying were affected by the insecticide. The 9 fields sprayed between May 29 and June 2 contained an estimated 1,156,000 beetles on May 29; the 2 fields sprayed between June 5 and June 9 contained an estimated 234,000 beetles on June 5. Survival (S) from time t to time t+1 was computed from

$$S = \frac{N_{t+1} + (\tfrac{1}{2})K}{N_t - (\tfrac{1}{2})K} \times 100\%$$

where N_i is the number of adults present at time i, and K is the number killed by pesticide between time t and time t+1. This equation adjusts survival close to what it would have been if no pesticide had been used in the region. Table 13 presents the results of applying the above equation to the 1971 data after averaging the results for each week. The average mortality rates for 1971 in Table 13 are 11.23% per day and .71% per degree-day. In all 4 years, the mortality rates as measured by the survey are higher than the average rates measured in the caged studies. As seen in Fig. 3, the survey mortality rate is based primarily on the period from late May through mid-June when cage studies indicate the lowest rate. This fact indicates

Table 13. Spring adult mortality as computed from the 1971 regional population survey.

Date				Mortality (%)		
From	To	Days	dd_{48}	Total	Per day	Per dd_{48}
May 20	May 27	7	61	28.7	4.7	.55
May 27	June 3	7	93	61.1	12.6	1.01
June 3	June 11	8	163	66.1	12.7	.66
June 11	June 19	8	208	72.4	14.9	.62

that the difference between the field and cage results is probably underestimated since the comparison is between the lowest field rate and the average cage rate.

To determine the rate of mortality on a degree-day scale, the 1972-74 survey results in Tables 11 and 12 were plotted on a degree-day scale (Fig. 4) and analyzed in the same manner as the calendar date results in Fig. 3. By fitting regression equations to the transformed data, mortality rates for 1972-74 were determined to be .710, .368, and .446 %/degree-day > 48, respectively. As with daily mortality rates, these field rates based on degree-days are generally greater than the results of the caged studies.

Survey Results vs. Overwintering Emergence

In the 3 years (1971-73) that both survey results and densities of overwintering adults are available, it seems worthwhile to compare the 2 estimates of regional densities. For this analysis, individual CLB adults are assumed to die at the rates measured by the surveys from the time they emerge from overwintering. Thus, the total emergence estimates (Table 7) are not directly comparable to the survey densities of Tables 11 and 12. To make this comparison, the equations for each year's emergence were solved on the computer in degree-day increments and the field mortality rates appropriate to each year were applied to the emerging densities in a simulation. The results of this simulation are shown in Fig. 4. The logistic curves show cumulative emergence of beetles from overwintering. The positively skewed curves show the number of alive beetles in the region throughout the season as determined by the emergence cage densities and the survey mortality rates. These simulated regional densities were determined for each survey date and compared to the regional densities from the survey. Since

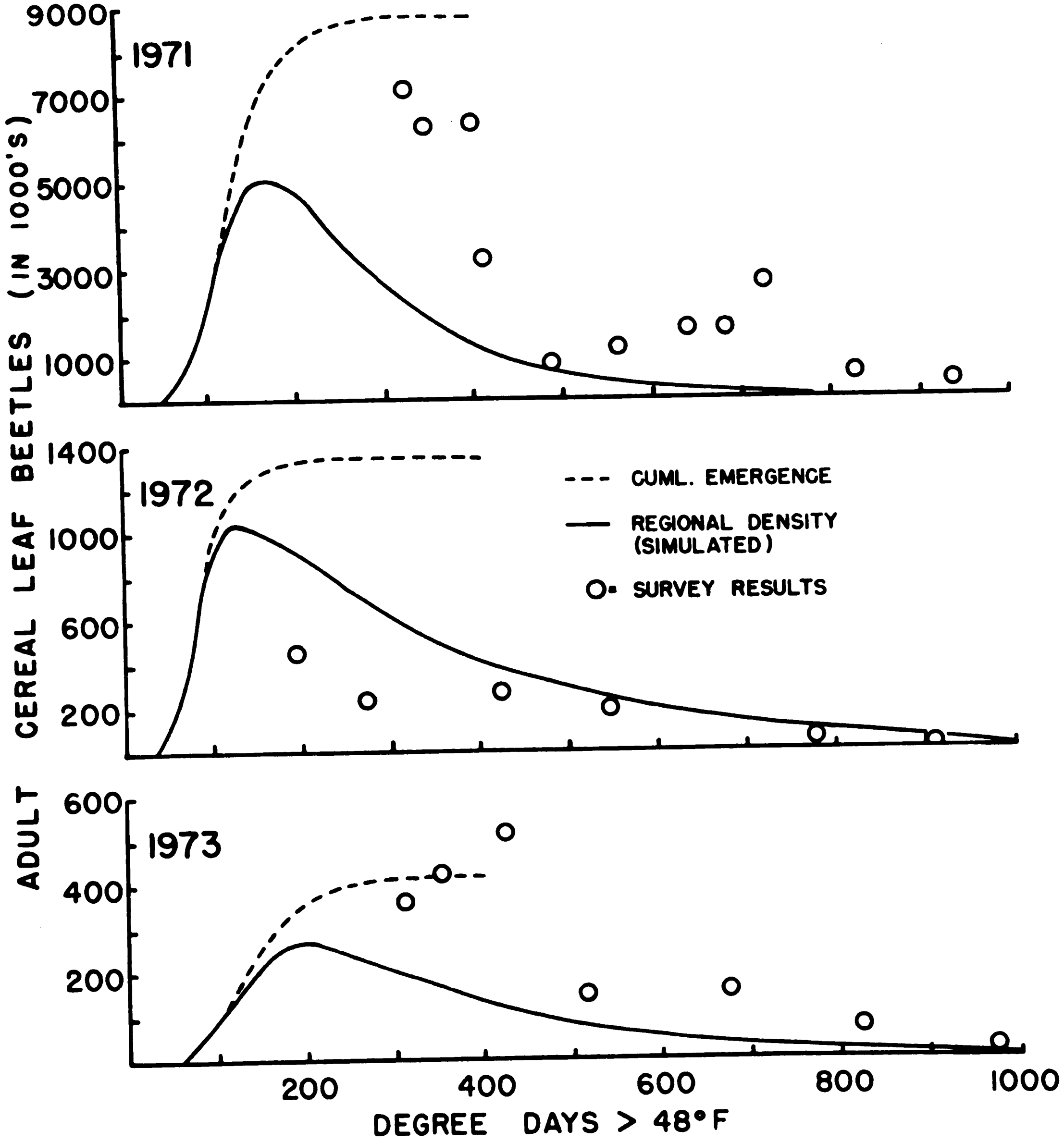

Fig. 4. Regional densities as measured by a sweepnet survey compared to densities simulated by applying annual mortality rates to emergence cage results. (Neg. 752244-22)

the results of the 1973 and 74 surveys indicated that an average of about 75% of the regional CLB density was found in the grain fields, the densities measured in grains in 1971 and 72 were divided by .75 to determine the regional density on each sample date. The average ratios of simulated regional density to survey estimates for 1971 to 1973 are .226, 1.46, and .328 respectively.

These ratios are based on several estimates, all of which are subject to considerable error. Since the survey densities and mortality rates are based on a large number of samples, it is probable that the largest errors are associated with the estimates of spring emergence which were based on small samples and relatively few beetles.

Cereal Leaf Beetle Behavior

In the 4 years of survey results in Fig. 3, it appears that there is a shift between years of beetles from spring grains (primarily oats) to winter grains (primarily wheat). In 1971 most of the beetles were in spring grains; however, by 1973 and 74 only a small part of the population was found in spring grains. Since the acreages of these crops changed a great deal between years, the 4 years' data were standardized somewhat by determining the density per square foot in spring and winter grains at peak densities in each crop for each year (Table 14). The ratios of densities in spring/winter grains for 1971-74 are 9.13 : 2.00 : 0.73 : 0.84.

To determine whether this shift between spring and winter grains is related to the condition of overwintering wheat plants, the average height of wheat for all fields was determined for the date of 99% emergence from overwintering in each year. These average heights for 1971-74 are 19, 13, 12.5, and 20.5 inches, respectively. These heights do not correspond in

Table 14. A comparison of peak regional densities of cereal leaf beetle adults in spring and winter grains at Gull Lake from 1971 to 1974.

Year	Crop	Peak Density	Acres	CLB's/Acre	Spring Density / Winter Density
1971	Spring grains	6,380,000	95.1	67,087	9.13
	Winter grains	1,571,000	213.7	7,351	
1972	Spring grains	140,983	25.6	5,507	2.00
	Winter grains	582,306	211.0	2,760	
1973	Spring grains	71,928	47.9	1,523	.73
	Winter grains	376,988	180.5	2,089	
1974	Spring grains	195,859	38.8	5,048	.84
	Winter grains	1,340,309	224.3	5,976	

any consistent manner with the relative CLB densities in spring and winter grains. Also, there is no consistent relationship between the relative densities in the spring and winter crops and the amount of oats available in early spring. In 1971-74 the percentage of oat fields out of the ground on the date of 99% emergence was 37, 30, 50 and 91%, respectively.

Thus, neither the maturity of oats nor wheat seem adequate to explain the apparent shift of beetles from oats to wheat which was associated with a decline in regional density.

Effect of Crop Age on CLB Density

In 1972 and 73 the planting dates of wheat and oat fields were adjusted to get a range of plant maturities to determine the effect on CLB's. In analyzing the survey results for these years, a regression of CLB densities vs. plant height was performed for each survey date. In 1972 there was initially no relationship between plant height and density in wheat, but by May 18 there was a significant ($P > .98$) negative relationship between CLB density and increasing plant height. Subsequent samples also revealed lower densities in higher wheat, but the slopes were not significant ($P > .90$ and $P > .70$ on May 25 and June 2). In 1973, there was not as great a range in plant heights and there was never any significant relationship between plant height and densities in wheat.

In oats in 1972 results were somewhat similar to wheat in that the first sample after all oats were germinated and out of the ground (May 25) indicated an insignificant relationship between crop height and beetle density. Subsequent samples gave a significant ($P > .99$) positive regression of beetle density on crop height, indicating higher densities in the earlier planted, more mature oats. In 1973 and 74, there were also significant positive slopes relating crop height to beetle density.

DISCUSSION

Overwintering

Newly emerged summer adult beetles, after feeding for a few weeks in early July in grain fields and other suitable grasses, distribute themselves throughout the environment. During the late summer and fall, these beetles gradually move into overwintering sites where they are found in different densities in different habitat types and show a distinct tendency to aggregate.

The gin mill samples taken in mid-August of 1970 (Table 1) indicated that at that time beetle densities outside the croplands were about 3 times as high as densities in crops. Those beetles in croplands were randomly distributed, but those in non-crops were highly aggregated. Samples taken in late September and November indicated the beetles had left croplands but were also difficult to find in non-crops. The most reasonable explanation for this observation is the aggregation in non-crops which was observed in the August sample, as well as in the 5 years of emergence trap catches. Apparently the beetles in the non-crops continue to move after mid-August so that by November they are more aggregated than in August. In 1970 few of the fall gin mill samples were taken in favorable overwintering sites, and those that were taken did not contain an aggregation.

An alternative explanation would be that beetles overwintered out of the universe sampled, which could mean out of the 1842-acre area, above ground, or deeper than 3 inches. With diversity of habitats in the study area there is no apparent reason for the beetles to leave the area. The high mortality of beetles above ground seems to eliminate the second alternative. The third alternative has been tested by repeated efforts to bury adult CLB's at various depths in a variety of habitat types and measure their emergence in the spring. These efforts have not shown beetles able to emerge in any numbers when buried more than an inch in the soil. Thus, it appears that the beetles did in fact overwinter at the soil surface.

The emergence cages used in the springs of 1971-73 measured densities of beetles which successfully overwintered in various habitat types. On the basis of 3 years' results at Gull Lake, and 2 years' of similar results from Galien, it was concluded that CLB's successfully overwinter in the highest densities at the edge of woodlots. In agricultural areas such as those sampled, if a total of 100 beetles emerged from equal acreages of habitat types, about 40 would come from woods edges, 33 from sparse woods, 15 from fence rows, 11 from dense woods, and less than 1 from croplands.

The number of beetles overwintering in the different habitats in a given region depends on the acreages of the habitat types as well as the density of beetles. The large numbers of beetles which have been observed along the edges of fields of grain stubble would probably have been along the same edges of woods or fence rows even without the presence of stubble, although they would be more difficult to find. One criterion which seems to determine overwintering distribution to some extent is the presence of small crevices into which beetles can crawl for the winter. The abundance of such microhabitats in grain stubble, in broken weeds, in sumac patches, etc., apparently serves to concentrate beetles in specific locations within a habitat type and might be the cause of, or at least a factor leading to, the consistently measured tendency to aggregate in overwintering sites.

Because of the non-random distribution of beetles within habitat types, it takes a large number of samples and a large number of beetles to accurately estimate the density in a habitat. This factor is attributed as the cause of the discrepancy between estimates of emerging overwintering populations and populations measured in surveys later in the spring. Thus, the fact that the emergence cages considerably underestimated the spring population twice and overestimated it once, is seen not as a problem with the technique, but as a lack of sufficient samples of CLB densities to allow accurate estimates.

Density, Behavior and Mortality

Gage (1974), in summarizing 7 years of population measurements at Gull Lake, reported that after the spread of the beetle into the area, densities of eggs and larvae increased each year from 1967 to 1969, and decreased each year from 1969 to 1973. Although this population decline has corresponded with the increasing densities of the larval parasite Tetrastichus julis (Walker), similar declines have been observed in areas without parasites and, hence, the decline is not attributed entirely to parasitism (Gage, 1974). Since to date, no one has adequately explained the population decline at Gull Lake, it seems reasonable to examine adult behavior and mortality for a possible mechanism.

Only a few of the many sources of adult mortality were measured during 1971-74. The average mortality rate of 9.2% per day as measured in 1971 for the newly emerged summer beetles is a very important component that results in a very rapid decline in adult densities. If beetles die at this rate for the 2-week feeding out period reported by Castro (1964), densities of adults would be reduced by 75% before entering estivation. It is probable, however, that this mortality rate is overestimated since beetles were unnaturally confined to crops at a time when they would normally be moving throughout the environment and congregating in areas where food and microclimates were most suitable.

Yun (1967) and Wellso et al. (1970) report only 3-4% mortality during the first 60 days of lab storage of beetles following estivation. This low rate of mortality is not unrealistic for this period because the beetles clearly cannot continue to die at the rate measured prior to estivation.

Overwintering mortality is another important component of total adult mortality. Cereal leaf beetles apparently cannot survive Michigan winters in above-ground habitats. Castro (1964) measured 100% mortality at 4 and 12-foot heights in caged studies. In the stumps, grapevines, and other above-ground habitats examined at Gull Lake in the spring of 1971, mortality was estimated at 94%. These habitats no doubt afforded the beetles some degree of protection from low air temperatures as did the standing grain stubble in which Denton (1973) measured about 70% mortality in 1972.

Cereal leaf beetles can tolerate quite well, however, the temperatures they normally encounter on the ground as evidenced by the mortality estimates of Castro (1964) of 68% and 48% mortality in 1963 and 1964, and Denton's estimate of 49% in 1972. Of the 750 beetles held in screen packets during the winter of 1973-74 at Gull Lake, 349 were caught in emergence cages in spring, indicating about 52% winter mortality. Thus, it appears that beetles survive in the field at least as well as in lab storage at 38^0 where mortality averages about 77% by April 1 (Yun, 1964; Wellso et al., 1970). Hence, cold exposure may not normally be an important factor in winter mortality of beetles at the soil surface. What may be more important is the apparent predation in some habitats noted by Castro (1964). This predation may be a factor involved in the often observed tendency of beetles to overwinter in tight crevices which probably serve to limit predation. There is some evidence that habitats which are too warm may result in physiological depletion of fat reserves as discussed by Denton (1974), and the length of the winter may also be a factor in determining winter mortality.

The mortality of spring adults following emergence from overwintering sites is another mortality component which is documented for 1970-74. Since the mortality rates as measured by the cage studies were consistently lower than the rates calculated from the survey, it appears that the cages underestimate the regional mortality rate. This might be due to screening out predators. If the spring mortality rates, as determined by the survey, are compared to the regional densities of beetles in either the same year, or in the next year, no apparent relationship develops. These mortality rates of 11.23, 4.45, 6.83, and 5.93%/day in 1971-74, respectively, seem to neither cause, nor result from the peak measured densities in grains of 6,096,000, 582,306, 408,733, and 1,341,109 for 1971-74.

One factor which seems to be correlated with the regional population decline is the relative densities of beetles in spring and winter grains (primarily oats and wheat, respectively). In 1971 when there was a high regional density, most of the beetles went into oats (Table 14). In subsequent years the proportion of beetles in oats declined as did the regional density. This phenomenon is probably best understood in light of CLB behavior.

As CLB's move from overwintering sites, they initially distribute themselves fairly evenly throughout the environment and are found wherever there are suitable grasses for feeding, i.e., alfalfa fields, pastures, idle fields, roadsides, etc., as well as winter grain fields. In wheat fields there is initially no relationship between plant height and beetle density in a field. Likewise there was initially no difference between densities in resistant and susceptible wheat during this time as discussed in Appendix C. During the early spring, beetles apparently continue to move both between and within fields, and by the time oviposition begins (late May), beetle densities are significantly correlated with plant height in wheat fields, and there are significantly more beetles in susceptible than in resistant wheat (Appendix C). At that time, beetles are found in the highest densities in the shortest fields. This was particularly apparent in 1973 when 4 different ages of wheat were grown in adjacent strips in one field. Densities of adults were consistently negatively correlated with crop height as were densities of immature stages (Gage, 1974).

In viewing the survey results of Fig. 3 and Tables 11 and 12, it is not exactly apparent where the beetles come from that end up in oats. The early season samples, particularly in non-crops, are certainly pushing the limits of the sweepnet model of Ruesink and Haynes (1972) used in determining absolute density, and not a great deal of confidence is placed in these estimates. Some facts are apparent, however, as in 1971 when 7 times as many beetles were found in spring grains as were ever measured in winter grains, and in the experiment of Wells (1967) which showed that spraying all the wheat in a township for adults did not affect subsequent oat densities. Clearly, beetles do not follow a rigid sequence of movement from overwintering sites to wild grasses to winter grains, and then to spring grains. Instead there appears to be a period of mobility during which beetles are generally found in the most preferred host plants.

Those beetles found in a particular field on a given day may just be passing through as part of a continuous flow between several habitats. Differential flow rates between crops and within crops at different times of the season can account for the different adult densities observed in the habitats.

Those oat fields which have germinated and are out of the ground during this period of mobility get high densities of adult CLB's. However, the late planted oats which are unavailable during this time of beetle movement do not get high densities of beetles. Thus, later in the season there is a positive correlation between height of oats and densities within oat fields. The low densities in late planted oats result from several factors. First, because of the mortality of adults, there are fewer available to move into the late oats. Secondly, since oats is the preferred host for CLB's, it acts as a sink and beetles leave oat fields at a much slower rate than they arrive. Thus, there is relatively little movement of beetles between even adjacent oat fields (this is apparent in the strip sprayed field of Appendix A where the density in the sprayed field decreased dramatically, but the adjacent oat field was unaffected by the spray). The third factor involved is the reduced rate of movement as the season progresses. As seen in Appendix A, the beetles move at a greatly reduced rate after the oviposition period begins.

This combination of beetle mobility and preferences serves to explain the densities in crops in a particular year; however, it does not explain the observed differences between years. It was already noted that the shift of beetles from oats to wheat during the 4 years surveyed was not apparently related to the planting dates or synchrony of the 2 crops. This shift might be related to the decline in regional density, however, because CLB's are less productive in wheat than in oats. The fecundity and within-generation survival of CLB's are higher in oats than wheat (Helgesen, 1969; Wellso, pers. comm.). Thus, when a large proportion of the regional population is in oats, that population can increase more rapidly than it could if most beetles were in wheat.

In turn, if the proportion of beetles going into oats were determined by regional density, the behavior of the adults could have a profound effect on the regional density. There is some evidence that beetle adults resist crowding. Helgesen (1969) reported a maximum density of 5-7 adults/ft.^{2} which was never exceeded regardless of the regional density, and he speculated that the audible sound which beetles produce might be a factor in regulating adult densities in a field. It is possible that the crowding of CLB's in high density years results in more beetles leaving wheat and other habitats and ending up in oats. In years of low density this crowding would be less important and more beetles could remain in other habitats. This behavioral feature coupled with the higher productivity in oats could serve to greatly amplify, over several years, what might otherwise be a minor change in population density due to weather conditions, plant synchrony, or some other factor affecting the regional density. It is possible that this shift from oats to wheat could have been one of the factors contributing to the decline of the CLB population at Gull Lake between 1971 and 73. Other factors which apparently contributed are the reduced survival of eggs and small larvae in oats, the adverse weather conditions in the summer of 1971, and the incidence of parasitism--all recorded by Gage (1974).

Implications for Cereal Leaf Beetle Management

In light of the new information on the survival and behavior of adult CLB's described in this section and in Appendices A-C, it is important to re-evaluate CLB management practices.

Gage and Haynes (1975) suggest directing control programs against the adults instead of the current practice of spraying for CLB larvae which is detrimental to parasites. The effective use of an adult control program requires a method of predicting or monitoring adult densities and an understanding of the significance of these densities with respect to the dynamics of the insect and its relationship with its hosts and parasites. Secondly, should this density be determined sub-optimal, the economic and ecological impact of a variety of control options must be evaluated. These factors are best evaluated by ecosystem models, of which an assortment is available on many aspects of the CLB ecosystem. Before adult control is effectively incorporated into a CLB management program, new ecosystem models will be needed to evaluate the impact of several factors affecting adult beetles.

The first of these factors to be considered in a control strategy is the type of crop planted. Based on the shift of beetles between oats and wheat according to the regional population density, it seems reasonable that to the extent it is economically feasible, the planting of oats should be discouraged during times of epidemic population levels. The acreage normally planted to oats could be planted to wheat instead, thereby allowing a buildup of parasites on the wheat (which will probably go unsprayed) and suppressing a further population increase in the oats, on which beetles are more productive. Growers would save on insecticide costs and maintain parasite populations by precluding the necessity of insecticide applications on oats, the "preferred" host of epidemic populations.

Oat acreage could be increased during years of endemic populations for several reasons. First of all oats would probably go relatively undamaged because of the low population levels and the tendency of endemic populations to remain in wheat. Secondly, an ecologically sound management program would involve maintenance of CLB population levels as well as suppression. [CLB densities are best maintained at just below the economic threshold (Haynes, 1974).] Making oats available to beetles as an alternative host after wheat matures in a season and is no longer attractive should serve to increase regional CLB production. This switch between crops according to regional density would thus reduce the need for insecticides and tend to maintain population levels of hosts and parasites at more stable levels.

Another factor relating to planting is the planting date of the crops. By planting wheat late in the fall, its infestation can be increased. Similarily, oat damage can be reduced by planting it late in the spring. Thus planting dates can be adjusted as part of an overall regional management program although factors such as Hessian fly damage and yield reduction from late planting must be considered.

Resistant wheat has an uncertain future in CLB pest management in Michigan. The current understanding is that over a period of years CLB populations are maintained on wheat and occasional population surpluses move to oats, thereby precipitating a rapid regional density increase. During phases of declining regional densities, the oat population dimin-

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ishes rapidly and most beetles are again found on wheat. The release of resistant wheat could have a large impact on the CLB ecosystem. The widespread planting of wheat with the current level of resistance could almost eliminate wheat as a source of CLB and parasite production. It is possible that resistant wheat would greatly reduce what would otherwise be low regional densities, even driving parasites to local extinction. During epidemic CLB populations, resistant wheat would further the shift of beetles to oats. Thus it appears that resistant wheat would reduce the small degree of stability that presently exists in the CLB ecosystem and its release would be counterproductive. Wheat which was less resistant, or mixtures of resistant and susceptible wheat could be important, however, in reducing damage to wheat during epidemic populations, while minimizing the adverse effects described above. In areas where spring wheat is grown, resistance in wheat could be as advantageous in CLB management as resistant oats would be in Michigan.

All of the control features discussed to this point pertain to planting of crops and these decisions will generally be made in the fall. Between the fall and spring overwintering mortality can greatly affect the status of CLB populations. It appears that beetles in Michigan generally cannot survive winter temperature exposures above ground and they are not killed by temperatures encountered on the ground. It is possible, however, that a very mild winter or an extremely cold period without snow cover could affect this winter survival as could an early or late spring.

Insecticide applications can be used against adults to "fine tune" the system just before oviposition. If it is determined that a particular field will have an excessive egg input, the proper level of reduction can be achieved most economically by strip spraying. In light of the movement of CLB's between hosts the possibility of re-entry of adults into wheat or oats sprayed early in the season must be considered. When treatment is deferred until after the period of rapid movement, this problem is minimized, however the impact on subsequent oviposition is reduced.

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APPENDIX A

A COMPUTER ANALYSIS OF STRIP SPRAYING FOR THE CONTROL OF CEREAL LEAF BEETLES

Introduction

For the control of active arthropod pests, an alternative to blanketing a habitat with pesticides is the technique of applying the pesticide in selected areas where they contact it as a result of their mobility. Depending on the mobility and susceptibility of target and non-target species in temporal and spatial association, this approach can result in differential mortality between species and a reduction in the amount of pesticide applied.

Adults of the cereal leaf beetle (CLB) are quite active in early spring and when beetle densities are high they can readily be seen moving between host plants in frequent short flights in apparently random directions. This mobility, coupled with a high susceptibility to insecticides suggests that a relatively persistent organic phosphate insecticide such as malathion sprayed in narrow strips in a grain field could have an impact on the CLB population throughout the field. This section describes efforts to evaluate this control strategy through computer simulation.

When a field is sprayed in strips as in Fig. A1, there are several factors important in determining the mortality of its inhabitants. The first factor is the direct effect of the chemical on those arthropods in the path of the spray. After this initial effect, the movement of the

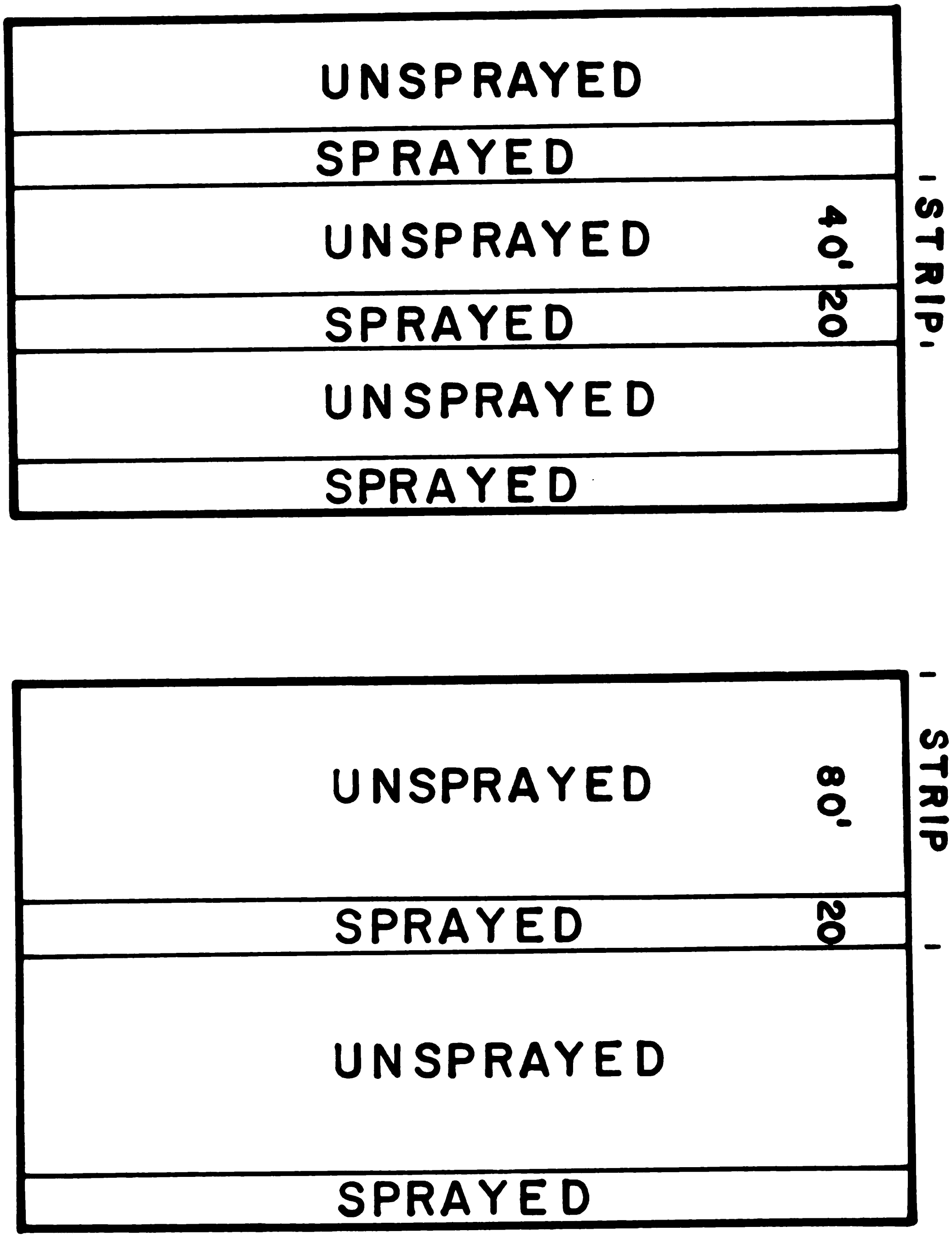

Fig. A1. Two strip spray patterns showing a strip comprised of a sprayed and an unsprayed portion. (Neg. 752244-14) the survivors and the residual effect of the pesticide determine the final level of mortality. In evaluating strip spraying for CLB's, the first of these factors is easily evaluated. The residual effects of the pesticide are quite complex however, and involve the degradation of the pesticide in the field, the movement into and out of the sprayed areas, the accumulation and degradation of the pesticide within the insects, and the effects of the pesticide on the insects. These factors were evaluated in field and laboratory studies. Their inclusion in a model allows computer simulations of experiments involving a variety of strip configurations, insecticide concentrations, and movement rates.

The evaluation and modeling of many of these factors was greatly simplified by imposing some constraints on the spray conditions. The model is intended to simulate application of malathion in narrow strips in a grain field by a tractor-drawn sprayer about 11 a.m. on sunny days when the temperature is above 60^{o}F and the wind speed less than 10 m.p.h. Although these constraints greatly simplify the modeling, they do not greatly hinder the applicability of the results as they reflect the standard or optimal conditions for application. Because strip spraying against CLB adults would normally be implemented in early spring before CLB parasites emerge from overwintering the effect on parasites is not included in this analysis.

Methods and Results

Beetle Movement

Cereal leaf beetle movement was studied by observing undisturbed individual beetles in grain at Gull Lake. The rows of wheat and oats were evenly spaced at 6 in. apart and the beetles were clearly visible on the small plants. On each of the dates listed in Table A1 several beetles were observed for about 5 minutes each, during which flight distances (total displacement in the x -y plane) were estimated (using the row spacing as an index) and the time between flights was measured with a stopwatch. These data were recorded on a cassette tape recorder to facilitate tracking the beetles. Table A1 summarizes the results of these observations collected over a 3-year period.

Theoretical Background and Analysis

A 1-dimension diffusion model was used to describe beetle movement in the strip spray model. The single dimension is justified because in a field sprayed as in Fig. A1, only movement in the x direction results in beetles getting into or out of or nearer or farther from the sprayed strips. Since the field lengths are very much greater than the strip widths, movement out the ends of the field is considered negligible. A diffusion model is justified because the movement of beetles within the fields involves flights of distances very much smaller than the dimensions of the field and the time interval between flights is measured in minutes compared to a scale of days for a strip spray experiment. Thus relative to the magnitude of the temporal and spatial dimensions of a strip spray experiment, the movement of beetles approximates continuous motion.

Pielou (1971) shows that for a 1-dimension diffusion model without drift, a diffusion coefficient (D) can be calculated by

$$D = \frac{(\Delta X)^2}{2\Delta t} \tag{1}$$

where ΔX is the displacement in the x direction. For a 2-dimension model D is calculated as

$$D = \frac{(\Delta \ell)^2}{4\Delta t} \qquad (2)$$

where $\Delta\ell$ is the displacement in the x-y plane. Using Pielou's assumptions that the x and y components of displacement (ℓ) are independent and identically, normally distributed with mean zero and unknown variance, it can be readily shown that the D for the 1- and 2-dimension models are identical, hence eq. 2 can be used to calculate D for 1-dimension diffusion. Thus D can be calculated as the mean of the individual observations of ℓ^2/t. Since not all observations on ℓ and t in Table A1 are paired, the mean ℓ^2 and mean of $\frac{1}{t}$ were used to calculate D for each sample date. The probability of an insect being at a distance X at time t is approximated by a normal probability density function with a mean of 0.0 and a variance of 2Dt. The equation

$$P(X,t) = \frac{1}{\sqrt{4\pi Dt}} \exp\left(\frac{-X^2}{4Dt}\right) \qquad (3)$$

uses the diffusion coefficient of eq. 1 to calculate the probability of an insect moving distance X in time t.

A multiple regression analysis of D vs. the main environmental factors listed in Table A1 indicated a highly significant correlation with the time of the season (in degree days). The diffusion coefficient decreases as the season progresses. There is no significant correlation between time of day, temperature, or solar radiation and diffusion rates; however, it should be remembered that consistent with the constraints discussed earlier, these observations were made under relatively uniform conditions. (Under more extreme conditions such as temperatures below 55^{o} and wind speeds over 10 m.p.h., CLB movement is greatly reduced). Cereal leaf beetles were observed to not move appreciably between sunset and sunrise. For purposes of simulation, a constant rate of diffusion was assumed for the day with no movement at night.

Table A 1. Observations on cereal leaf beetle movement, 1972-1974, in oats (O) and wheat (W) at Gull Lake.

Date	Time	°Days>48	Crop	Tem. (°F)	Wind (mph)	Solar Rad. (cal/cm^2/min.)	$\bar{X}$ (Dist)^2 (=ℓ^2)	Observations	$\bar{X}$ Freq. (=1/t)	Observations	Diff. Coeff. (ℓ^2/4t)
1972											
May											
22	3 PM	386	6" O	80	--	.85	341.32	22	5.835	14	497.90
24	4 PM	428	7" O	82	--	.80	120.80	50	4.759	39	143.72
24	8 PM	428	7" O	74	--	.05	749.13	18	1.771	9	331.64
25	10 AM	449	7" O	78	--	.95	151.18	55	2.830	42	106.96
June											
5	3 PM	623	9" O	76	--	1.15	59.56	32	3.026	26	45.06
6	11 AM	642	10" O	75	--	1.10	174.56	10	4.829	9	210.69
6	1 PM	642	10" O	77	--	1.20	21.12	26	2.968	17	15.67
7	10 AM	657	11" O	72	--	.85	39.87	25	4.492	22	44.77
7	2 PM	657	11" O	77	--	1.20	89.14	21	3.231	16	72.00
13	11 AM	746	10" O	77	--	.20	110.90	30	4.020	24	111.45
14	3 PM	776	11" O	86	--	.70	401.76	13	2.738	10	275.00
1973											
May											
20	1 PM	366	4" O	63	4.38	1.00	521.80	15	2.213	14	288.69
20	1 PM	366	11" W	63	4.38	1.00	284.31	39	5.444	33	386.95
26	4 PM	441	15" W	66	4.66	1.30	129.52	9	1.212	6	39.24
June											
7	2 PM	637	11" O	76	--	.80	15.68	6	2.396	5	9.39
7	6 PM	637	11" O	76	--	.80	250.79	33	4.719	30	295.87
8	11 AM	661	11" O	75	8.42	--	105.18	13	3.308	10	86.98
8	3 PM	661	11" O	83	8.42	--	370.85	14	4.835	13	448.26
9	11 AM	689	12" O	78	4.74	.90	68.06	8	0.585	6	9.95
10	1 PM	713	12" O	83	4.95	1.20	48.31	11	1.497	12	18.08
12	2 PM	763	13" O	85	9.00	--	28.90	7	0.611	7	4.41
15	1 PM	837	14" O	75	5.35	.90	76.51	16	2.038	13	44.15
1974											
June											
4	11 AM	590	-- O	76	3.64	1.10	41.82	15	5.339	15	55.82
4	1 PM	590	-- O	81	3.58	1.10	30.87	9	7.665	9	59.15
25	11 AM	985	-- O	66	7.15	.90	38.70	12	2.713	6	26.25
27	11 AM	1023	-- O	78	5.83	1.20	58.80	12	0.994	6	14.61

Insecticide Features: Decay Rate and Insecticide-Induced Mortality

One set of experiments was conducted to evaluate the decay rate of malathion on cereal grain plants, the dose-mortality response of CLB's from exposure to residual malathion, and the mortality of beetles directly exposed to the malathion spray. In these tests a laboratory spray apparatus was used to simulate application of 1.5 lbs/acre of active malathion E.C. in 40 gal. water by a tractor-drawn sprayer. Twenty adult cereal leaf beetles from Galien, Michigan, were placed on 4" pots with 6" barley seedlings spaced at least ½ inch apart and the plants were sprayed at 11 a.m. on June 2 and June 10, 1974. Ten minutes after application the beetles were removed from the plants and the plants were placed outdoors on grass. In both tests, the sprayed beetles were all knocked down by 10 minutes, and at 24 hours mortality was measured to be 100%.

At the time intervals indicated in Table A2, some of the treated plants were moved indoors to a 70^{o} room where 20 adult CLB's were confined on each plant by means of a glass lantern globe with a screen top. With some occasional encouragement to keep a few off the glass, the beetles remained on the plants for the duration of the exposures. After the various exposure times indicated in Table A2, a sample of 40 beetles was removed from two pots of plants and held for 24 hours before mortality was counted. The results of these tests show the relationship between time of exposure and mortality as the malathion decayed during the two days following application.

It can be seen that as the insecticide decays, the exposure time required to cause a given level of mortality increases. When percent mortality is plotted against the log of exposure time for each test a series

Table A 2. Results of two tests on exposing adult cereal leaf beetles to malathion treated plants.

JUNE 2, 1973

Hours After Application									
.75		4.25		9.50		22.50		31.75	
Exposure Time (Min.)	% Mortality	Exposure Time (Min.)	% Mortality	Exposure Time (Min.)	% Mortality	Exposure Time (Min.)	% Mortality	Exposure Time (Min.)	% Mortality
10	20.51	20	22.50	30	37.50	30	8.33	80	0
20	68.42	45	70.73	90	35.29	100	20.93	165	13.16
30	62.50	90	70.00	120	58.54	130	43.90	235	35.90
40	82.50	120	83.78	150	72.50	150	73.17	300	51.16
60	76.92	180	90.24	180	90.00	200	51.28	840	57.14

JUNE 10, 1973

Hours After Application					
6.25		11.50		26.00	
Exposure Time (Min.)	% Mortality	Exposure Time (Min.)	% Mortality	Exposure Time (Min.)	% Mortality
15	20.00	30	7.50	60	8.33
30	27.50	60	20.51	120	21.62
50	52.50	90	23.08	240	23.68
80	57.50	120	30.77	420	50.00
125	83.78	330	95.12	540	61.11

of sigmoid graphs results and, hence, in Fig. A2 the time and mortality axes are expressed in log and probit scales, respectively. Regression equations were fit to each of the data sets in Table 2 (average $r^2 = .811$) and Table A3 was generated by solving each of these equations for percent mortality. Table A3 serves as a standardized data set, allowing analysis of both the insecticide decay rate and the dose-mortality response.

The mortality levels in Tables A2 and A3 are determined by the dose of insecticide that the beetles were exposed to. In these exposures, the amount of this dose is determined by the concentration of insecticide at the time of the exposure [$C(t)$] and the duration of the exposure (T). The product $C(t) \times T$ is the amount of insecticide accumulated during an exposure and is termed exposure level. Actually, the pesticide decays somewhat during an exposure period, but since the decay rate is very slow compared to the duration of exposures in this experiment, the concentration is assumed constant during exposures. Thus, the concentration during the time interval (t_1, t_2) is approximated by $C(t_1)$.

Saini and Durough (1970) showed an exponential decay of malathion on cotton plants. Assuming a similar decay on small grains, the residual concentration of malathion can be expressed by an equation of the type

$$C(t) = C_0 e^{-Kt} \qquad (4)$$

where C_0 = initial concentration = 1.5 lbs/acre in the lab experiments,

K = decay constant,

t = hours after application.

Thus, the exposure level of an insect exposed from time t_1 to t_2 is

$$El = C_0 e^{-Kt_1}(t_2 - t_1) = C_0 e^{-Kt_1} T \qquad (5)$$

where T = duration of exposure.

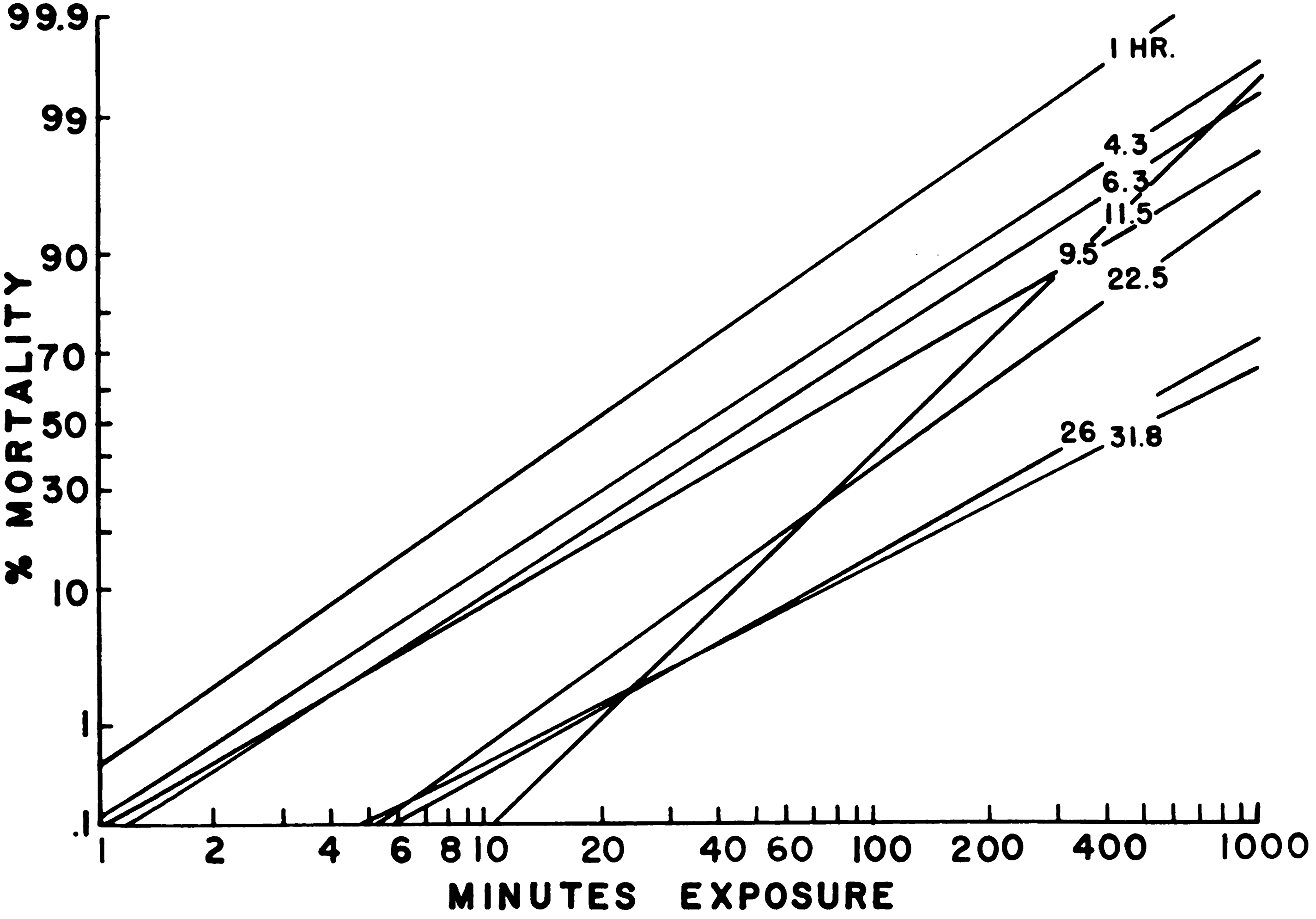

Fig. A2. The relationship between exposure time of CLB's on treated plants and mortality at various times after applying the insecticide to the plants. (Neg. 752244-27)

Table A 3. Minutes exposure required for various mortality levels at different times after insecticide application.

MINUTES EXPOSURE REQUIRED

%	.75	4.25	6.25	9.50	11.50	22.50	26.00	31.75	Hours After Spray
Mortality	.75	4.25	6.25	9.50	10.50	13.50	17.00	22.75	Hours Daylight
10	4.49	8.33	10.51	11.99	44.59	37.56	72.33	77.74	
20	7.34	14.05	17.96	21.62	63.36	60.65	133.64	147.50	
30	9.39	20.54	26.50	33.18	81.82	85.93	208.86	235.00	
40	14.21	28.30	36.80	47.63	101.51	115.31	304.42	384.13	
50	18.80	38.07	49.88	66.56	123.94	151.40	431.48	500.92	
60	24.87	51.22	67.60	93.03	151.33	198.79	611.58	720.78	
70	33.65	70.57	93.88	133.54	187.75	266.75	891.38	1,067.77	
80	48.14	103.16	138.55	204.96	242.43	377.98	1,393.07	1,701.20	
90	78.78	173.90	236.60	369.42	344.52	610.36	2,573.95	3,227.68	

Since the exposure level of a population uniquely determines the mortality level, it follows that groups of beetles with similar mortality levels experienced similar exposure levels. Thus, for a particular mortality level in Table A3, for instance 50%, all the combinations of C(t) x T that cause 50% mortality must result in the same exposure level (El50). This can be expressed as:

$$El50 = C(t) \times T = C_0 e^{-Kt_1} T$$

or in linear form:

$$\ln El50 = \ln(C_0 T) - Kt_1$$

or:

$$\ln(C_0 T) = Kt_1 + \ln El50 \qquad (6)$$

which may be recognized as the linear equation relating C_0T to t_1. Thus, if $\ln(C_0T)$ is plotted against t_1, the resulting straight line has a slope = K and an intercept = log(El50).

Fig. A3 shows plots of $\ln(C_0T)$ vs. t_1 for the 10 to 90% mortality values in Table A2. In this plot, the X axis has been converted to hours daylight after spray application (using a 15-hour day) as this transformation resulted in a better fit of the data to equation 4 (average r^2 = .939). The results of the regression equations fit to these data are shown in Table A4. Ideally, the 9 values for K in Table A4 should all be identical since they all reflect the decay rate of the insecticide. As seen in Fig. A3 and in the table, these slopes do not differ greatly; however, because of the observed differences, the 50% mortality values were used to develop the equation expressing the residual concentration of malathion as:

$$C(t) = C_0 e^{-.1585td} \qquad (7)$$

where td = hours daylight since application. This residual concentration is shown graphically in Fig. A4.

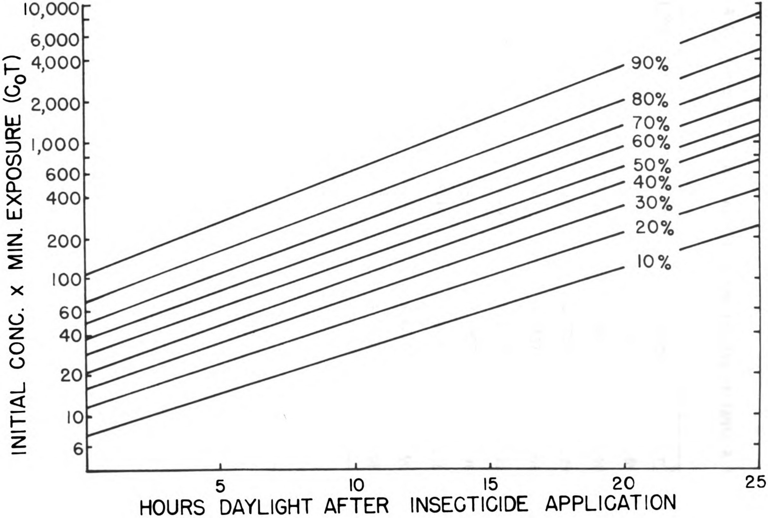

Fig. A3. Insecticide exposures required for various mortality levels as the insecticide decays in time. (Neg. 752244-25)

Table A 4. Regression equations fit to the results in Table A 3.

% Mortality	Slope	Intercept	r^2
10	.14142	1.9763	.867
20	.14730	2.4423	.909
30	.15466	2.7344	.930
40	.15855	3.0437	.954
50	.15850	3.3315	.955
60	.16183	3.5967	.960
70	.16544	3.8826	.962
80	.16970	4.2216	.961
90	.17557	4.6875	.952

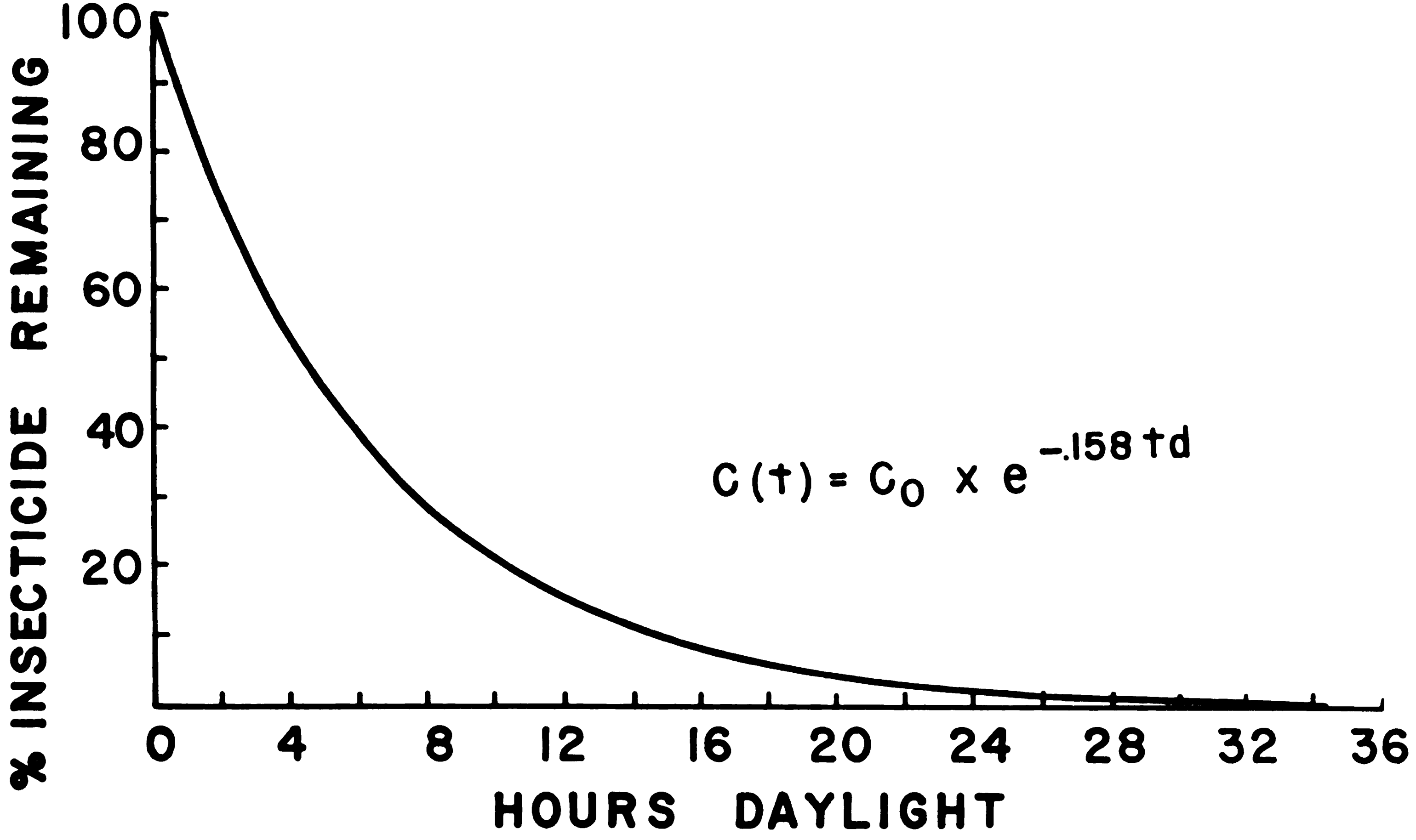

Fig. A4. Insecticide concentration as a function of time (in hours daylight). (Neg. 752244-15)

The intercepts in Table A4 correspond to the natural logarithms of the exposure levels required for various mortality levels at $t = 0$ (before any insecticide decay has occurred). A plot of mortality levels vs. the ln of the corresponding exposure levels from Table A4 reveals a sigmoid curve of the type generally associated with dose-response curves. Thus, in Fig. A5 mortality is plotted on a probit scale vs. the corresponding exposure levels on a log scale. The linear regression equation

$$P = .9412 \times \ln El + 1.8713 \qquad (8)$$

was fit to these values ($r^2 = .999$) where P = probits mortality.

Equations 7 and 8 serve as the basis for an insecticide model by providing a decay function for the insecticide and an equation relating CLB mortality to doses (exposure levels) of malathion. When the observed mortality levels of Table A2 are plotted against the mortalities predicted by eqs. 7 and 8 for similar exposures, an r^2 of .782 results. This value can be compared to the average r^2 of .811 for the 8 individual regression lines fit to the data in Table A2.

Insecticide Features: Effects on Behavior

It was expected that malathion might affect the behavior of CLB's by repelling them or affecting their mobility. This was difficult to observe in the field because of low densities in sprayed strips so observations on these factors were limited to the lab. Preliminary observations were made on CLB behavior during the experiments described previously wherein CLB adults were confined to 6" potted barley seedlings which had been sprayed with malathion at the rate of 1.5 lbs/acre. During these exposures some individuals fed on the plants and others walked between the leaves, but the majority remained motionless after crawling up a leaf.

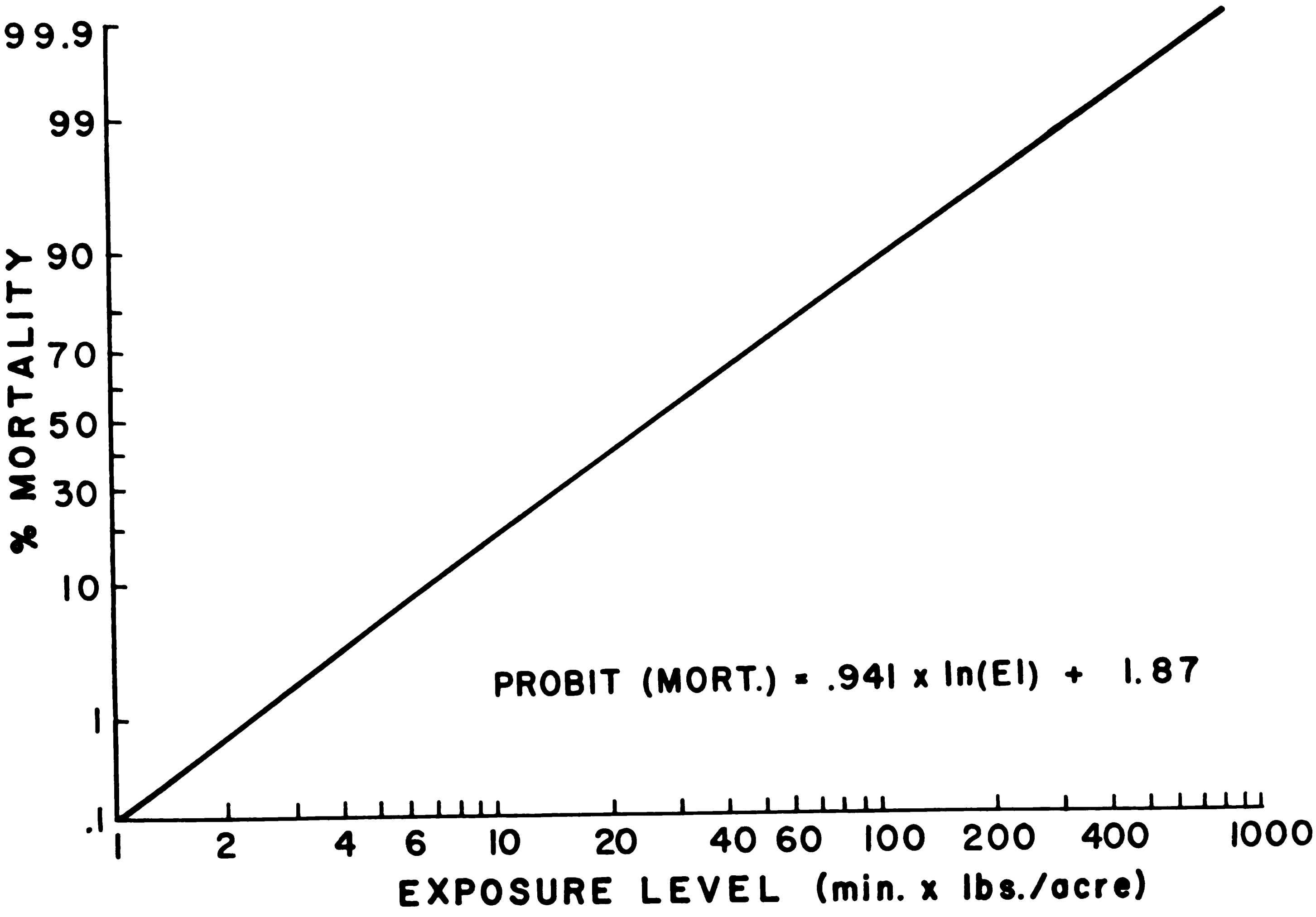

Fig. A5. The relationship between exposure levels and mortality. (Neg. 752244-26)

No behavioral differences were observed between the CLB's confined to treated plants and those controls confined to untreated plants. Also, no change in behavior was observed as the insecticide exposure of the beetles increased. Without any outward indication of stress, beetles which had accumulated a high enough dosage would fall from the plants and remain on their backs kicking.

To test for a possible repellent effect of the malathion on CLB's, 4 treated (1.5 lbs/acre) and 4 untreated plants were placed in a 2 x 2 x 2 ft. screened cage and 20 CLB's were introduced at various times after the insecticide application. At the time the first CLB fell from insecticide exposure (first knockdown), the distribution of the 20 CLB's in the cage was determined. The numbers on the sprayed and unsprayed foliage as well as the number elsewhere in the cage were recorded. The results shown in Table A5 show no significant difference at the .05 level in the numbers on the sprayed and unsprayed plants.

The effect of the insecticide on activity was measured in an experiment where 8 treated plants (1.5 lbs malathion/acre) were placed in one 2 x 2 x 2 ft. cage and 8 untreated plants were placed in a second similar cage. One hour after the spray was applied, 20 CLB's were introduced into each cage. At 2-minute intervals, each cage was observed for 10 seconds and the number of CLB's which moved during that time was recorded. Observations were ended with the first CLB knockdown at 25 minutes. The results shown in Table A6 indicate no difference in activity between the CLB's in the 2 cages. Although the experiment was terminated at 25 minutes with the first knockdown, the beetles' exposure was fairly large. According to the insecticide model of eqs. 7 and 8, the exposure they

Table A 5. Distribution of 20 cereal leaf beetles in a cage at the time of first knockdown.

Minutes After Application	First Knockdown (Min.)	No. on Sprayed Plants	No. on Unsprayed Plants	No. Elsewhere in Cage
15	5	9	6	5
30	25	7	13	0
60	25	9	11	0
90	30	7	7	6
$\overline{X}$		8.00	9.25	2.25
S.D.		1.15	3.30	3.20

Table A 6. The number of cereal leaf beetles moving out of 20 during a 10 second observation period at various times after introduction into cages of sprayed and unsprayed plants.

Time After Introduction	No. Moving in 10 Sec.	
	Sprayed	Unsprayed
2 minutes	1	3
4 "	2	0
6 "	3	1
8 "	2	3
10 "	2	3
12 "	2	4
14 "	2	5
16 "	5	3
18 "	3	3
20 "	2	2
22 "	5	3
24 "	2	3
$\overline{X}$	2.583	2.750
S.D.	1.240	1.288

received should have ultimately resulted in 55.3% mortality. These two experiments, while somewhat preliminary in nature, indicate that malathion applied at the rate of 1.5 lbs/acre does not affect CLB behavior and, thus, behavioral effects were not incorporated into the strip spray model.

Temporal and Regional Changes in Insecticide Tolerance

An important factor in determining the applicability of a strip spray model is the uniformity of the insect response to the insecticide. Regional differences in tolerance levels, the development of insecticide resistance, and within season changes due to beetle age must be considered before widespread utilization of the technique is accomplished.

Monroe and Polityka (1965) collected adult cereal leaf beetles near Galien, Michigan, in the spring of 1965 and gave them topical applications of malathion in 1 microliter of acetone. Using a range of insecticide concentrations varying from .01 to .04 micrograms/microliter, they determined the LD50 to be .018 µg./beetle.

In 1973 and 1974, adult cereal leaf beetles were collected at two sites near Galien and also at Gull Lake, 77 miles N.E., and Laingsburg, Michigan, 135 miles N.E. of Galien, in order to measure possible differences from the baseline established by Monroe. The results shown in Table A7 were fit with log-probit regression equations which were solved for 50% mortality. The resulting LD50 values indicate that the beetles collected in the spring of 1974 from site 1 (3 miles southeast of Monroe's collection site) had about the same tolerance level (LD50 = .0184) as Monroe measured 9 years earlier. The newly emerged summer adults collected at the same site in July of 1973 and 1974 and similarly tested, were found to have LD50's of .0444 and .0442, respectively. The 2.5-fold

Table A 7. Temporal and regional differences in response to topical applications of malathion.

GALIEN SITE 1
JULY 18, 1973

Dose (µg)	% Mortality	Replicates
.010	7.5	2
.020	7.5	2
.040	25.5	3
.045	31.6	2
.050	45.0	3
.055	50.0	2
.060	81.1	2
.080	80.0	2
.100	97.4	2

LD50 = .0444 µg

GALIEN SITE 1
JULY 8, 1974

Dose (µg)	% Mortality	Replicates
.010	0	2
.020	12.5	2
.030	17.9	2
.040	30.8	2
.050	37.5	2
.060	79.5	2
.070	87.2	2
.080	95.0	2
.090	85.0	2
Control	0	2

LD50 = .0442 µg

GALIEN SITE 1
MAY 21, 1974

Dose (µg)	% Mortality	Replicates
.010	10.0	2
.020	70.3	2
.030	80.0	2
.040	92.5	2
.050	92.5	2
Control	0	1

LD50 = .0184 µg

GALIEN SITE 2
MAY 16, 1974

Dose (µg)	% Mortality	Replicates
.01	20.0	2
.02	55.0	2
.03	92.2	3
.04	93.8	3
.05	100.0	1
Control	20.0	2

LD50 = .0157 µg

GULL LAKE
MAY 21, 1974

Dose (µg)	% Mortality	Replicates
.01	5.0	2
.02	57.5	2
.03	85.0	2
.04	97.5	2
.05	100.0	2
Control	0	1

LD50 = .0185 µg

LAINGSBURG
MAY 29, 1974

Dose (µg)	% Mortality	Replicates
.010	2.5	4
.018	13.2	2
.020	20.0	4
.030	48.8	4
.040	87.5	4
Control	0	1

LD50 = .0275 µg

increase was found to be highly significant by covariance analysis, indicating a shift in malathion tolerance during the year. Beetles from a second site near Galien, 1.5 miles southeast of Monroe's collection site, had an LD50 of .0157 μg./beetle which was determined by covariance analysis to be not significantly different from the spring population from site 1. The Gull Lake beetles had almost exactly the same LD50 as those from site 1 near Galien; however, the Laingsburg beetles had an LD50 of .0275 which is almost 1.5 times greater than site 1, and this difference is highly significant.

It is not apparent why the Laingsburg population is less susceptible to malathion than the Galien population. Cereal leaf beetles have been established at Galien for a longer time and have been exposed to more insecticide than at Laingsburg. It was thought that perhaps a size difference could account for this discrepancy; however, in Table A8 it is seen that the Galien beetles were actually larger than the Laingsburg population, although these differences proved insignificant. It is interesting to note that the average weight of the Galien beetles, 6.945 mg., is considerably less than the 8.22 mg./beetle that Monroe determined as the average for 500 beetles of mixed sexes in 1965. Again, this difference is not significant.

Recovery from Insecticide Exposure

Equations 7 and 8 describe CLB mortality from continuous exposure to malathion; however, in a field sprayed with strips of malathion, beetles can repeatedly move into and out of the sprayed strips. This brings up the question of additivity of sequential exposures. If CLB's are able to "recover" when out of the sprayed strips, this could have a large impact on mortality from subsequent exposures and should be included in the model.

Table A 8. Live weights of cereal leaf beetles collected in May of 1974 at Galien and Laingsburg.

Source	Galien			Laingsburg		
Sex	♂	♀	♂ + ♀	♂	♀	♂ + ♀
$\bar{X}$ Weight (mg)	5.609	8.282	6.945	5.310	7.970	6.640
SD	.718	.980	1.604	.597	.710	1.507
N	11	11	22	10	10	20

A literature review reveals that relatively little is known about the recovery of insects from non-lethal doses of malathion. Apparently at least two factors are involved in this recovery. The first factor is the degradation of the insecticide within the insects. Krueger and O'Brien (1959) showed that malathion and malaoxon (its activation product) are degraded to a considerable extent during a 24-hour period following malathion application to American cockroaches. It is noteworthy that at 24 hours, the malaoxon level remained at nearly 30% of the maximum level.

A second factor involved in recovery from malathion exposure is the recovery from esterase inhibition. It is generally accepted that malathion, as an organophosphate insecticide, exerts its toxic effects through the inhibition of acetylcholinesterase (AChE) (Tripathi and O'Brien, 1973). Insects can recover from this inhibition by enzyme synthesis and by reversal of inhibition. O'Brien (1961) measured the level of AChE and other esterases in house flies surviving a topical LD50 of malathion. He found all 4 esterases tested to decrease during the first 60 to 90 minutes after application, but after that they all increased at a slow rate. By 21.3 hours the flies had not fully recovered as none of the esterases had returned to its normal level. Tripathy and O'Brien (1973) point out that inhibition of thoracic AChE is better correlated with degree of poisoning than head AChE. They show in house flies that the 3 isozymes of thoracic AChE all increase in activity after a maximal inhibition at 20 minutes after malathion application but, again, none returned to its normal level in 21.3 hours.

On the basis of these studies, it appears that malathion and its activation product can be degraded in insects and insects can also "recover" from esterase inhibition. Furthermore, both of these processes occur on a time scale of significance to strip spraying. To measure the extent of recovery of CLB's from malathion exposures, a lab experiment was conducted on June 3, 1974, using CLB's collected 3 days earlier at Galien, Michigan. In this test, a group of 60 beetles was given a topical application of .02 μg. malathion in 1 μl of acetone. A second group of 300 beetles was given half of that dose (.01 μg.) on June 3 and given a second dose of .01 μg. at various times after the initial application as indicated in Table A9. Mortality from each test was counted 24 hours after applications and at the completion of the entire test at 9 days. During the test, beetles were stored in petri dishes, 20 per dish. Each day they were fed clipped leaves from barley seedlings and were given a fresh dental wick saturated with water.

The initial applications of .01 and .02 μg. caused 1.3% and 30.4% mortality, respectively, at 24 hours after application. In Table A9 and Fig. A6, it can be seen that the mortality from the second dose decreased with time, indicating recovery of the beetles from the first dose. A regression analysis of these data reveals the slope of this line to be significantly different from 0 at the .01 level.

Because the mortality of the controls and the beetles given the first dose increased substantially during the 8 days of the test, it could be argued that the population exposed to the second dose shifted in time. If, as a result of this mortality, the most susceptible beetles were selected out before the second exposure, the resulting decrease in mortality from the second exposure could appear to indicate recovery. By measuring the total mortality from both exposures at the 9th day, this problem was accounted for. In Fig. A6, it can be seen that this total mortality decreased

Table A 9. Additivity of sequential malathion applications.

Day When 2nd Dose Applied	Mortality From 1st Dose When 2nd Applied	Mortality From 2nd Dose at 24 Hours	Total Mortality at 9 Days
0	1.3%	30.4	45.0
1	3.3%%	24.1	33.3
2	5.0%	26.3	50.0
4	16.7%	18.0	38.3
6	9.8%	10.9	28.3
8	11.7%	13.5	25.0

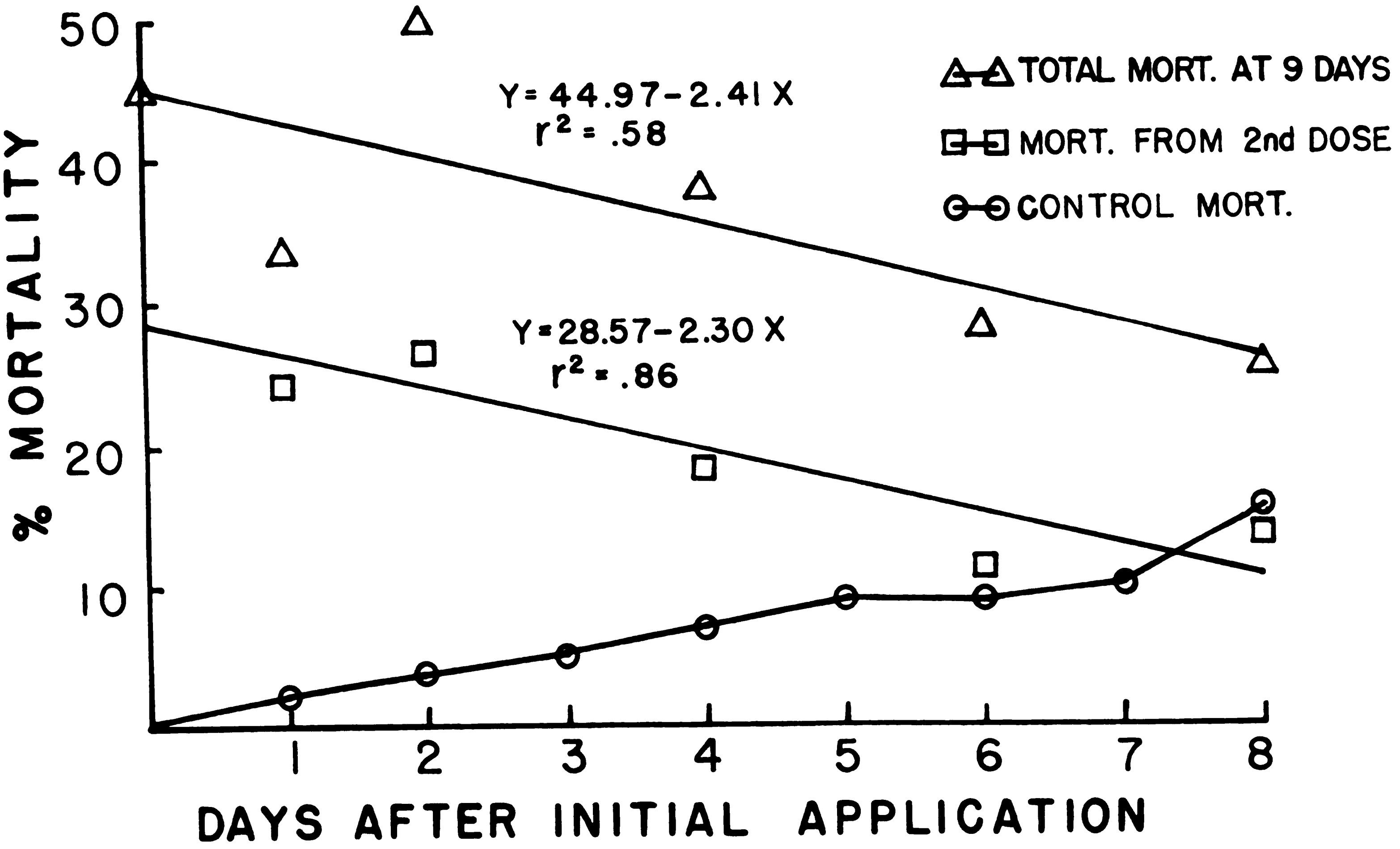

Fig. A6. Mortality resulting from applying 2 equivalent doses of insecticide to CLB's with different time intervals between applications. (Neg. 752244-29) in time, despite the increase of mortality in the controls. This clearly demonstrates that recovery occurred, although the slope of this line is only significantly different from 0 at the .1 level.

It is difficult to quantify the recovery rate from this experiment, especially since the population tolerance to malathion apparently decreased during the test. A dose of .01 μg./beetle which caused 1.3% mortality to previously unexposed beetles on the first day of the test was found to cause 11.1% mortality to surviving previously unexposed beetles on the 8th day. This apparent loss of tolerance means that by the 8th day the beetles had recovered from the first exposure because the mortality from the second dose (about 10% from the regression line) is less than the 11.1% mortality caused by a single dose on the same day.

This recovery rate is very much slower than the degradation of the insecticide in the field which, according to equation 6, is over 90% complete in one day and 99% complete in two days. It appears that recovery is not very significant in strip spraying with malathion for CLB's and this factor was not included in the model.

Strip Spray Model

A Fortran program was developed to simulate strip spraying with malathion against the cereal leaf beetle. The program (Appendix D) simulates a single strip which consists of a sprayed part and an unsprayed part as shown in Fig. A1. It is assumed that a field would contain a large number of these strips so that field boundaries would not be a significant factor. In the simulations beetles moving out of one side of the strip were allowed to move back into the strip from the other side (toroidal symmetry).

Equation 3 was used to calculate the percent of beetles moving various distances in a unit of time for the particular diffusion coefficient used in the simulation. Consistent with the observation that all beetles actually sprayed with malathion at a rate of 1.5 lbs/acre are killed, an initial density of 0 beetles/ft^2 was assumed in the sprayed part of the strip. A uniform distribution of any density was assumed as an initial condition for the unsprayed strip since there are no density dependent factors in the model. CLB's were characterized by two parameters: their location, and their exposure level. Initially, all beetles had an exposure level of 0.0; however, whenever beetles moved into the sprayed strips they increased in exposure level according to the duration of the exposure and the concentration of the insecticide from eq. 7. Beetles outside of sprayed strips did not "recover" or reduce their exposure level.

In the simulations, beetles moved and the insecticide decayed only during daylight hours. During the night those beetles on sprayed strips continued to increase their exposure levels throughout the night. At the end of the simulation, the number of beetles of all exposure levels were totalled and percent mortality was determined from eq. 8.

Field Validation

A field experiment was conducted at Gull Lake during June of 1973 to provide a data set for validating the model. An 11" high oat field measuring 88 ft. by 1250 ft. was divided into four stips (20 x 1250 ft.). Of each strip, 8.5 ft. was sprayed at a rate of 1.5 lbs/acre at 11 a.m. on June 7. At various times after insecticide application, the treated field (designated as field 1) was sampled with a sweepnet and the density of

CLB's/ft^2 was calculated using the model of Ruesink and Haynes (1973). Two control fields (designated as fields 2 and 3) were also sampled at the same times. Both control fields had about the same dimensions as the sprayed field and both had 11" high oats on June 7. Field 2 was located 100 ft. west of the sprayed field, and was separated from it by a freshly plowed field. Field 3 was 1875 ft. east of field 1, separated by a road and several fields of alfalfa and grains.

The results of these samples (Table A10) show that the density in the sprayed field (#1) declined dramatically during the first 24 hours after spraying, and then began to increase slightly. The densities in the two control fields (#2 and #3) appeared to fluctuate considerably between samples. What is more probable is that the actual densities in these fields remained approximately constant and the apparent differences in density reflect the inability of the sweepnet model to adequately account for changing environmental conditions. This model was not intended for use at 7 p.m. or 9 a.m. (when the 8 and 94 hour counts were taken). It is important to note that the density in field 2 did not decrease with respect to field 3. This indicates that relatively few CLB's moved the 100 ft. from field 2 into field 1 where they would likely have been killed.

Because there was apparently no significant movement of CLB's among the 3 fields in this experiment, the control fields were used to evaluate the density reduction in the sprayed field. For each of the samples in Table A10, the relative density in the strip sprayed field was determined by dividing the strip sprayed density by the average density of the two controls. Since the initial density in the strip sprayed field was 1.2% greater than the average of the two controls at the same time, each of the relative densities for this field were multiplied by .98814 to determine the

Table A 10. Results of a field test of strip spraying.

Time	Field No.	No. Sweeps	No. Caught	No./Acre	% of Initial Density
1.5 hours before spraying	1	1,500	151	1,441.8	100.00
	2	700	89	1,825.2	
	3	700	50	1,023.7	
8 hours after spraying	1	2,700	72	389.8	11.76
	2	350	95	3,545.8	
	3	350	81	3,023.1	
24 hours after spraying	1	2,700	4	27.2	2.08
	2	350	23	1,058.5	
	3	350	32	1,472.3	
49 hours after spraying	1	2,700	17	79.6	6.03
	2	350	42	1,463.6	
	3	350	33	1,145.6	
75 hours after spraying	1	2,700	15	66.1	7.92
	2	350	21	705.7	
	3	350	28	940.9	
94 hours after spraying	1	2,700	32	141.5	7.02
	2	350	58	2,016.8	
	3	350	56	1,951.5	

percent of the initial density. These results, included in Table A10, are corrected for sampling error and for the natural mortality which occurred during the 4 days of the test. The same results are plotted in Fig. A7.

At several times during the strip spray experiment of June 7-10, 1973, observations were made in the nearby control field on the rate of movement of the cereal leaf beetles. From these observations which are included in Table A1, a weighted mean diffusion coefficient of 207.46 in^2/min. was calculated. Using this diffusion coefficient, the strip spray model was run to simulate the field test and the results are graphed in Fig. A7. The close agreement between the model predictions and the field observations serves as a reasonable validation of the model. The slight increase in densities in the sprayed field observed in the last two days of the test might be due to the immigration of some beetles, although no emigration was detectable from the nearest grain field (#2).

Model Analysis: Sensitivity

Simulations were run with the model to investigate its sensitivity to the diffusion rate of the beetles, the strip configuration, and the initial insecticide concentration. All the simulations resulted in either 100% mortality or a stable mortality level after the insecticide completely degraded. These final mortality levels were used to evaluate the effects of changing the three parameters.

The diffusion coefficients calculated from the field data on flight frequencies and distances have a wide range of values (4.41 to 497.90 in^2/min.). In Fig. A8 it is seen that varying D over this range has a large impact on the final mortality level, however the model is less sensi-

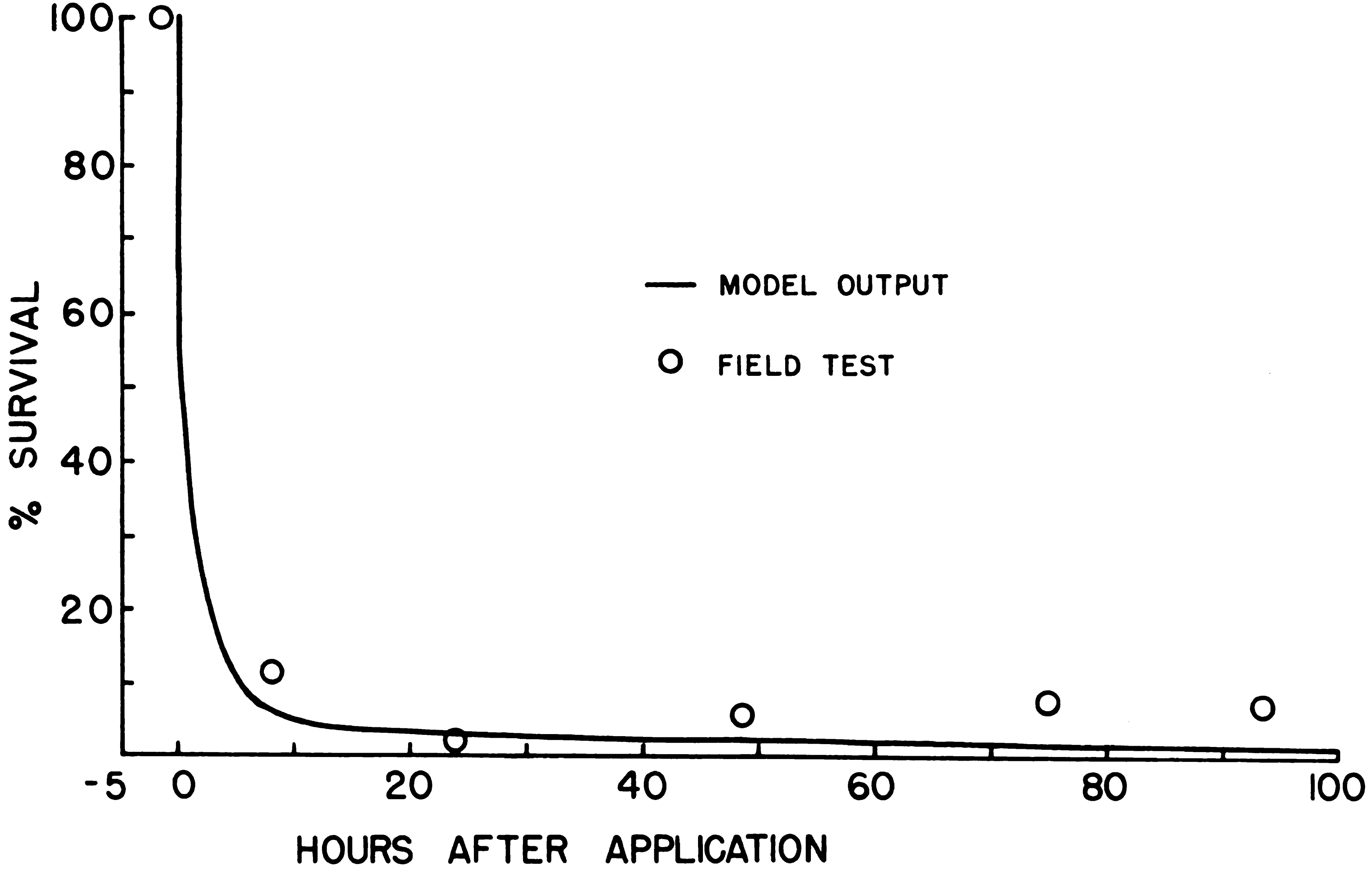

Fig. A7. A comparison of a field experiment and a computer simulation involving spraying 8.5 ft. of 20 ft. strips with malathion at a rate of 1.5 lbs./acre. (Neg. 752244-28)

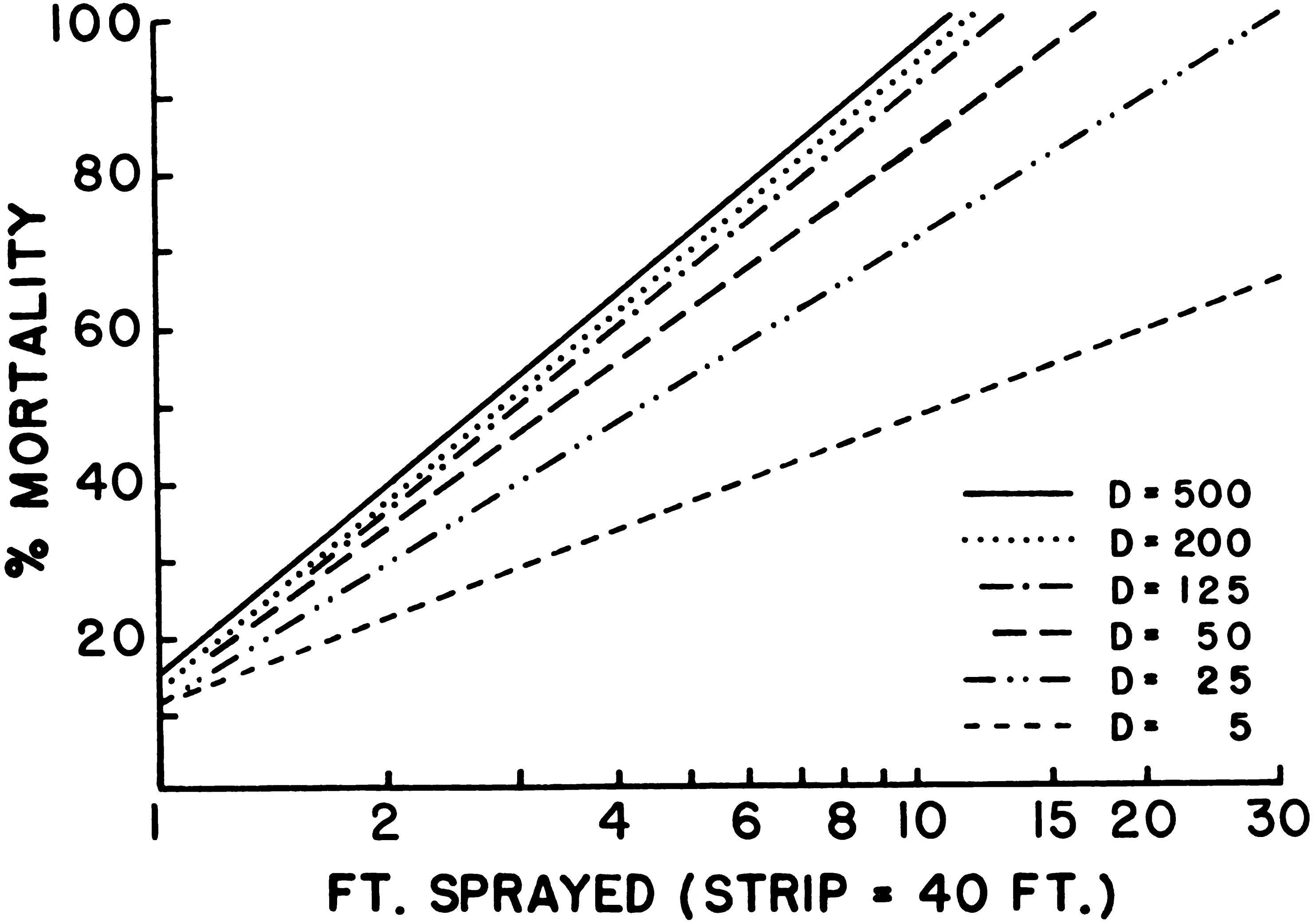

Fig. A8. Results of computer simulations showing the relationship between the width sprayed of a 40 ft. strip and the resulting final mortality level for various diffusion rates. (Neg. 752244-16) tive to high diffusion rates than to low. Since the objective of an adult control program would be to reduce oviposition, it would be desirable to spray early in the season. At that time diffusion rates are high, thus decreasing the sensitivity of the model to the parameter. An arbitrary diffusion rate of 125 in^2/min. was used to evaluate the impact of other parameters of the model. Although this rate is lower than the mean, increasing it to even the maximum observed level has little effect on the final mortality.

Fig. A9 shows the sensitivity of the model to varying initial concentrations of insecticides. It is apparent that for the range of concentrations at which malathion is effective, the model is more sensitive to the amount of the strip sprayed than it is to the concentration of the insecticide. For example, when spraying 2 feet of a 40-foot strip, doubling the application rate from 1 lb. to 2 lbs/acre results in an increase of 14.3% in the final mortality. However, by keeping the rate constant at 1 lb/acre and doubling the width sprayed from 2 ft. to 4 ft., an increase of 22.7% mortality is realized. Thus, for a given amount of insecticide it is more effective to spray more of the strip than to concentrate the insecticide. In light of this, an application rate of 1 lb/acre was selected for subsequent simulations as the minimal application rate consistent with the assumption of 100% kill of those beetles actually sprayed.

Economic Considerations

Strip spraying has economic advantages over normal spray practices in reducing both application and insecticide costs. Because both of these

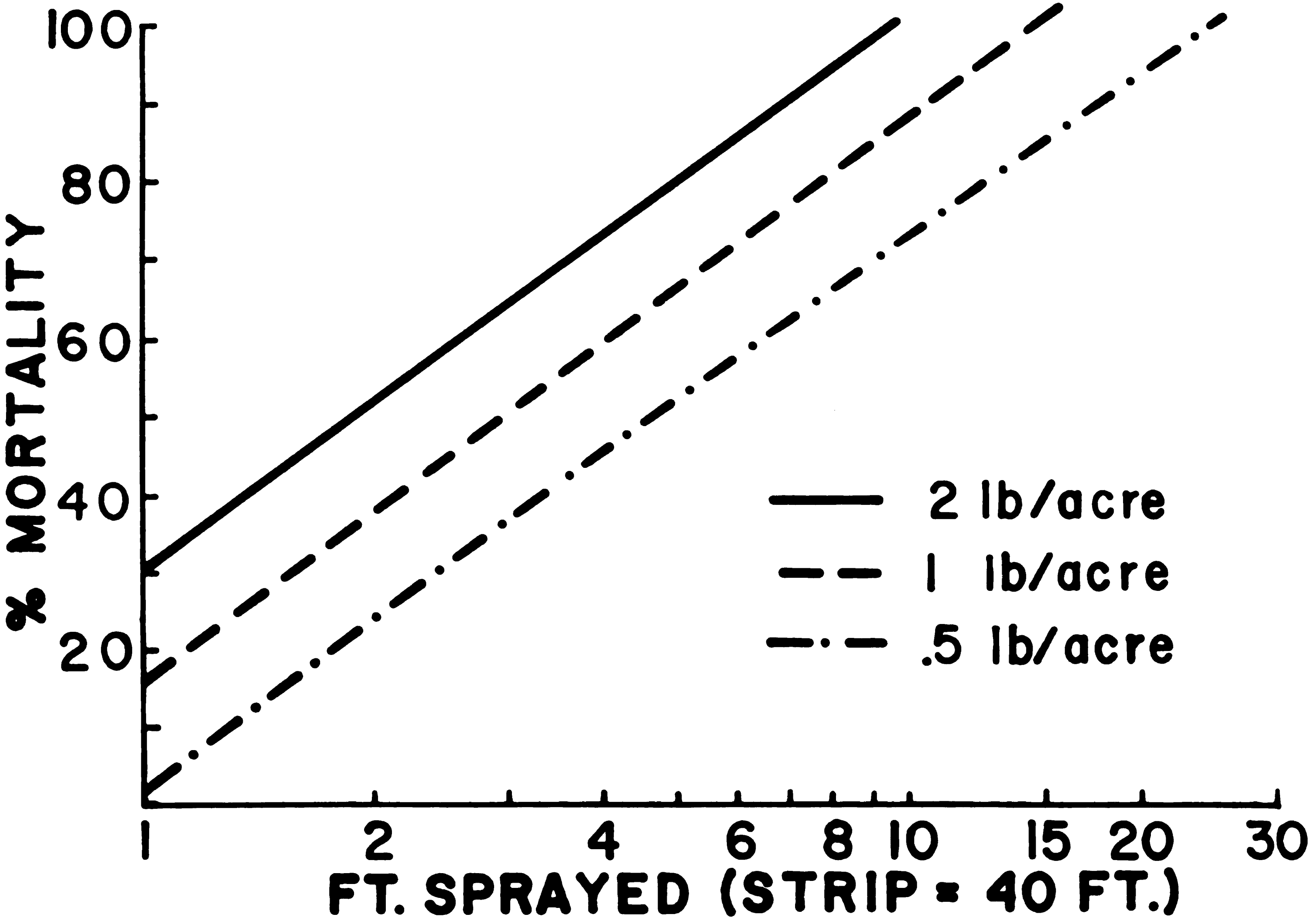

Fig. A9. Results of computer simulations showing the relationship between the width sprayed of a 40 ft. strip and the resulting final mortality level for various diffusion rates. (Neg. 752244-13) costs are extremely dynamic, it is difficult to accurately assess these advantages with any degree of generality or permanence. Furthermore, in spite of a reasonable field validation of the model, there is a certain element of uncertainty associated with its predictions and this is difficult to evaluate in terms of economics. For the purpose of evaluating one strategy relative to another, however, it seems that the model should be quite effective and for that purpose using relative costs for material and application should be adequate.

In evaluating the economic aspects of strip spraying, it was assumed that the costs of the insecticide equaled the application cost. Using a 1975 retail price of $2.75/lb. for single container lots of malathion and an advertised cost of $2.75/acre for custom application, a cost of $5.50/acre was used for applying 1 lb. of malathion/acre by a tractor-drawn sprayer. In Table A11, these costs were used to evaluate three strategies, all of which involve spraying 25% of the field at a rate of 1 lb/acre for a total application of 4 oz/acre. It is seen that increasing the total strip width from 20 to 80 ft. results in a decrease of 22% mortality and, thus, represents a less efficient use of the insecticide. However, by utilizing the full capacity of the 20-ft. spray boom, a 75% reduction in application time is achieved in spraying the 80-ft. strip and as a result total cost of application and material is reduced by 60%. Thus, for short-term economic advantage, it is most efficient to utilize the 20-ft. capability of the sprayer.

In Table A12, various strip widths are simulated of which 20 ft. were sprayed in each. These patterns represent the most economically advantageous use of the insecticide to achieve a given mortality level. All of the strategies represent a considerable reduction in both cost and material applied from the recommended application of 1 lb./acre.

Table A 11. An evaluation of 3 strategies which involve spraying 25% of a field.

Application Rate = 1 lb/acre
Diffusion Coefficient = 125 in^2/min.

Sprayed	Unsprayed	% Mortality	Cost	oz./acre
5'	15'	100	3.44	4
10'	30'	89	2.06	4
20'	60'	78	1.38	4

Table A 12. An evaluation of 5 strip configurations in which 20 ft. widths are sprayed.

Application Rate = 1 lb/acre
Diffusion Coefficient = 125 in^2/min.

Sprayed	Unsprayed	% Mortality	Cost	oz./acre
20'	20'	100	2.75	8
20'	40'	90	1.83	5.3
20'	60'	78	1.38	4
20'	80'	66	1.10	3.2
20'	100'	59	.92	2.7

Discussion

It is apparent from the computer simulations and field test that strip spraying can be effective in reducing populations of adult cereal leaf beetle in grain fields. The technique is not without limitations, possibly the most severe of which is deciding how much to reduce adult densities (if at all) to get the optimal egg input in a field. Once that decision is made and the approximate time decided upon, it is necessary to have two days of satisfactory weather to effectively use strip spraying with malathion.

Clearly, the technique of strip spraying for CLB control needs additional investigation before it can be generally implemented. Despite an encouraging field validation it is probable that the computer simulations are more valuable in choosing between alternative spray strategies than in accurately predicting a final level of mortality. Much of the work on recovery, behavior, insecticide degradation, and beetle movement is rather preliminary and could greatly benefit from additional experimentation. The present results, however, are quite adequate to design field experiments, and leave no doubt that strip spraying can be an economical means of controlling CLB's.

There are many other pest ecosystems where the technique and this model are of value, the primary requisites being mobility of pests and susceptibility to insecticides. Strip spraying can be advantageous in these cases in (1) effecting a differential kill of favorable and unfavorable species; (2) reducing the amount of pesticide applied; (3) reducing the cost of control; and (4) achieving a predetermined level of control.

APPENDIX B

A PREDICTIVE MODEL FOR CEREAL LEAF BEETLE MORTALITY FROM SUB-FREEZING TEMPERATURES

Introduction

Insects can survive exposure to temperatures below their freezing points by either supercooling or by tolerating freezing (Asahina, 1969). According to Salt (1961), the majority of insects are freezing susceptible, meaning they can supercool to some extent; however, with extreme or prolonged cold exposure they freeze and die. The remainder are able to survive freezing and are called freezing resistant or freezing tolerant. The cereal leaf beetle (CLB) is among the former group.

Salt (1950) showed that freezing of insects depends not only on the temperature, but also on the duration of cold exposure. Nonetheless, the majority of published low temperature survival studies since that time have been based on supercooling point determinations. Most researchers have specified cooling rates and have attempted to use supercooling points as an index of cold tolerance.

Preconditioning

Many authors (Green, 1962; Pantyukov, 1964; Sullivan, 1965; Sullivan and Wallace, 1972) have observed a change in mean supercooling points throughout the winter attributable to preconditioning or cold hardening at moderately cold temperatures (near 32°F). Somme (1967) in summarizing some of these results noted that diapausing insects can cold harden at relatively moderate temperatures and do not lose that hardening until after diapause termination.

Wellso (1974) found cereal leaf beetles to estivate from mid-July to mid-November in Michigan. Dickler (unpublished) collected cereal leaf beetles in the field in July and held them at 38°F throughout the winter. He found the mean supercooling point of these beetles to decrease from 4°F in July to -10°F in October. It did not change significantly from October to February (-9.7°F), but it again increased to -3°F in April.

Several authors (Atwal, 1960; Green, 1962; Sullivan, 1965; Greenbank, 1970; Sullivan and Wallace, 1972) have investigated the effects of short-term exposures at low temperatures on the supercooling point of insect populations and have found these treatments to generally lower mean supercooling points. Three investigations have compared these acute preconditioning treatments to long-term, gradual preconditioning treatments and found differing results. Sullivan and Wallace (1972), working with gypsy moths (Lymantria dispar, L.), found acute exposures of 7 days at -13°C to -22°C (8.6 to -7.6°F) to significantly lower the mean supercooling point below that resulting from 18-25 days conditioning at 0°C. However, when compared to long-term conditioning of 59-74 days at 0°C, this acute conditioning did not significantly lower the mean supercooling point. Working with the European pine sawfly [Neodiprion sertifer (Geoffroy)], Sullivan (1965) found 1 week exposures at -13 to -23°C (8.6 to -9.4°F) significantly reduced supercooling points beyond the level resulting from 8 weeks conditioning at 0°C. Similarly, Green (1962), working with the European pine shoot moth [Rhyacionia bouliana (Schiff)], found a 1-week exposure to temperatures lower than 7.5°F to cause a significant decrease in the mean supercooling points beyond that caused by two month's storage at 32°F.

Regional Differences in Supercooling Ability

Many investigators have been concerned with the gradual development of an increased level of cold tolerance in insect populations which could result in greater overwintering survival or an increased range for an insect. To date, this phenomenon has not been investigated directly, but by comparing supercooling points of populations in different regions. Several authors (Somme, 1965a,b; Taksdal, 1967; Pantyukov, 1964; Sullivan, 1965) have reported regional differences in cold tolerance where the more cold-hardy populations were from the area with the colder winter. Other authors (Sullivan and Wallace, 1972; Green 1962) have found insignificant differences in supercooling points between populations from regions differing in cold exposures. In evaluating regional differences in cold tolerance, complicating factors such as differences in preconditioning, food quality, quantity, and contaminants such as dust particles must be considered as they influence supercooling points (Salt, 1958).

There is no good evidence of regional differences in cold tolerance of the cereal leaf beetle. Dickler (unpublished) showed no consistent differences among populations collected at 3 different sites on a N-S gradient through lower Michigan. Logan (unpublished) found cereal leaf beetles from northern Michigan (Petoskey) to be consistently but insignificantly more cold tolerant than beetles from southern Michigan (Galien).

Predictive Models for Cold-Induced Mortality

There are several examples of predictive models for winter mortality in the literature. Greenbank (1970) developed a model for winter mortality of the balsam woolly aphid by placing minimum thermometers on tree trunks next to groups of aphids. At the end of the season he correlated percent mortality with the minimum temperature of the season. This simple linear regression model described with reasonable accuracy ($r^2 = .72$) the relationship between minimum temperature and winter mortality.

Green (1962) and Sullivan (1965) developed models to predict mortality of European pine shoot moth larvae and the European pine sawfly, respectively. Both models were based on supercooling point determinations and consisted of regressions of cumulative percent mortality on decreasing temperatures. Allowances were made in both models for changes in cold-hardiness throughout the season and both gave remarkably accurate predictions of mortality based on the lowest winter temperature and the extent of cold-hardening of the insects at that time.

Since all 3 of these insects overwinter in highly exposed locations (on the bark of firs, in new pine needles, and in buds, respectively), it seems likely that their cold exposure closely approximates that of the air temperature both in extent and duration. Thus, for relatively extreme exposures of relatively short duration, a model based on supercooling points may be adequate. Neither of the latter two models account for the mortality of those insects beneath snow cover, however, and it is questionable whether a supercooling point model could be accurate in predicting mortality from the milder and longer exposures in protected habitats.

Several authors have considered the aspects of time and temperature in developing models for mortality from cold exposure. Raske (1975) gave forest tent caterpillar larvae exposures to 6 temperatures of 3 durations and developed multiple regression models expressing survival as a function of time and temperature. The models he presented for pharate larvae (fully developed 1st instars within eggs) and for unfed 1st instars gave r^2 values of .721 and .884, respectively. He used these models to conclude that low temperature exposures in the field cannot account for the high spring mortality occasionally observed in Alberta.

Salt (1961) felt that under ideal conditions, freezing of insects is a probabilistic event and, hence, the same percent of a surviving population should freeze in one time unit as another. Thus, the compound interest formula $Y(t) = Y(o)e^{-rt}$ should describe survival at one temperature as a function of time. A consistent departure from this model (Salt, 1950, 1966) led to a conclusion that differences among individuals was a significant factor in determining mortality.

Chaing _et al_. (1962) held adult Drosophila at various temperatures for exposures of various durations and found a sigmoid relationship between mortality and exposure time at a constant temperature. They pointed out the similarity of this response to that of insects exposed to insecticides, yet found their results at variance with those of Salt (1950) who used a logarithmic curve to describe the same response. Actually, in a subsequent paper, Salt (1958) acknowledged a sigmoid relationship between percent mortality and the logarithm of time and he indicated that a log-probit transformation (common to insecticide tests) linearized this relationship.

Salt (1966) suggested developing a model for mortality due to chronic cold exposures by holding insects at various low temperatures and determining mortality as a function of time. Field temperatures are, of course, not constant so Salt suggested dividing daily exposures into a chronological sequence of constant temperature exposures. During each of these exposure periods, mortality is determined from the time-mortality curve for the temperature during the period.

This algorithm requires time-mortality curves for many temperatures to reduce the errors due to approximating field temperatures. Salt approached this problem by determining the relationship between temperature and time required for 50% mortality (LT50). By plotting the logarithms of LT50 values vs. temperatures, he developed a linear relationship between these variables allowing the continuous expression of LT50 as a function of temperature.

Salt did not complete this model by including mortality levels other than 50%, nor did he discuss the validity of his implicit assumption of additivity of sequential exposures. He did clearly show a method for developing a predictive model for mortality due to exposures of various temperatures and durations and he further showed that supercooling points are readily derived from such a model.

Methods

Temperature Control

In order to study the effects of long-term exposures at various temperatures on cereal leaf beetle adults, a series of 8 constant temperature water baths were set up in a walk-in freezer using aquarium heaters and motorized stirrers for temperature control. Standard 10-quart plastic pails were placed in 15" high round trash cans and the air space was filled with vermiculite. The pails were filled with a mixture of water and ethylene glycol and were fitted with plexiglass covers with holes for heaters, stirrers, thermocouples, and tubes of beetles. A series of baths was set up at temperatures ranging from -5^{o}F to 30^{o}F at 5-degree intervals using aquarium heaters of 25 watts for temperatures of 20^{o}F and less and 50 watts for temperatures over 20^{o}F. Temperatures were monitored by placing a thermocouple from a recording potentiometer in each bath.

Temperatures within the baths were reasonably constant despite the fact that the air temperature in the freezer fluctuated in a 8^{o}F cycle every 20 minutes and defrosted once/day. During a typical 48-hour period the average maximum fluctuation for all baths was $\pm .52^{o}$F (range = $\pm .35^{o}$ to $\pm .75^{o}$F). Since the maximum deviations occurred during the defrost cycle, a more informative statistic on the consistency of temperature control is the standard deviation from the mean temperature. These standard deviations averaged $.36^{o}$ among the 8 baths during a 12-hour period (range = $.27^{o}$ to $.47^{o}$F). With rapid stirring, the temperature distribution within the baths was constant to within $\pm .1^{o}$F at all depths below 1" from the surface.

The mean temperatures within the baths were found to drift in time (generally decreasing) and whenever a perceptible drift from the desired mean was observed (about $.2^{o}$F) the heater control was adjusted. The average time between such adjustments during a 4-month period was 22.4 days (SD = 14.1).

Cereal Leaf Beetle Treatment

The cereal leaf beetles used in these tests were collected when newly emerged in early July near Galien, Michigan. They were fed in the lab on barley seedlings until estivation, then stored at $40^{o}F$ (100% R.H.). Beetles taken from storage were warmed to $70^{o}F$ for about 2 hours during preparation and then refrigerated at $40^{o}F$ again for an average of 3 days until tests were run. Twenty CLB's were individually placed in 12-inch long 9 mm. glass tubes where they were separated from the glass and from each other by a capsule of filter paper. Each tube was furnished with a few drops of water at the bottom before sealing the bottom with a cork and the top with a cotton plug.

In setting up the tests, tubes were dropped in the baths and periodically samples of 3 tubes (60 CLB's) were removed and held at $70^{o}F$ for 24 hours before counting mortality. The cooling rate of the beetles in the tubes was very rapid (about 40^{o}/min.) and, hence, the cooling period is considered a negligible part of the exposure periods which varied from 1 hour to 15 weeks. In counting mortality, those beetles incapable of coordinated movement when on their feet were included among the dead.

Results

Mortality From Constant Exposures--Preliminary Model Development

Table B1 lists the results of a test set up on Jan. 31, 1974* which served as a data set for the development of a preliminary model. These data show that as temperature decreases below $25^{o}F$, the time required for

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*The -5^{o} results are from a test conducted on December 17, 1974.

Table B1. Mortality levels resulting from exposures of various temperatures and durations.

-5°F		0°		5°		10°	
Hours	% Mortality	Hours	% Mortality	Hours	% Mortality	Hours	% Mortality
1	18.3	1	11.9	1	1.7	1	3.4
2	27.1	6	8.3	20	18.3	100	21.7
3	55.0	10	15.0	32	31.7	174	49.2
4	67.5	14	56.7	48	21.7	240	61.7
5	83.7	15	44.1	62	33.9	308	87.9
		18	60.0	88	65.0	360	94.9
		22	60.0	112	48.3	431	100
		26	78.3	120	100		
		32	89.0	140	81.7		
		40	100	160	88.3		
				188	100		

15°F		20°		25°		30°	
Hours	% Mortality	Hours	% Mortality	Hours	% Mortality	Hours	% Mortality
240	5.0	360	12.1	360	11.7	120	3.3
360	23.3	744	25.0	744	20.3	360	28.3
480	42.4	960	34.1	960	28.8	744	10.0
744	75.0	1320	81.4	1320	37.3	960	30.0
984	98.3	1800	91.3	1800	73.3	1320	30.0
		2160	100	2160	59.3	1800	72.9
				2496	80.0	2160	72.9
						2496	88.3

mortality greatly decreases. When percent mortality is plotted against time at a temperature, a sigmoid curve results and, hence, probit regression lines were fit to each of the data sets in Table B1 (average r^2 = .934, SD = .0508). The resulting curves (Fig. B1) were found to have an average intercept of 3.8% (range = .3 to 8.2%) mortality from 0 hours exposure. This initial mortality was verified by measuring the mortality due to handling alone without any cold exposure, and it was found that of 180 beetles checked just prior to exposure, 6 had already died (3.3%).

To develop the relationship between temperatures and mortality rates, the equations fit to the data in Table B1 were solved for the number of hours required for mortality levels of 10 to 90%. The resulting values (Table B2) provided a standardized method of comparing temperature treatments.

It can be seen in both Fig. B1 and Table B2 that the results at 25°F and 30°F are quite similar and beetles do not survive longer at 30° than at 25°. There is, however, a large difference in survival times between 20° and 25°. This may be interpreted as demonstrating that 25°F is near the upper threshold for low temperature-induced mortality.

In Fig. B2, the times required for 50% mortality (LT50) values from Table B2 are plotted against temperatures on the upper X-axis and degrees below 25°F on the lower X-axis. This transformation of 25-T allows the development of a model based on degree hours below a somewhat arbitrary threshold of 25°F. A linearized transformation of this graph is shown in Fig. B3 where the logarithm of the Y-axis is plotted against the X-axis squared. A linear regression equation $Y = 7.088 - .0067425X$ was fit to the transformed points in Fig. B3 with an r^2 of .996. This equation gives

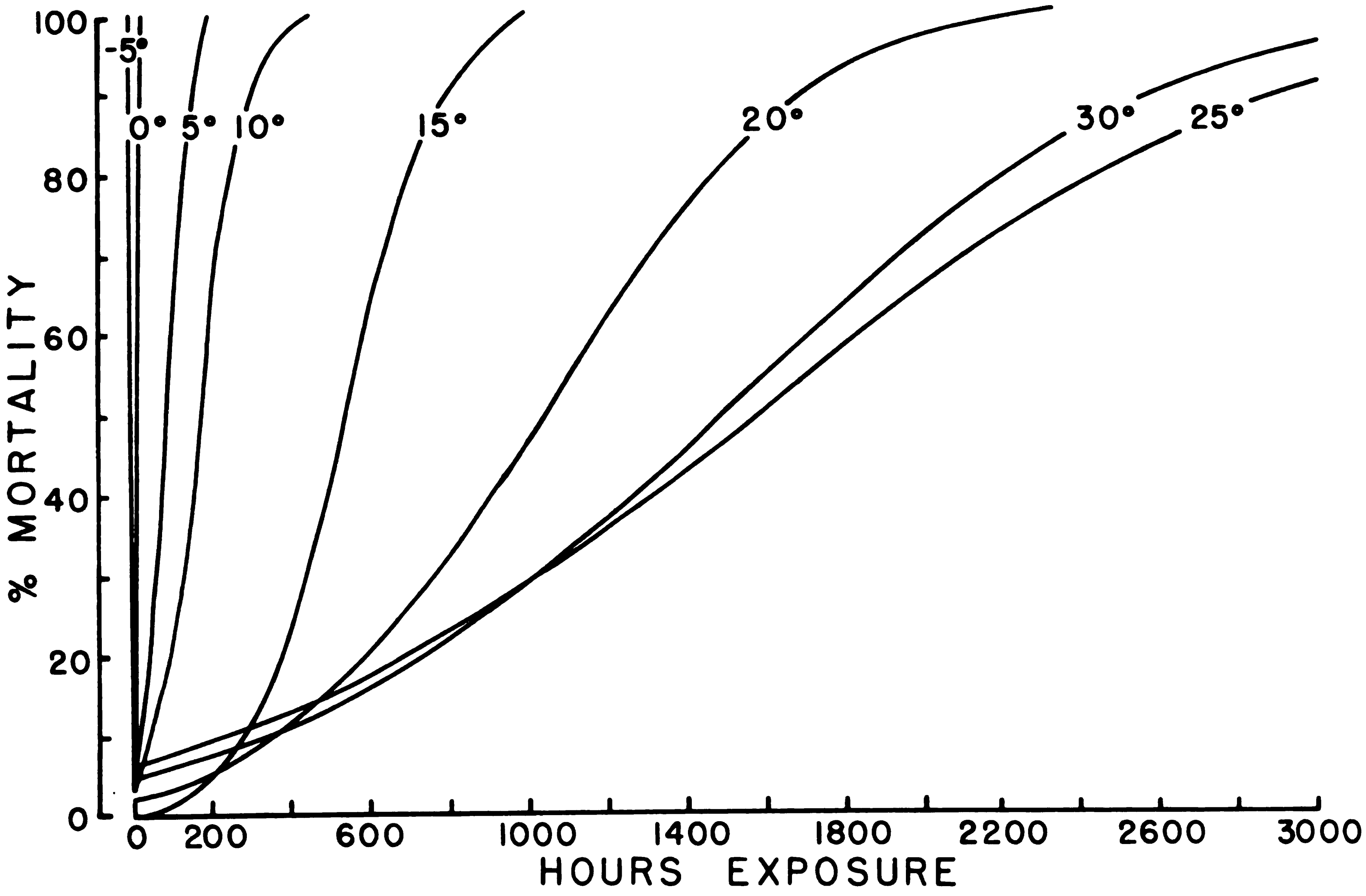


Fig. B1. Mortality levels resulting from continuous exposures of CLB's to various temperatures. (Neg. 752244-24)

Table B2. Hours exposure required for various mortality levels at various temperatures.

%	Temperature (°F)							
Mortality	-5	0	5	10	15	20	25	30
10	.33	2.92	13.47	53.02	286.86	365.61	220.36	366.01
20	1.24	7.85	39.22	99.48	375.49	596.42	691.16	753.97
30	1.90	11.43	57.94	133.28	439.94	764.28	1033.57	1036.13
40	2.46	14.46	73.74	161.79	494.33	905.91	1322.47	1274.21
50	2.98	17.26	88.37	188.19	544.68	1037.06	1589.97	1497.64
60	3.50	20.06	102.99	214.59	595.03	1168.20	1857.47	1715.08
70	4.05	23.08	118.79	243.11	649.42	1309.83	2146.38	1953.15
80	4.72	26.67	137.51	276.90	713.87	1477.69	2499.48	2235.31
90	5.62	31.60	163.26	323.36	802.50	1708.50	2959.59	2623.28

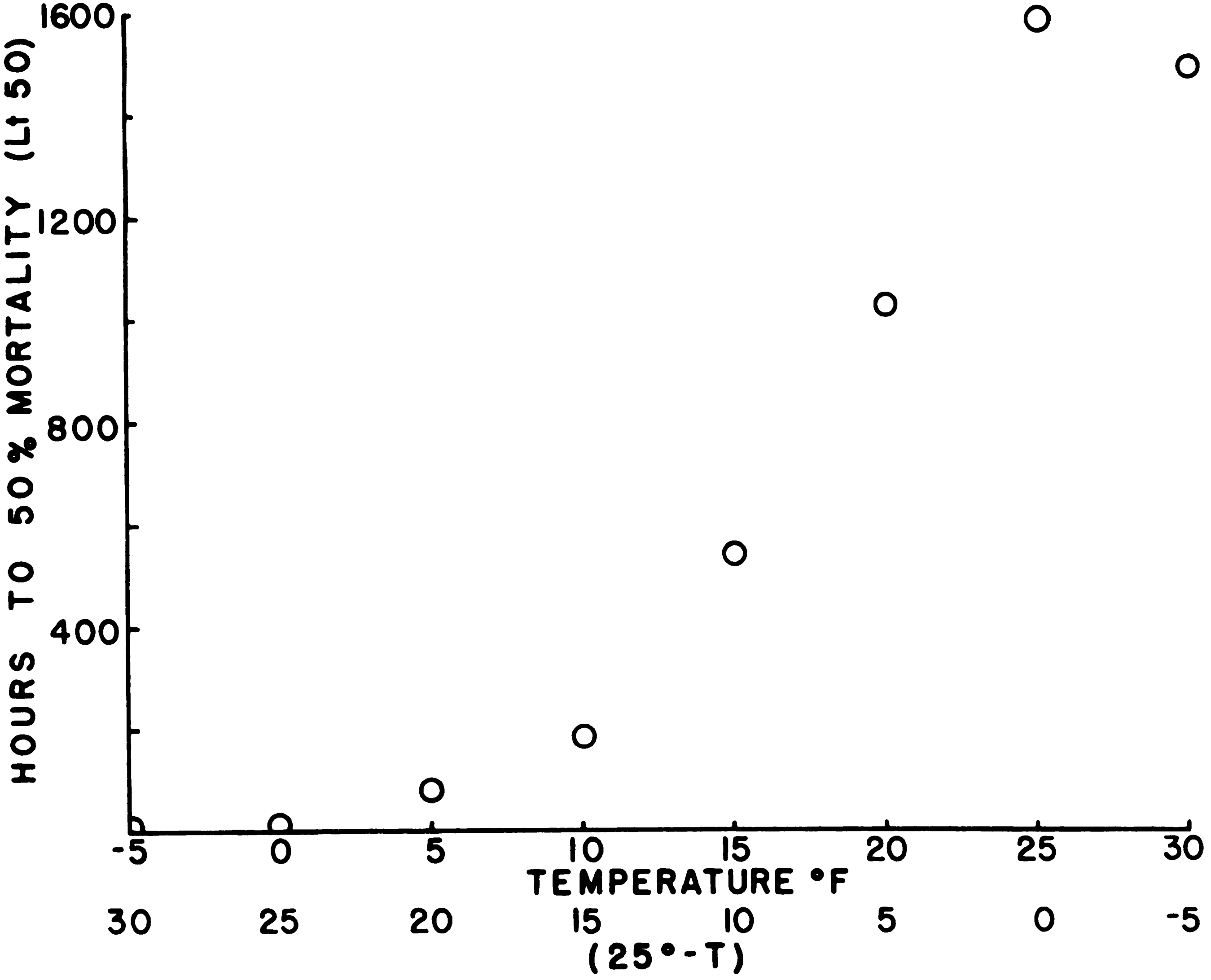


Fig. B2. Hours exposure required for 50% mortality (LT50) at various temperatures. (Neg. 752244-1)

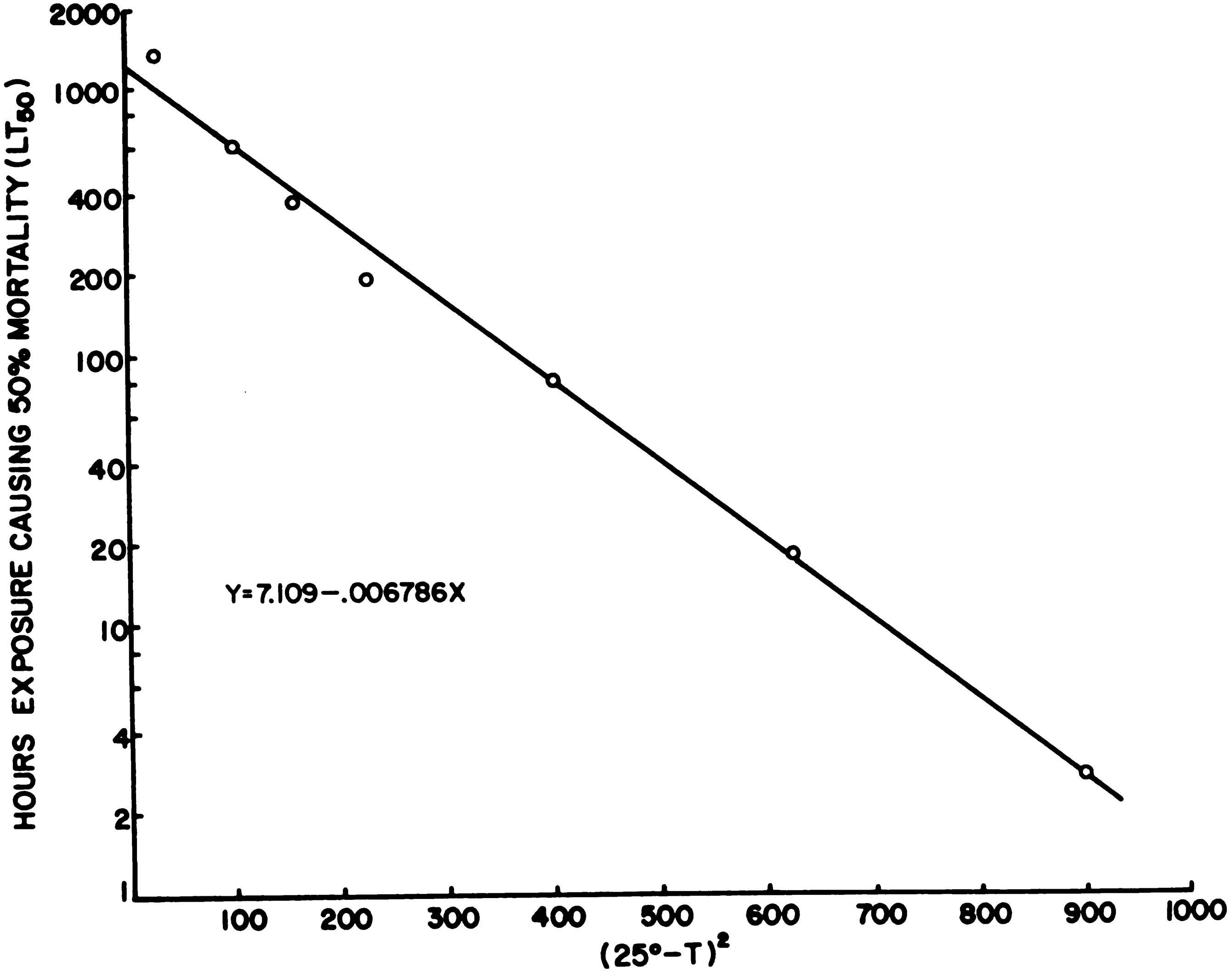


Fig. B3. A linearized relationship between LT50 and temperature where the y-axis has a logarithmic scale and the x-axis is converted to $(25^0 - \text{Temperature})^2$. (Neg. 752244-2) all the time-temperature combinations resulting in 50% mortality. The slope of this line shows the rate of loss of effectiveness of increasing temperatures in causing mortality of the CLB and, thus, gives the relationship needed to combine time and temperature into a standardized dose of cold exposure (called exposure level). Exposure level is thus calculated by the equation:

$$El = t \cdot e^{.0067425\ T^2} \tag{1}$$

where El = exposure level; t = hours exposure; and T = 25^{o} - temperature (in ^{o}F).

Given equation 1 relating exposure level to time and temperature, it is necessary to develop the relationship between exposure level and mortality. This was accomplished in Table B3 by solving equation 1 for the time-temperature combinations in Table B2. The average exposure levels corresponding to mortality levels of 10 to 90% in Table B3 were plotted in Fig. B4 showing a sigmoid relationship between exposure level and mortality. By transforming the Y-axis to probits, the following linear regression equation was fit to the transformed points (r^2 = .998):

$$M = .001376\ El + 3.328 \tag{2}$$

where M = probits mortality; El = exposure level. The curve in Fig. B4 results from solving equation 2 for percent mortality. Equations 1 and 2 together form a model for predicting mortality due to constant exposures of various durations at various temperatures.

To determine how well this model fit the original data set on which it was based, the results in Table B1 were compared to the model predictions for similar treatments. A linear regression analysis of the observed and predicted mortalities gave a slope of .963 and an r^2 of .859. This slope is very close to the 1-to-1 ratio of a perfect prediction, and the

Table B3. Exposure levels corresponding to the time-temperature treatments in Table B2.

%	Temperature (^oF)								
Mortality	-5	0	5	10	15	20	25	$\overline{X}$	S.D.
10	142.54	197.48	199.83	241.71	562.25	431.42	220.36	285.08	152.52
20	535.60	530.90	581.84	453.51	735.96	703.42	691.16	604.63	106.50
30	820.67	773.01	859.56	607.60	862.28	901.85	1033.57	836.93	129.73
40	1062.55	977.93	1093.95	737.57	968.89	1068.97	1322.47	1033.19	175.16
50	1287.16	1167.29	1310.99	857.93	1067.68	1223.73	1589.97	1214.96	226.05
60	1511.76	1356.66	1527.89	978.28	1166.26	1378.48	1857.47	1396.69	280.73
70	1749.32	1560.90	1762.28	1108.30	1272.86	1545.60	2146.38	1592.23	341.57
80	2038.72	1803.69	2040.00	1262.34	1399.19	1743.67	2499.48	1826.73	418.68
90	2427.46	2137.11	2422.01	1474.14	1572.90	2016.03	2959.59	2144.18	518.67

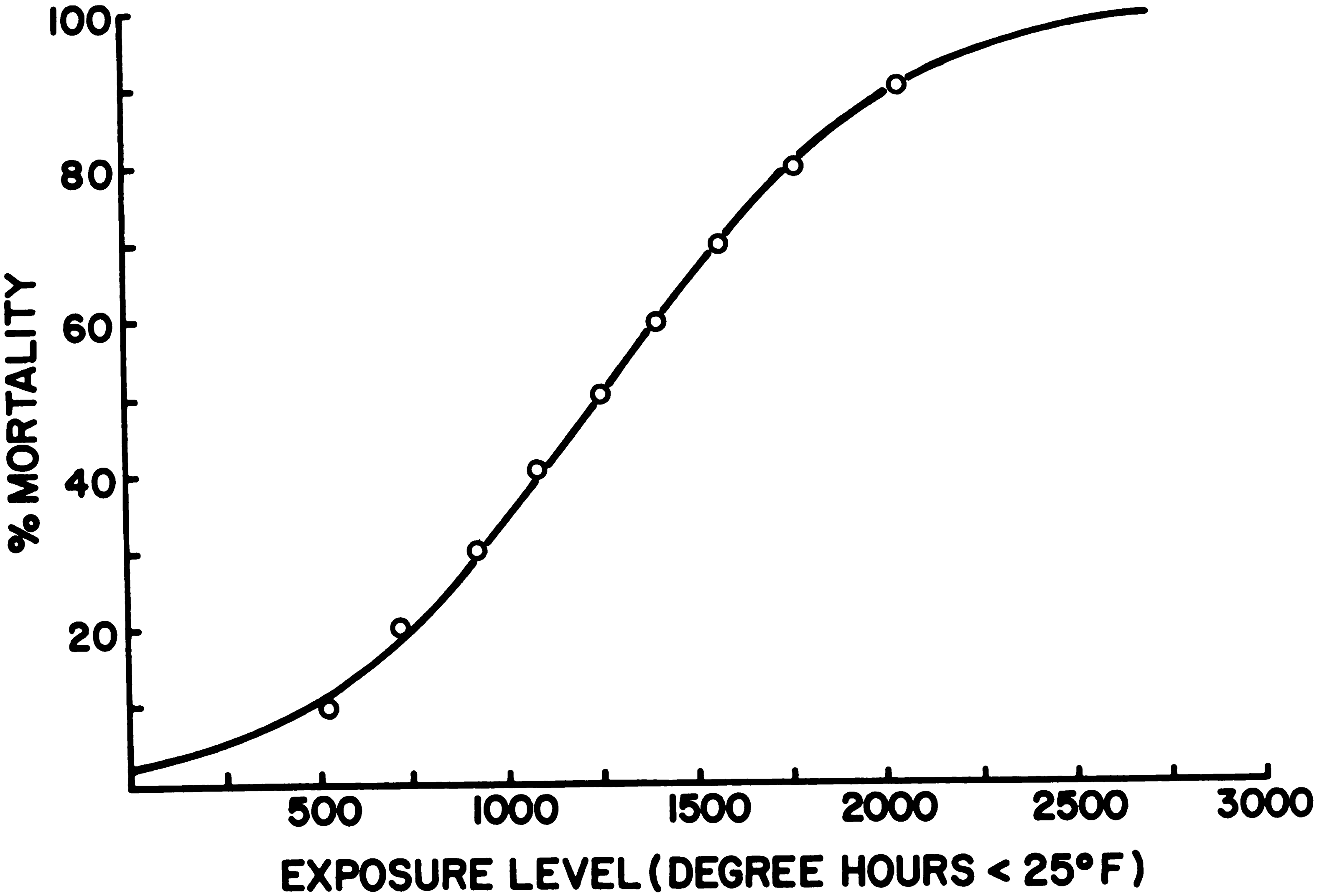


Fig. B4. The relationship between exposure level and mortality. (Neg. 752244-11)

r^2 indicates that the model explains 85.9% of the variation associated with all the observations in Table B1. The model is more accurate at the low temperatures and a similar regression of observed and predicted mortality levels for -5^0 and 0^0 gives r^2 values of .985 and .932, respectively. If the overall coefficient of determination (r^2) of .859 is compared to the average r^2 of .934 for the individual probit lines in Fig. B1, it can be seen that the inclusion of temperature effects in the model resulted in a loss of .075 in the r^2.

Seasonal Change in Cold Tolerance

As previously discussed, Dickler (unpublished) found the cold tolerance of adult cereal leaf beetles remained relatively constant from October to February, but decreased considerably by April. To further investigate this phenomenon, adult CLB's were given cold exposures (as described in the previous section) at various times during the winter. The results of these exposures are presented in Table B4 along with predicted results from the model described previously. The LT50 values were calculated by fitting a probit regression line to the results of each test and solving the equations for the exposure time causing 50% mortality.

It is apparent from Table B4 that there are no large or consistent differences between the mid-December and the early February tests. By mid-March, however, cold tolerance decreased considerably as indicated by the smaller LT50 values at both temperatures. These results seem consistent with Dickler's observations that cold tolerance remains approximately constant from October until February, but decreases by mid-March. In light of this fact and the smaller sample size of the test at -5^0 run on February 7, 1974, the December 17, 1974, results were presented in Table B1 and used in developing the model.

Table B4. Mortality levels resulting from cold exposures at different times of the year.

Date	Hours Exposure at -5°F 1	2	3	4	5	6	LT50 (Hours)
Dec. 17, '74	16.7	27.1	70.0	75.0	86.3	--	2.73
Feb. 7, '74*	30.0	12.0	44.4	61.9	95.0	95.0	2.98
Mar. 11, '74	29.4	43.3	61.7	83.1	95.0	--	2.20
Model Prediction	14.06	31.45	54.43	75.98	90.32	97.09	2.81

Date	Hours Exposure at 0°F 1	6	10	12	14	15	18	22	24	26	30	32	40	LT50 (Hours)
Dec. 17, '74	--	--	--	--	--	35.0	48.0	36.7	60.0	--	--	74.6	--	21.73
Jan. 31, '74	11.9	8.3	15.0	--	56.7	44.1	60.0	60.0	--	78.3	--	89.0	100.0	17.27
Mar. 17, '75	--	22.7	--	64.0	--	--	86.7	--	83.3	--	95.0	--	--	10.59
Model Prediction	5.72	13.27	22.92	28.94	35.61	39.13	50.13	64.64	71.29	77.28	86.87	90.43	97.99	17.97

* Mortality estimates based on samples of approximately 20 beetles instead of 60 as in all other tests.

Preconditioning

A series of tests were conducted in February, 1975, to determine if a brief, non-lethal exposure to low temperatures caused any additional cold hardening beyond the level achieved from 6 months' storage at 40°F. In these tests, beetles in glass tubes were exposed in a low temperature bath for a period of time and then immediately transferred to a bath of an even lower temperature, and the mortality from the two exposures was determined. The time required for the beetles to change from one temperature to the other is considered a negligible part of the exposures. Table B5 shows the results of a test run on February 7, 1975, where 9 samples of 20 CLB's each were exposed to each treatment. In analyzing these results, two important factors are apparent. First, the constant exposures and controls showed mortality levels consistently higher than was predicted by the model, although only 1 of these differences was significant. This may, in part, be due to the seasonal tolerance change described in the last section.

The other observation is that all 3 exposures with preconditioning resulted in less mortality than the unconditioned counterparts. The 2 exposures with 5 days preconditioning at 15^{0} differed significantly (.01 level) from the unconditioned tests. However, the 6-hour exposure at 0^{o} did not result in a significant decrease in mortality from the subsequent exposure at -5^{o}. Since the mortality levels of the preconditioned CLB's in Table B5 include both the mortality due to the preconditioning exposure and the subsequent exposure, it must be concluded that the initial exposure was beneficial to the beetles in significantly reducing total mortality from the 2 expsoures. Thus, preconditioning can be important in determining CLB mortality from cold exposure.

Table B5. Mortality levels resulting from cold exposures with and without preconditioning. Each treatment consisted of 9 samples of 20 CLB's per sample.

	$\bar{X}$ % Mortality	S.D.	Model Prediction	Observed vs Model	Preconditioned vs Unconditioned
Conditioned Treatments					
6 hours at 0^o, 3 hours at -5^o	55.56	31.96	--	--	NS
5 days at 15^o, 3 hours at -5^o	20.50	8.54	--	--	**
5 days at 15^o, 18 hours at 0^o	26.29	14.59	--	--	**
Unconditioned Treatments					
3 hours at -5^o	61.67	16.58	54.43	NS	--
18 hours at 0^o	86.67	16.20	50.13	**	--
Controls					
5 days at 15^o	11.69	8.66	8.88	NS	--
6 hours at 0^o	22.78	15.23	13.27	NS	--
No exposure	5.56	5.27	4.72	NS	--

** Significant at the .01 level

A second series of preconditioning tests was run on February 11, 1975, to determine whether the amount of preconditioning is influenced by the time and temperature of the preconditioning. Table B6 summarizes the results of these tests, each of which consisted of 9 samples of 20 CLB's. An analysis of the results in Table B6 shows the following:

1) 5 days preconditioning at 15°F was not significantly different from 6 hours at 25°F, but it was significantly different from 6 hours at 15°F.

2) 6 hours at 25° was significantly more effective in preconditioning CLB's than 6 hours at 15°.

3) 6 hours at 25° did significantly precondition the CLB's. Thus, significant preconditioning did occur in some treatments and the extent of preconditioning was determined by the time and temperature of preconditioning.

Additional tests were run during the period of February 6 to February 11, 1975, to further quantify the relationship between time and temperature of preconditioning and the subsequent mortality resulting from a 3-hour exposure to -5°. Table B7 contains a summary of these additional tests as well as those already presented in Tables B5 and B6. These results, graphed in Fig. B5, show that there is apparently an upper level for preconditioning resulting in 20-25% mortality from the combined exposures. Additional preconditioning beyond the time needed to achieve this level resulted in greater mortality due to the increased lethality of the preconditioning treatment. Preconditioning occurs most rapidly at 25° where the maximum level is reached in 6 hours. At 15° it takes 5 days to reach about the same level. At 5°, preconditioning seems to occur at

Table B6. Mortality levels resulting from various preconditioning treatments. Each treatment consisted of 9 samples of 20 CLB's per sample. Means followed by the same letter are indistinguishable at the .01 level.

Treatment	$\bar{X}$ % Mortality	S.D.	
6 hours at 15°, 3 hours at -5°	46.67	16.39	a
6 hours at 25°, 3 hours at -5°	24.44	13.10	b
1 hour at +5°, 3 hours at -5°	47.22	17.87	a
5 days at 15°, 3 hours at -5°*	20.50	8.54	b
Unconditioned, 3 hours at -5°*	61.67	16.58	a

* From Table 5

Table B7. Mortality level resulting from various preconditioning treatments. Each sample consisted of 20 CLB's.

Preconditioning Treatments		$\bar{X}$ % Mortality	S.D.	Samples
1 hour at 5°, 3 hours at -5°		47.22	17.87	9
6 hours at 5°, 3 hours at -5°		45.00	10.95	6
24 hours at 5°, 3 hours at -5°		81.67	15.27	3
1 hour at 15°, 3 hours at -5°		50.00	22.80	6
6 hours at 15°, 3 hours at -5°		46.67	16.39	9
72 hours at 15°, 3 hours at -5°		36.67	5.77	3
120 hours at 15°, 3 hours at -5°		20.50	8.54	9
192 hours at 15°, 3 hours at -5°		33.77	14.07	3
6 hours at 25°, 3 hours at -5°		24.44	13.10	9
72 hours at 25°, 3 hours at -5°		23.33	5.77	3
120 hours at 25°, 3 hours at -5°		48.33	7.64	3
192 hours at 25°, 3 hours at -5°		53.33	20.21	3
Controls:	24 hours at 5°	21.67	5.77	3
	120 hours at 15°	11.69	8.66	9
	192 hours at 15°	21.67	7.64	3
	72 hours at 25°	6.67	7.64	3
	120 hours at 25°	23.33	11.55	3
	192 hours at 25°	26.67	18.93	3
	3 hours at -5°	61.67	16.58	9
	No exposure	5.56	5.27	9

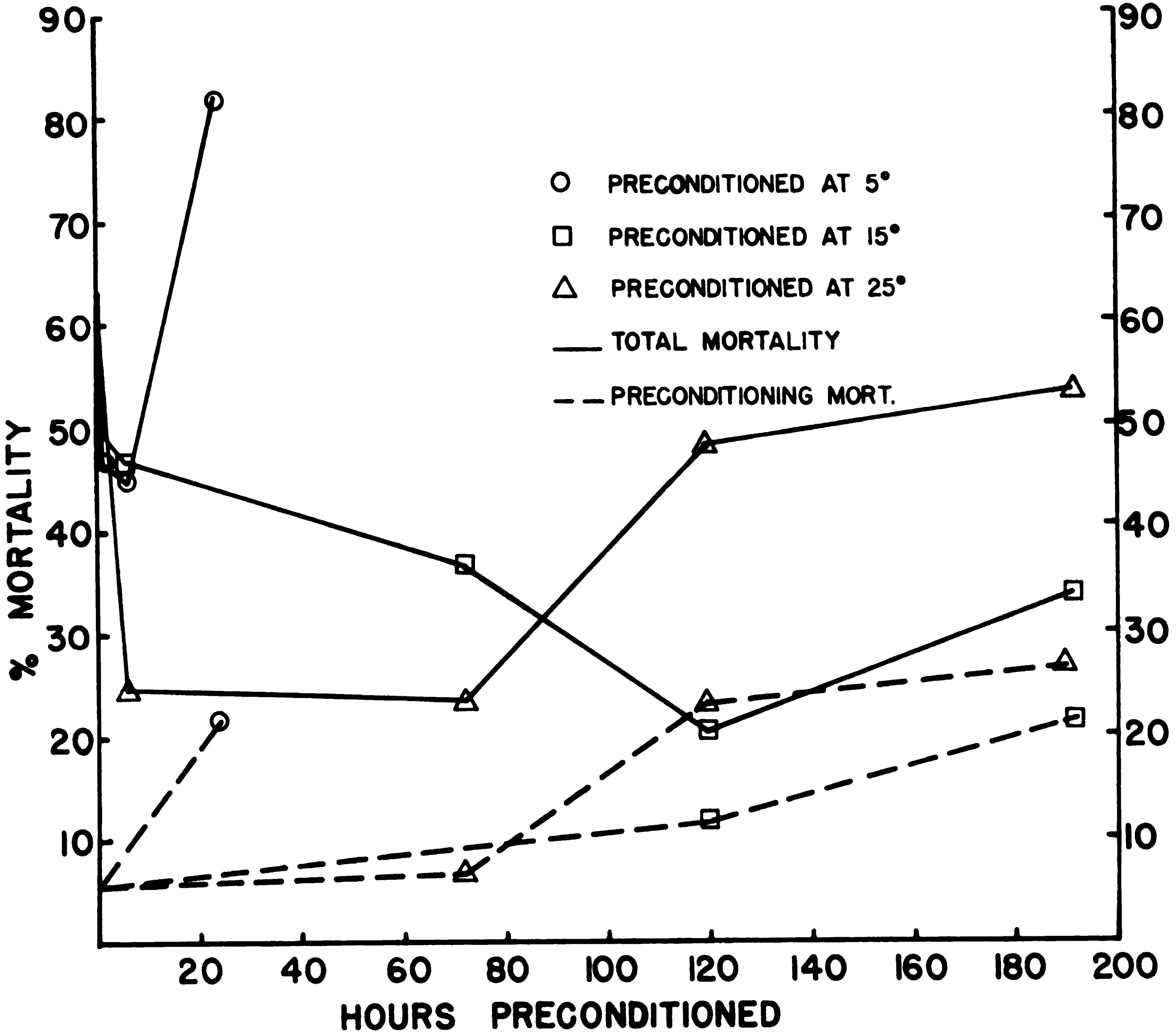


Fig. B5. Mortality resulting from an exposure of 3 hrs. at -5° following preconditioning treatments at different times and temperatures. (Neg. 752244-3) approximately the same rate as at 15^{0}; however, the lethal effect of the preconditioning period is quite apparent by 24 hours, and thus a high level of preconditioning is never reached.

Three tests were conducted in January and February of 1975 to determine how preconditioning affects the mortality levels resulting from subsequent exposures at various temperatures and of various durations. These results presented in Table B8 were fit with probit regression lines to determine the displacement caused by preconditioning. This displacement is evidenced by the increased LT50 values in the preconditioned treatments.

Recovery

In adapting the constant temperature model for field conditions, it is necessary to consider the additivity of sequential exposures. Cereal leaf beetles are able to recover from a non-lethal exposure to cold if allowed to warm up a few degrees. The clearest demonstration of this recovery is that the beetles are generally better able to tolerate two cold exposures with a warm-up period between them than they can tolerate one equivalent continuous exposure.

A comparison of preconditioning and recovery was made on February 11, 1975, when 1 group of 9 samples of 20 CLB's was given a preconditioning treatment of 6 hours at 15^{0}, and another group was given 6 hours at 25^{0} before both groups were exposed to 3 hours at -5^{0}. Equivalent recovery experiments consisted of 1.5 hours at -5^{0}, 6 hours at 15 or 25^{0}, and then another 1.5 hours at -5^{0}. In recovery experiments, as in all preconditioning experiments, the temperature of the insects was changed by moving the glass tubes containing the beetles between water baths. All

Table B8. The effects of preconditioning on mortality levels from various cold exposures.

	PRECONDITIONING TREATMENTS							
	7 days at 15°	None		12 hrs at 25°	None		12 hrs at 25°	None
Hours at -4°	% Mortality	% Mortality	Hours at -4.5°	% Mortality	% Mortality	Hours at 0°	% Mortality	% Mortality
0	11.7	11.7	2	28.3	--	12	28.3	48.3
2	26.2	53.3	3	--	43.3	19		85.0
4	23.3	43.3	4	35.6	63.3	24	51.7	83.3
6	53.3	70.0	5	31.7	78.3	34	98.3	
8	81.7	90.0	6	47.5	--	LT50	18.93 hrs	10.95 hrs
10	98.3	96.7	8	95.0	--			
LT50	4.70 hrs	3.58 hrs	LT50	4.91 hrs	3.33 hrs			

mortality counts in all experiments were made 24 hours after the completion of the exposure. The results of the comparisons of preconditioning and recovery are presented in Table B9, along with the mortality from an unconditioned group of beetles tested 5 days earlier. An analysis of these data reveals that in both cases the recovery treatments resulted in lower mortality levels than the 3 hours at -5° treatment, although only 1 of these differences is significant above the .05 level. A comparison of the recovery treatments with the equivalent preconditioning treatments reveals that at 15° the beetles recovered significantly more than they preconditioned, and at 25° there was no significant difference between the 2 treatments. Recovery, unlike preconditioning, seemed to occur at the same level at 15° and 25° (31.11 vs. 32.22 % mortality). Thus, on the basis of this experiment, it seems that CLB's can recover from interrupted exposures; and, therefore, exposures are not necessarily additive. Furthermore, recovery and preconditioning are apparently different processes in the cereal leaf beetle.

A series of experiments was conducted during January and early February of 1975 to quantify the aspect of recovery so that it might be included in the model. In these tests, CLB's were exposed to a repeated sequence of exposures and, periodically, samples of 60 beetles were removed and mortality was counted. Most of the recovery periods were intended to cause minimal mortality, even when repeated several times. Thus, the mortalities shown in Tables B10 and B11 are caused primarily by the lower temperature exposures, and the recovery periods generally make an insignificant contribution to total mortality. The recovery periods are usually quite advantageous to the beetles, as even a 15-minute re-

Table B9. A comparison of preconditioning and recovery in reducing mortality caused by a standard exposure. Means followed by the same letter are indistinguishable at the .05 level.

Treatment	$\bar{X}$	S.D.
6 hrs at 15°, 3 hrs at -5°	46.67	16.39 a
1.5 hrs at -5°, 6 hrs at 15°, 1.5 hrs at -5°	31.11	8.58 b
6 hrs at 25°, 3 hrs at -5°	24.44	13.10 b
1.5 hrs at -5°, 6 hrs at 25°, 1.5 hrs at -5°	32.22	15.63 a,b
3 hrs at -5°	61.67	16.58 a

Table B10. Recovery from exposures at -5^{o} (F).

	HOURS AT -5^{o}										
Exposure Sequence	1	2	3	4	5	6	8	9	10	Recovery (Degree-hours)	Recovery Rate (^{o}Hours/hour)
Constant exposure	18.3	27.1	55.0	67.5	83.7	--	--	--	--		
Model prediction	14.06	31.45	54.43	75.98	90.32	97.09	99.90	99.99	100.0		
1 hr -5^{o}, 15 min 0^{o}	--	20.3	--	53.3	--	78.0	98.3	--	100.0	120.76	483.04
1 hr -5^{o}, 30 min 0^{o}	--	15.0	--	57.6	--	49.2	83.3	--	85.0	283.28	566.56
1 hr -5^{o}, 1 hr 0^{o}	--	11.7	30.0	20.0	20.3	--	--	--	--		
2 hrs -5^{o}, 1 hr 0^{o}	--	49.2	--	63.3	--	66.7	91.7	--	95.0	546.31	
3 hrs -5^{o}, 1½ hrs 0^{o}	--	--	--	--	--	91.7	--	100.0	--	471.70	
1 hr -5^{o}, 30 min 5^{o}	--	35.0	--	30.5	--	43.3	66.7	61.7	--	326.62	653.2
2 hrs -5^{o}, 30 min 10^{o}	--	--	--	60.0	--	88.1	80.0	--	88.3	580.18	1160.2
2 hrs -5^{o}, 1 hr 5^{o}	--	--	--	81.4	--	86.7	98.3	--	91.7	518.03	

Table B11. Recovery from exposures at 0° (F).

	HOURS AT 0°														
Exposure Sequence	8	10	16	20	24	30	32	36	40	48	50	56	60	Recovery (Degree-hours)	Recovery Rate (°Hours/hour)
Constant exposure	--	15.0	--	--	--	--	89.0	--	100.0	--	--	--	--		
Model prediction	17.68	22.92	42.75	57.51	71.29	86.87	90.43	95.34	97.99	99.74	99.86	99.98	100.00		
8 hrs 0°, 16 hrs 15°	32.0	--	22.0	--	20.0	--	--	--	25.0	43.0	--	36.0	--	492.88	
8 hrs 0°, 16 hrs 30°	--	--	35.0	--	35.0	--	47.0	--	32.0	--	--	--	--	451.68	
10 hrs 0°, 2 hrs 5°	--	--	--	56.7	--	82.5	--	--	98.3	--	99.2	--		106.40	53.2
10 hrs 0°, 2 hrs 10°	--	--	--	35.6	--	44.8	--	--	47.5	--	75.0	--	83.3	441.80	220.9
10 hrs 0°, 2 hrs 15°	--	20.0	--	30.5	--	40.0	--	--	53.3	--	61.0	--	--	488.80	244.4
10 hrs 0°, 2 hrs 25°	--	--	--	68.3	--	83.3	--	--	66.1	--	73.3	--	53.3	593.1	
12 hrs 0°, 12 hrs 5°	--	--	--	--	50.0	--	--	78.3	--	100.0	--	--	--	503.64	
16 hrs 0°, 8 hrs 5°	--	--	--	--	--	--	98.4	--	--	94.9	--	--	--	483.21	

covery at 0^o seems to increase survival from repeated 1-hour exposures at -5^o. This is in spite of the fact that 0^o is itself a lethal temperature to CLB's given longer exposures.

The results in Tables B10 and B11 show that recovery is important, but require considerable analysis to determine to what extent and at what rates recovery occurs. For the purpose of including recovery in the model described in equations 1 and 2, the data in Tables B10 and B11 were analyzed in light of this model. It is because of recovery that the state variable "exposure level" was included in the model. This variable is linearly related to time at any temperature and is deterministically related to mortality by a probit regression equation. Thus, if after a sequence of exposures, a certain level of mortality is measured, the maximum exposure level that the beetles were exposed to can be determined from the mortality level.

In analyzing each experiment presented in Tables B10 and B11, exposure levels were calculated for each expressed mortality level. Then the additional exposure level increase during the recovery period was calculated from equation 1 and subtracted from the "observed" exposure level. After developing a regression of this corrected exposure level vs. hours at the lower temperature, the slope of this line with recovery was subtracted from a similar slope without recovery, with the difference reflecting the amount of recovery (in degree-hours).

Consider as an example the first treatment in Table B10. These beetles were given a 1-hour exposure at -5^o, a 15-minute recovery period at 0^o, then another -5^o exposure, another recovery period, etc. It is apparent that this treatment resulted in greater survival than either the experimental data or model predicted for constant exposure at -5^{o}. To determine the extent of this recovery, the analysis shown in Table B12 was performed. The "observed" exposure level was calculated from the observed mortality using equation 2. The exposure level from the recovery period was calculated from equation 1. In determining the amount of recovery, this additional exposure level resulting from the recovery period was subtracted from the "observed" exposure level and a regression equation was calculated relating exposure level to hours at -5^{o}. The slope (311.18 degree-hours/hour) was then subtracted from the corresponding slope of 431.94 degree-hours/hour which was calculated from equation 1 for constant exposure at -5^{o}. The difference between these slopes (120.76) is the amount by which exposure level decreased during the recovery period. Similar calculations were made for recovery from each of the treatments in Tables B10 and B11, and these results are presented in both tables.

When the calculated recoveries are plotted in Fig. B6 against recovery time, it appears that there is an upper level for recovery between 450 and 600 degree hours which is not exceeded by increasing the time or temperature of recovery. A comparison of the maximum recovery levels for -5^{o} and 0^{o} (considering all points between 450 and 600) reveals an insignificant difference between the means of 529.06 and 502.22 for -5^{o} and 0^{o}, respectively. Thus, the two temperatures can be grouped to give an average upper limit of 515.64 degree-hours for recovery. It is apparent from Fig. B6 that recovery rates are determined by the temperature at which, and from which, the beetles recover. With higher recovery temperatures, the beetles recover more rapidly. They also recover more rapidly from -5^{o} than from 0^{o}. Additional data would be required to properly ana-

Table B12. Determining the amount of recovery during a repeated sequence of expsoures of 1 hour at -5° and 15 minutes at 0° (F).

Hours at -5°	Observed Mortality	Observed Exposure Level	Hours at 0°	Exposure Level from 0°	Observed Exposure Level - Exposure Level from 0°
1	--	--	0	0	431.94
2	20.3	611.18	.25	16.91	594.27
4	53.3	1275.11	.75	50.72	1224.39
6	78.0	1776.00	1.25	84.54	1691.46
8	98.3	2775.33	1.75	118.36	2656.97
10	100.0	--	2.25	152.17	--

$y = 12.86 + 311.18x$ $r^2 = .98$

431.94 - 311.18 = 120.76°Hours = Recovery

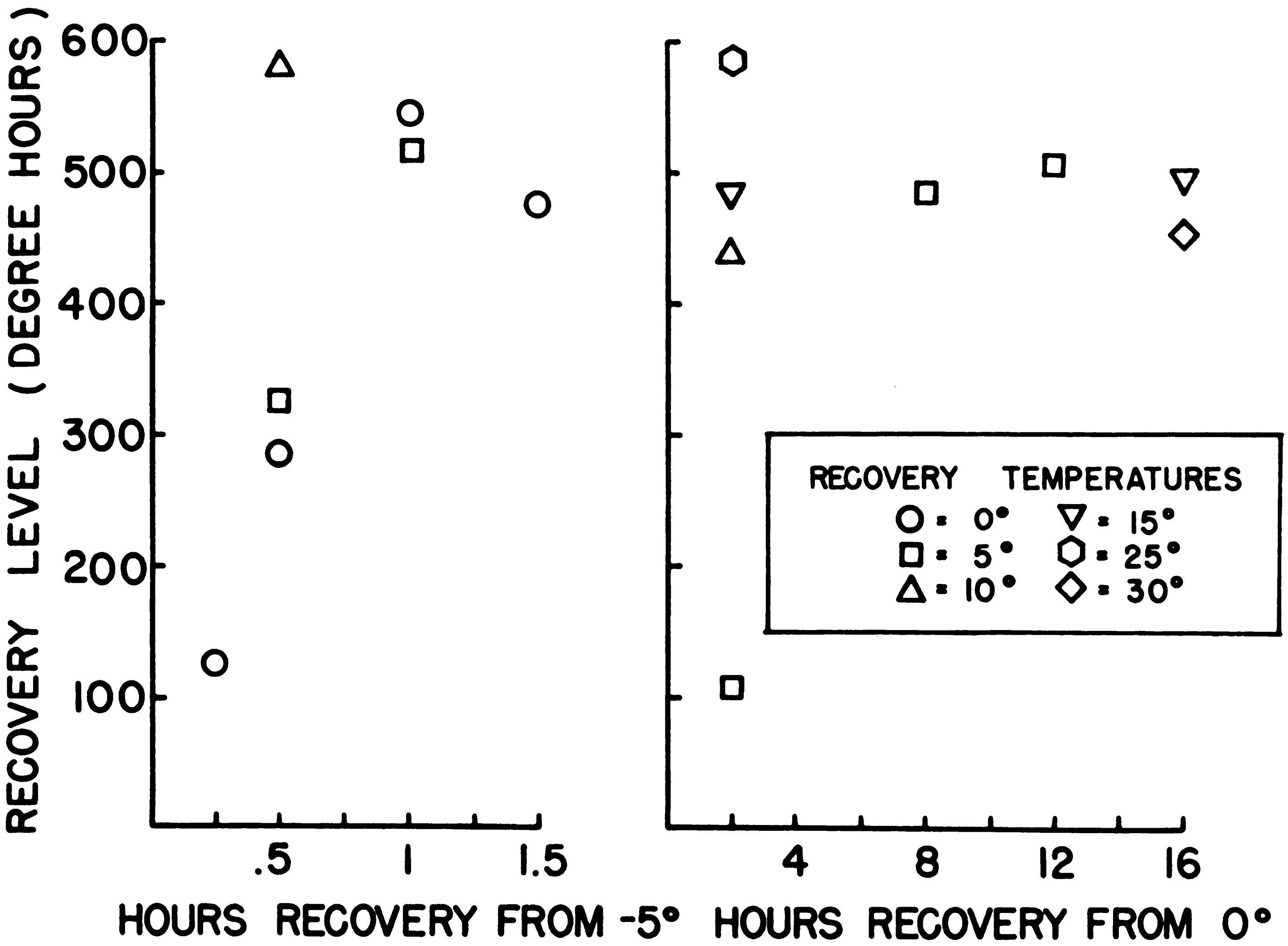


Fig. B6. Recovery levels as a function of time and temperature of recovery from initial exposures at -5 and 0°F. (Neg. 752244-8) lyze recovery rates, however, for purposes of demonstration, this analysis will be continued.

Since the maximum recovery level is reached in a relatively short time, it is only possible to calculate recovery rates from those treatments in Tables B10 and B11 which apparently did not exceed the time required to reach this level. By dividing recovery levels by recovery times, recovery rates (degree-hours/hour) were calculated and plotted in Fig. B7 vs. recovery temperatures. These plots resulted in reasonably linear relationships for recovery from both -5^{0} and 0^{0} exposures. By sight-fitting lines to these points, a slope of 77.35 was calculated for -5^{0} and 18.50 for 0^{0}. These slopes reflect the rate at which the recovery rate increases with increasing temperature and, thus, have units of degree-hours/hour/degree. By plotting these slopes as in Fig. B7 and fitting an equation to them, it is possible to determine a continuous expression for recovery rates from any temperature at any temperature. In this case two points are insufficient for an adequate functional relationship so two likely alternatives are plotted. A mathematical expression for this relationship would give recovery rate as a function of recovery temperature and initial exposure temperature. It should be noted that although the data and statistical analyses show recovery to be a real biological phenomenon which exists independent of the model of eqs. 1 and 2, the quantification of this phenomenon is entirely dependent on the model.

Discussion

The sequence of developments in this modeling effort is apparent from the amount of data available on the various aspects. Initially, it was

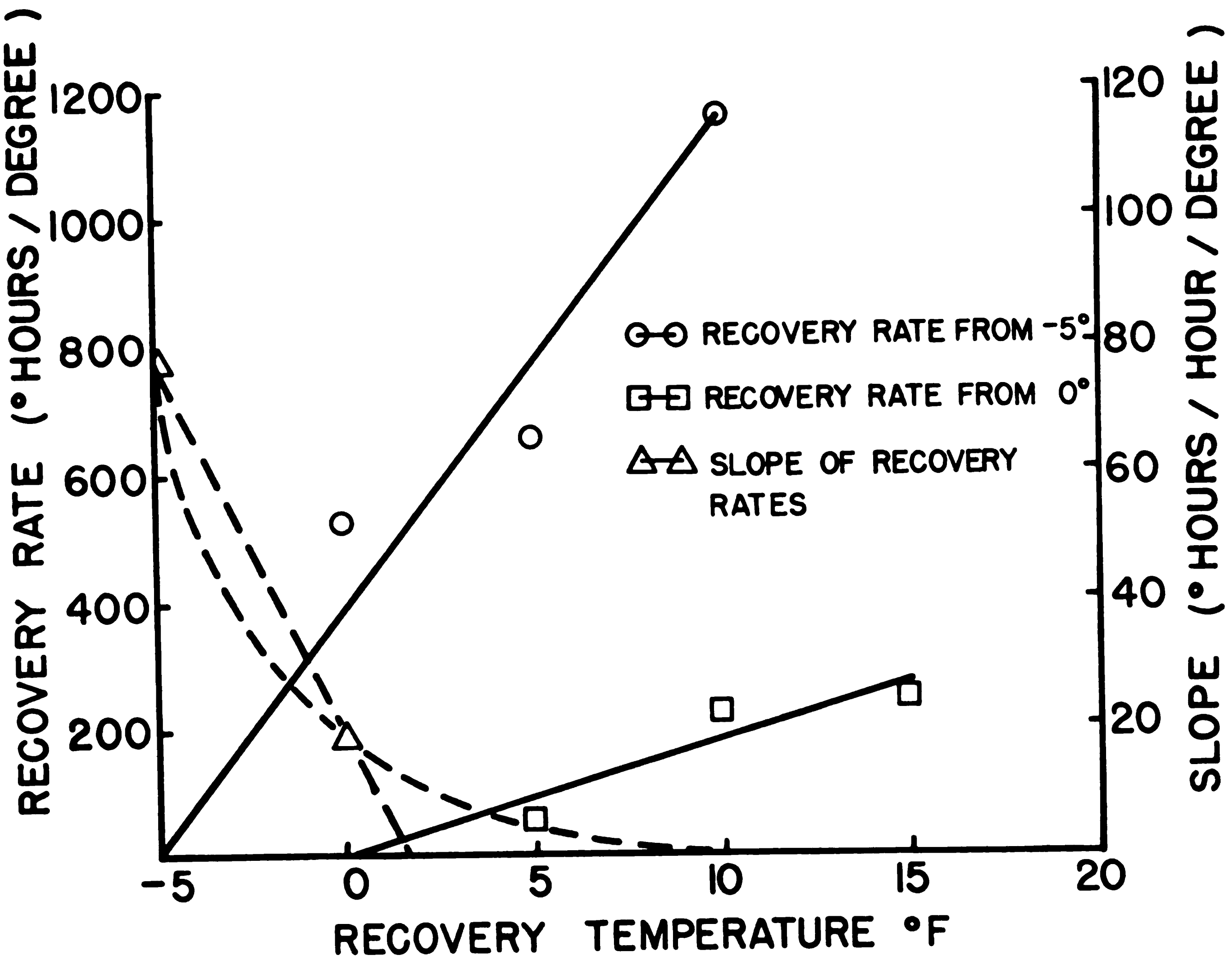


Fig. B7. Rates of recovery from -5 and 0^{0}F as a function of recovery temperature and 2 possible alternatives for a general function relating the rate of recovery per degree of temperature increase to the initial temperature exposure. (Neg. 752244-6) thought that a constant temperature model would be adequate when used with short time intervals in the field. In this light, considerable data was collected and the model of eqs. 1 and 2 was developed. It soon turned out, however, that exposures were not all additive and recovery had to be considered.

This aspect of recovery requires a great deal of data as three factors are involved: the temperature from which beetles recover, the duration of the recovery period, and the temperature of recovery. With additional data this aspect can be further quantified and a more comprehensive low temperature exposure model can be developed, however it is difficult with the present data to develop even a preliminary model for recovery as it has not been discussed elsewhere in entomological literature, and there is no theory or experience to draw upon in modeling it.

Preconditioning is an aspect which is more easily understood and modeled. It is apparent from Fig. B5 that 6 hours of preconditioning at 25° is adequate to fully precondition CLB's and, thus, it seems reasonable to assume that in the field beetles are normally fully preconditioned when exposed to temperatures below 25°. In light of the preconditioning results of Fig. B5, it appears that some of the higher temperature exposures in Table B1 resulted in preconditioning of the beetles before any appreciable mortality occurred, but the lower constant temperature exposures killed them before they preconditioned. Thus, in its present form, the model is based on both preconditioned and unconditioned exposures. Additional tests should be conducted to develop time-mortality curves for fully preconditioned CLB's at temperatures from -5° to +5°F. These could then be used to recalculate eqs. 1 and 2 so they would predict mortality for preconditioned beetles.

The seasonal change in cold tolerance is an important factor which also requires additional data. Although it is now apparent that cold tolerance is reduced after February, this needs further quantification to determine to what extent tolerance is reduced and whether a further loss occurs as the season progresses.

In the absence of these additional data, the model in its present form is limited in its utility. It is useful, however, in showing that even without preconditioning, CLB's can tolerate long exposures to moderately cold temperatures. Even at 5^o it takes over three days exposure to reach 50% mortality. With the additional survival caused by preconditioning and recovery, it is apparent that CLB's are well able to tolerate the cold exposures they experience at the soil surface. Those beetles that overwinter above ground do receive low temperature exposures in the lethal range, and the current model might prove useful in determining upper limits for mortality in that its predictions are higher than occur in nature where preconditioning and recovery occur.

APPENDIX C

THE IMPACT OF RESISTANT WHEAT ON POPULATIONS OF THE CEREAL LEAF BEETLE AND ITS PARASITES

Introduction

The cereal leaf beetle (CLB) has been observed by several authors to have preferences among its host crops of the family Gramineae. For oviposition, beetles generally prefer oats to wheat plants of the same age, and both crops are more suitable as host plants than native grasses. However, this preference is complicated by age of the plant, and if oats are as much as 10 days older than wheat, the preference is reversed (Wilson and Shade, 1966).

In Michigan, fields of oats and wheat, the primary host crops of the CLB, and acreages of native grasses, are frequently in close proximity and adult beetles distribute themselves among these three hosts. The synchrony of insect and plant development, which is determined by planting date and weather, is important in determining the distribution of the beetles. This synchrony, combined with the innate preference of the beetles, usually results in a relatively low CLB density in native grasses, a somewhat higher density in wheat (most of which is fall-seeded) and a much higher density in spring oats. An indication of the outcome of this interaction is the fact that between 1969 and 1971, an average of 26.4% of the oats in Michigan were sprayed for CLB control, while only 1.7% of the wheat was sprayed (Ruppel and Guyer, 1972; and Anonymous, 1974).

Shortly after the discovery of the CLB in North America at Galien, Michigan, in 1962, a program was developed to breed crops resistant to the beetle. To date there has been little success in developing resistant oats; however, pubescent wheat has been found highly resistant to the CLB by inhibiting oviposition, increasing developmental times, and increasing within-generation mortality.

Schillinger and Gallun (1968) and Webster et al. (1973) compared the oviposition of CLB's on resistant and susceptible spring wheats in uncaged field studies and found reductions of 98.2% and 82.4% respectively, associated with the resistance. In a similar comparison with winter wheat, Webster et al. (1973) found a 96.3% reduction in oviposition on resistant wheat (CI 8519) compared with the susceptible Genesee variety.

Of those eggs laid on the pubescent wheat, a high egg mortality was observed by Schillinger and Gallun (1968), and a high larval mortality was also observed by Schillinger and Gallun (1968) and Wellso (1973). Webster et al. (1973) did not observe a decrease in within-generation survival associated with pubescent wheat in the field. This observation was at variance with the other published work, and they attributed this discrepancy to probable contamination of their seed with a small amount of susceptible wheat, and to the decreased density of pubescence on the larger plants in their study.

On the basis of these studies, it appeared that large-scale plantings of highly resistant wheat should greatly reduce or totally eliminate CLB production in wheat. This could have several important consequences on a CLB management program. If all wheat were effectively removed from the CLB environment, it is possible that those beetles which would normally infest wheat, where they generally do not cause serious damage, would instead move into oats, thereby increasing the likelihood of spraying for their control. This could also work to the detriment of a biological control program since the wheat, which is seldom sprayed, serves as a sanctuary for CLB parasites. On the other hand, resistant wheat could work to the advantage of a biological control program if it encouraged additional oviposition in wild grasses where the larval parasites would be less subject to the deleterious effects of plowing while diapausing in the ground in CLB pupal cases. The object of this study, conducted in 1972 and 1973, was to determine the impact of large plots of resistant wheat on the cereal leaf beetle and its larval parasites. Since it was felt that the high level of resistance provided by the pubescent wheat was possibly more than was needed in a CLB management program, plantings of mixtures of resistant and susceptible wheat were also evaluated.

Methods

Plot Description

On October 16, 1972, a field at the Michigan State University Kellogg Biological Station Research Farm in Kalamazoo County, Michigan, was planted with resistant (R) and susceptible (S) wheats and mixtures of R and S as shown in Fig. C1. The resistant germplasm called Vel (alluding to its velvet-like pubescent surface), was developed and propagated at Purdue University CI 15890 where it was selected for pubescence. The susceptible wheat, Genesee (CI 12653), is a soft white winter wheat commonly grown in Michigan. All the plots were seeded at a rate of 2 bu/acre and fertilized with 250 lbs/acre of 6-24-24 at planting. The combinations of resistant

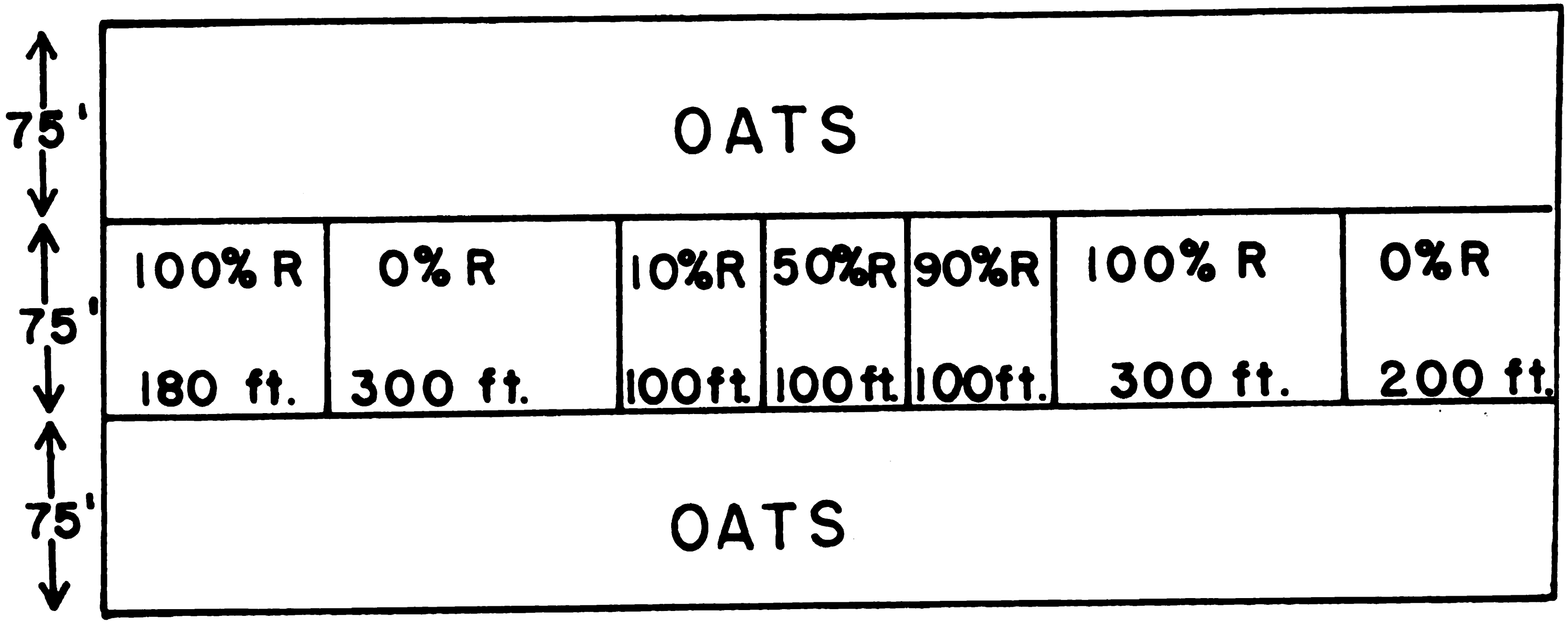


Fig. C1. Layout of the resistant wheat plots studied showing the location of the resistant (100% R) and susceptible (0% R) plots and the plots containing mixtures of R and S seed. (Neg. 752244-17) and susceptible wheats were obtained by mixing seed before planting. The plots are labeled in terms of % R. The remaining portion of the plant composition (100% - % R) reflects the susceptible complement.

Survey

As part of a weekly survey to determine the distribution of CLB larvae and adults in all the acreage in the 4-square mile area surrounding the Kellogg farm, the plots were sampled once a week by taking 4 groups of 25 sweeps with a 15" diameter sweepnet in each plot. Absolute densities of adult and larval CLB's were determined using the model of Ruesink and Haynes (1973). In all samples other than the sweepnet survey, only the innermost 5 plots were studied. At weekly intervals, 5 samples of all foliage in 2 linear feet (ca. 1 ft^2) were taken in each of the 5 plots to determine the egg density, the amount of adult CLB feeding, and the relative number of R and S plants in the plots.

Cereal Leaf Beetle Behavior

Schillinger and Gallun (1968) observed that female beetles were abnormally active on R wheat. To determine the significance and extent of this activity paired observations were made on beetles in the R and S plots on two occasions. The results in Table 6 were obtained by observing individual beetles in each plot for about 5 minutes each during which the flight distances and the time intervals between flights were recorded. The flight distances were estimated using the 6" row spacing as an index, and the time between flight was measured with a stopwatch.

Caged Studies

Since adult densities were low early in the season, adult CLB's were introduced into milliacre cages (6.6 ft. square and 6 ft. high) on May 19

to get sufficient oviposition to monitor within-generation survival and parasitism rates. One cage was set up near the middle of each of 5 plots (100% R, 90% R, 50% R, 10% R, and 0% R) and stocked with 400 CLB's. An additional cage was set up in both 100% R and 0% R and stocked with 800 CLB's. The beetles released in the cages were collected a few hours earlier about 22 miles northwest of the research plots by sweeping wheat. The wheat in the cages averaged 12 inches in height at the time the beetles were introduced, and 18 inches when the cages were removed (and CLB adults allowed to escape) 7 days later. Oviposition occurred in the cages between May 19 and 26. In Fig. C2, it can be seen that the peak egg density outside the cages was observed slightly earlier (May 14). Following oviposition and cage removal, the milliacre plots where adults were confined were each divided into 4 subplots of 4 sq. ft. each, with 1 subplot in each corner of the caged area. Egg densities were determined in each of the 28 subplots on May 28 by counting the eggs in 1 ft^2 in the center of each subplot. Eggs were not removed from the plants. Larvae were similarly counted on June 12. To measure adult CLB and parasite production, the soil surrounding each subplot was removed to a depth of 6 inches and a 1 yd^2 emergence cage, described by Gage and Haynes (1975) was placed over each of the subplots and left in place throughout the emergence period.

At the completion of CLB and parasite emergence, the soil was removed from each subplot to a depth of 4" and washed through a screen as described by Helgesen and Haynes (1972) to recover CLB pupal cells. All cells were examined and dissected to determine CLB pupal mortality and parasitism rates for the 3 larval parasites, _Tetrastichus julis_ (Walker), _Lemophagus curtus_ Townes, and _Diaparsis_ n. sp.

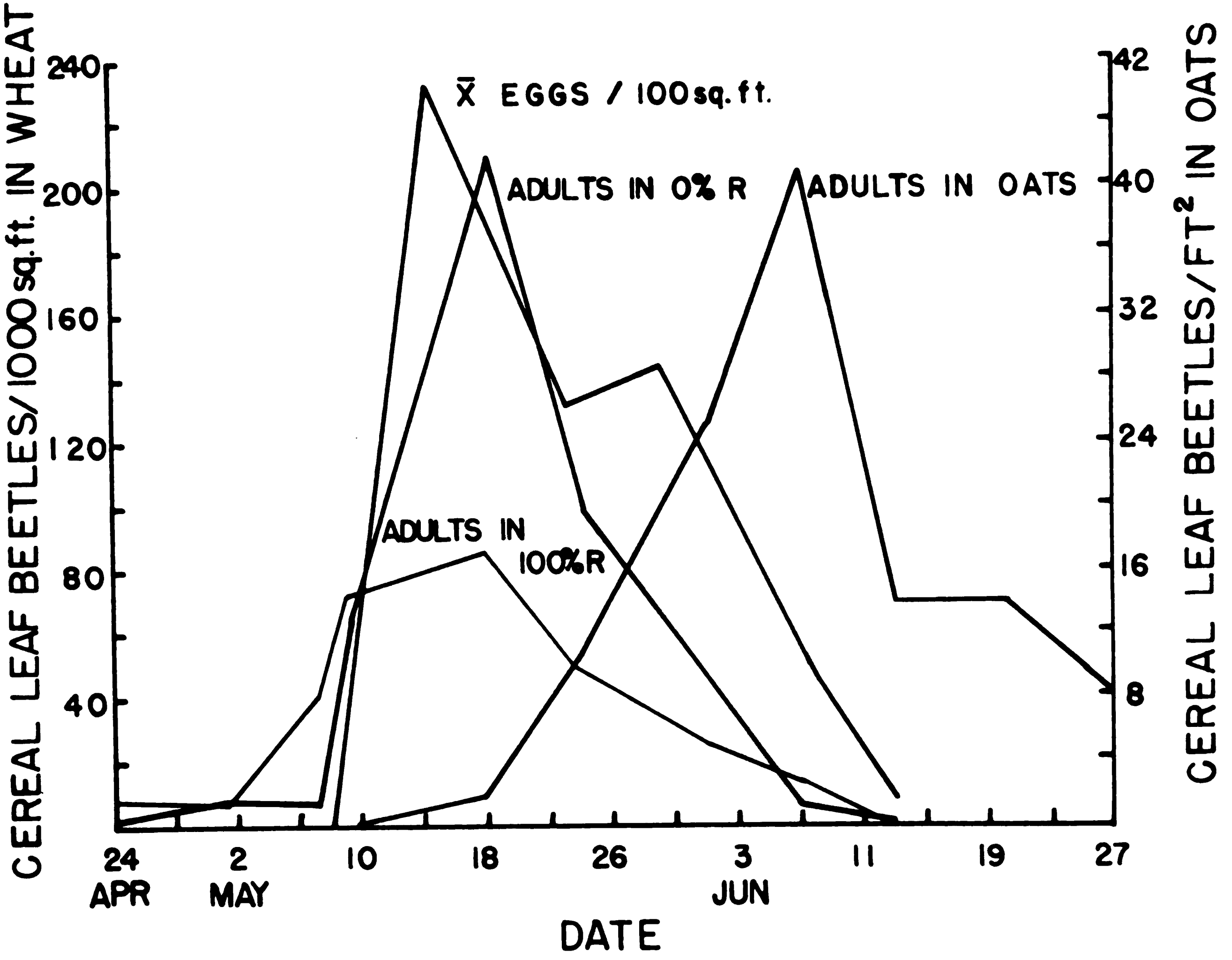


Fig. C2. Densities of CLB adults in pure resistant and pure susceptible wheat throughout the season. Densities of eggs in wheat and adults in oats are included as a reference. (Neg. 752244-5)

Results

Survey

The results of the foliage samples, Table C1, indicated ratios of R and S plants somewhat different than those attempted. Even the "pure" R and S plots had some plants of the other category, so that throughout the season the average % resistant plants in the 5 categories was 99.7: 90.8:50.3:15.1:0.3 compared to the intended ratios of 100:90:50:10:0. These imperfect ratios reflect the errors involved with mixing R and S seeds of differing sizes and germination rates. To a lesser extent, they may also result from contamination of the grain drill and impure germplasm. The stem densities and the relative proportions of R and S in the plots did not systematically change during the season.

The egg densities as determined from the foliage samples (Table C2) indicate a strong non-preference for R wheat for oviposition. Of the 140 eggs found in all the foliage samples, only 2 (1.4%) were found on the resistant wheat plants. There is also a strong negative relationship between the % of R plants in a plot and the total egg input.

The adult feeding on the wheat plants (Table C3) also indicates a non-preference for R wheat for feeding. Adult CLB's feed on leaves by eating strips about .5 mm wide. Although there were no significant differences in the average feeding hole size of adults on S and R wheat (6.51 vs. 5.93 mm long, respectively), the adults preferred to make holes in the S wheat. As a result, in all plots except 0% R the average % of total feeding on the S plants exceeds the average percentage of S plants in the plot (from Table C2).

Table C1. Stem densities and relative amount of resistant wheat in foliage samples on different dates.

	PLOTS									
	100% R		90% R		50% R		10% R		0% R	
Date Sampled	Stems /ft^2	% R	Stems /ft^2	% R	Stems /ft^2	% R	Stems /ft^2	% R	Stems /ft^2	% R
May 1	42.3	99.5	40.8	94.1	52.4	45.0	35.2	13.6	61.2	0
May 8	54.8	100	55.8	88.9	76.4	47.6	45.8	17.0	39.8	2.0
May 14	49.4	100	61.0	89.2	60.2	52.5	61.0	5.2	65.2	0
May 23	43.6	98.6	63.8	93.7	53.6	49.3	45.6	12.7	45.2	0
May 29	49.0	100	53.0	95.5	59.0	55.9	50.0	13.6	50.0	0
June 8	58.4	100	65.2	85.3	63.4	54.6	56.4	19.9	40.8	0
June 13	59.8	100	71.6	89.1	48.8	47.1	36.6	24.0	52.0	0
$\bar{X}$	51.03	99.73	58.74	90.83	59.11	50.29	47.23	15.14	50.60	0.29

Table C2. Total eggs found on susceptible and resistant plants in 5 foliage samples of 1 ft^2 in each plot.

Sample Date	Plant Type	PLOTS 100% R	 90% R	 50% R	 10% R	 0% R
May 8	S	0	0	0	0	0
	R	0	0	0	0	0
May 14	S	0	5	6	23	24
	R	0	0	0	0	0
May 23	S	0	0	4	15	12
	R	2	0	0	0	0
May 29	S	0	1	5	18	12
	R	0	0	0	0	0
June 8	S	0	2	0	9	0
	R	0	0	0	0	0
June 13	S	0	2	0	0	0
	R	0	0	0	0	0
Total		2	10	15	56	48

Table C3. Adult feeding damage (mm. removed/ft^2) on resistant and susceptible wheat in each plot.

	PLOTS									
	100% R		90% R		50% R		10% R		0% R	
Sample Date	Total Feeding	% Feeding On S	Total Feeding	% Feeding On S	Total Feeding	% Feeding On S	Total Feeding	% Feeding On S	Total Feeding	% Feeding On S
May 1	4.80	12.5	33.40	29.94	39.98	97.50	21.95	91.80	50.4	100.0
May 8	18.61	0	39.22	19.38	120.15	77.04	101.16	90.91	116.68	97.77
May 14	29.98	0	117.08	31.72	80.61	88.09	306.06	99.67	444.90	100.0
May 23	36.80	21.74	92.90	22.19	177.55	96.85	356.08	99.16	343.00	100.0
May 29	101.17	0	101.74	9.23	178.85	86.03	313.46	97.15	647.74	100.0
June 8	77.46	0	137.36	22.42	327.34	72.10	272.92	94.94	--	--
$\overline{X}$		5.71		22.48		86.27		95.61		99.55
% S Plants		.27		9.17		49.71		84.86		99.71

The densities of adult CLB's in the 100% R and 0% R plots as determined from the sweepnet survey are presented in Table C4. The densities in the 100% R and 0% R plots are plotted in Fig. C2 along with the average egg density in the 5 plots. This graph indicates that the densities in these plots of almost pure susceptible or resistant wheat did not differ appreciably until after the onset of oviposition. Samples taken at peak adult density on May 18 indicated significantly more CLB adults in the 0% R than 100% R plots (.05 level with a t-test). Subsequent samples indicated no significant differences between the plots as densities diminished with time. The average adult CLB densities in the 12 oat fields on the Gull Lake Farm are included in Fig. C2. This graph of oat densities indicates that at the time the differential adult density in the 100% R and 0% R plots developed, CLB's were beginning to move into the new oats, their preferred host. Thus, the beetles which would normally have gone into the R wheat could have instead gone into nearby oats.

The larval densities measured with the sweepnet survey are presented in Table C5. In Fig. C3, these densities are plotted against degree-days > 48^oF. The area under each of these curves was divided by 240^o days, the larval developmental time used by Tummala et al. (1975), to determine the number of individuals produced per unit area for the season (Southwood, 1966). From these numbers included in Table C5, the % reduction from the 0% R plot was calculated for each plot. The "pure" R wheat caused a reduction of 83.2% in the number of larvae produced and the mixed plots (90% R, 50% R, and 10% R) caused reductions of 74.4%, 47.5%, and 1.3%, respectively. Wellso (1973) observed an increased larval developmental time of about 10% on seedlings of a pubescent wheat (CI 8519) compared to the S variety,

Table C4. Adult cereal leaf beetle densities per 1000 square feet as determined by a sweepnet survey.

	PLOTS				
Date	100% R	90% R	50% R	10% R	0% R
April 24	8.3	8.3	12.4	0	1.7
May 1	7.6	7.6	7.6	30.6	7.6
May 7	40.0	32.0	48.0	40.0	8.0
May 9	72.5	46.4	98.6	116.0	64.0
May 18	86.0	16.8	63.0	67.2	210.0
May 24	47.5	34.8	92.8	63.8	98.6
June 1	27.5	36.0	55.4	0	34.3
June 7	14.0	0	10.4	23.4	4.7
June 13	0	0	0	0	0

Table C5. Cereal leaf beetle larval densities per 100 square feet as determined by a sweepnet survey.

		PLOTS				
Date	$^oD>48^oF$	100% R	90% R	50% R	10% R	0% R
June 1	510	1	1	0	0	0
June 7	641	27	37	78	105	140
June 13	796	15	33	62	149	116
June 20	968	3	3	0	4	9
June 27	1119	0	0	1	0	1
July 5	1292	0	0	0	0	0
# Produced/100 ft^2		28.3	43.1	88.3	166.1	168.3
% Reduction from 0% R		83.2	74.4	47.5	1.3	0

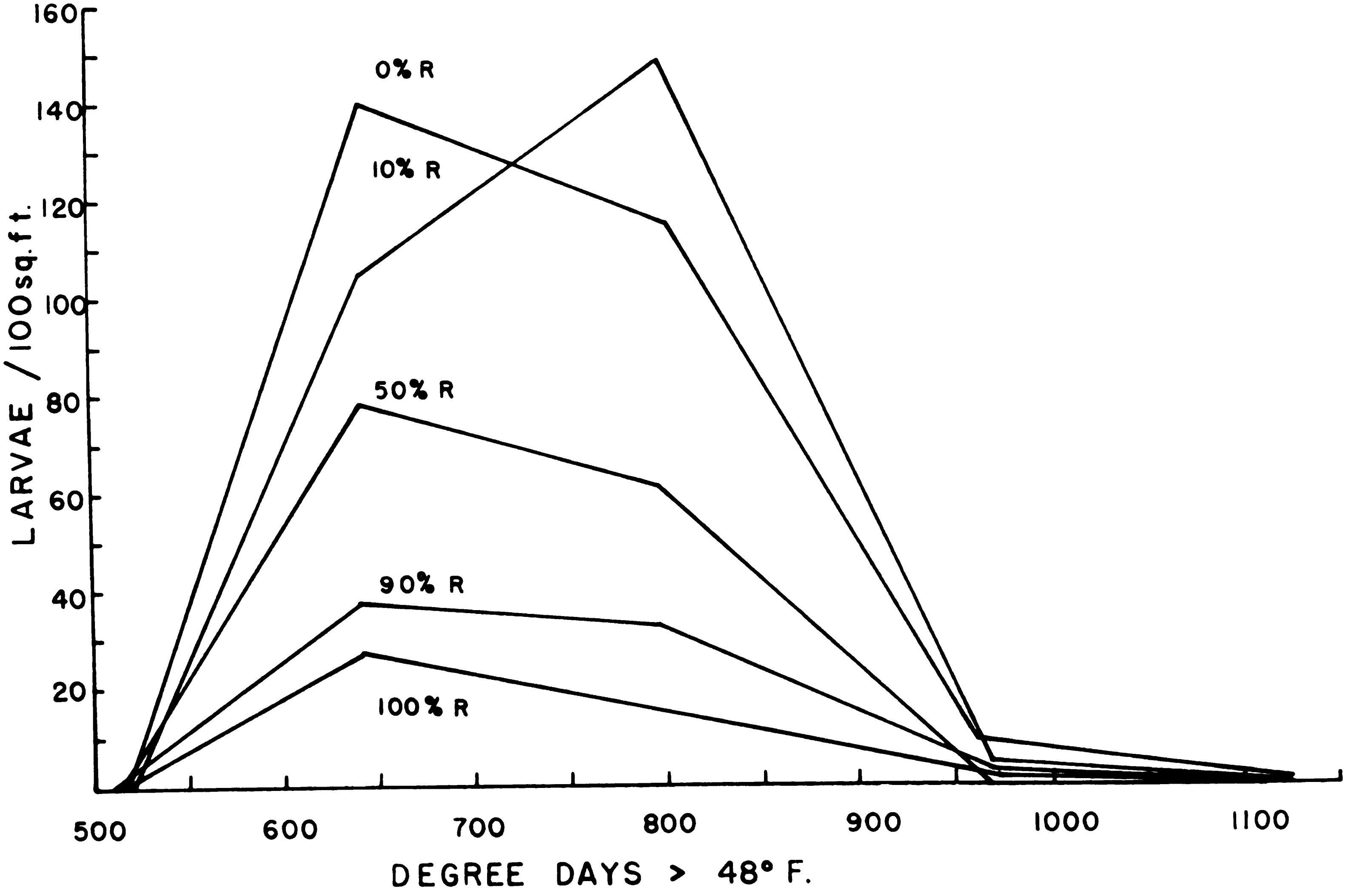


Fig. C3. Larval densities in the pure and mixed plots of resistant and susceptible wheats throughout the season. Neg. 752244-4)

Genesee. If the developmental time for larvae on R wheat is adjusted by 10% to 264 $^{o}D > 48$, the % reduction in the 100% R increases to 84.7%.

Cereal Leaf Beetle Behavior

The results of the observations on beetle movement (Table C6) indicate behavioral differences between the beetles in the 2 wheats. On May 20, the beetles were found to fly farther in the susceptible than in the resistant wheat. This difference was found to be significant ($P < .99$) with a t-test. None of the other behavioral differences between beetles in the two plots in Table C6 proved to be significant, but the beetles consistently took shorter, more frequent flights in the 100% R plot than in the 0% R plot.

From these data on flight distances and frequencies, a diffusion coefficient can be calculated as:

$$D = \frac{(\Delta \ell)^2}{4\Delta t} \qquad \text{(Pielou 1971)}$$

where ℓ = displacement in a plane (= flight distance). The diffusion coefficients in Table C6 describe the rate of spread of a population across a field as a result of random movement. Clearly, the more rapidly a population distribution is spreading out, the more rapidly individuals are getting out of a field of finite size (assuming non-reflecting boundaries). Since on May 20 the beetles had a higher diffusion rate in the resistant wheat and 6 days later it was higher in the susceptible, it appears that additional work will be required before generalizations can be made about the impact of resistant wheat on diffusion rates. On the basis of Fig. C2 it appears that this might be a dynamic relationship and differential diffusion might only be apparent during oviposition.

Table C6. Observations on flight distances and intervals between flights for cereal leaf beetles in susceptible and resistant wheat.

Date	Plot	$\overline{X}$ Distance (in.)	Observations	$\overline{X}$ Time (min.)	Observations	Diffusion Coefficient (in^2/min.)
May 20	0% R	11.21**	39	.92$^{n.s.}$	33	386.95
	100% R	5.98	51	.51	47	70.34
May 26	0% R	10.78$^{n.s.}$	9	1.09$^{n.s.}$	12	39.24
	100% R	7.50	12	.83	10	130.25

Caged Studies

By caging ovipositing adults in the 6.6 ft. square plots for 1 week, a pulse of eggs was introduced into the plots. While it is not very meaningful to compare the egg production of confined adults in different plots, certain aspects of egg distribution and within generation survival after the cages were removed are of interest.

Distribution within cages. On May 29, egg counts indicated a considerable variation in density within individual caged areas, but an average distribution that was almost equally divided among the 4 sq. ft. subplots in the corners of the milliacre caged areas (26%, 23%, 27%, and 24% in the NW, NE, SW, and SE corners, referred to as subplots 1-4 in Table C7). The non-preference for R wheat for oviposition, which was observed throughout the field (Table C2) was also indicated in the caged plots in mixed R and S wheat where 97%, 98%, and 92% of the eggs were laid on the susceptible plants in the 10% R, 50% R, and 90% R plots, respectively. This indicates that the beetles sought out the susceptible plants in the plots for oviposition and as a result deposited an average of 95.7% of the eggs on the S plants in the 3 plots.

The larval count of June 12, Table C7, indicates a shift in the distribution of beetles in the caged plots from the time of the egg count because an average of 84.7% of the larvae were found on the S wheat (100%, 79%, and 75% on the S wheat in the 10% R, 50% R, and 90% R plots, respectively), compared to 95.7% of the eggs. This redistribution could be due to higher mortality on the S plants or movement from S to R plants.

Within-generation survival. The number of adult CLB's produced in each caged subplot as determined from the emergence trap catch is presented in Table C7. These adult densities were divided by the egg densities in the same subplots to determine the within-generation survival. Helgesen and Haynes (1972) showed that CLB within-generation survival is inversely related to the log of egg density, so it is not possible to directly compare survival in plots with differing initial egg densities. Since in this test the 3 plots with mixtures of R and S plants had similar average densities of eggs [44.0, 49.5, and 43.5 in 10% R, 50% R, and 90% R, respectively (Table C7)], the within-generation survivals in these plots are directly comparable. The average survivals of .1059, .0896, and .1408 in these plots are not significantly different and show no consistent relationship between within-generation survival and relative percentage of S and R plants in the plots.

As a result of large differences in egg densities in the 0% R and 100% R caged plots ($\overline{X}$ = 53.3 and 24.9 per ft., respectively), the survival in these plots is compared by covariance analysis. In Fig. C4, a plot of survival vs. the log of initial egg density indicates that in both 100% R and 0% R plots survival decreased with increasing egg densities. The slopes of both lines differ from 0 ($P > .99$ in S and $P > .90$ in R). The slopes of the 100% R and 0% R lines are not significantly different from each other; however, the means of the covariate (egg densities) are significantly different ($P > .98$). Thus, % survival was adjusted for the effect of egg density. The adjusted means (8.33 and 12.98% survival in the 100% R and 0% R wheat plots, respectively) are not significantly different at the 5% level. In summary, there was density dependent mortality in the plots with "pure" R and "pure" S wheat; however, when corrected for differing egg densities, within-generation survival in the two wheats was

Table C7. Cereal leaf beetle egg and larval densities and total emergence trap catch in each of the 4 square-foot subplots (densities are per square-foot).

Plot	CLB's Introduced	Subplot	Eggs	Larvae	Adults	*T. julis*	CLB Adults/Egg
0% R	800	1	48	39	5.50	3.00	.1146
		2	28	19	4.25	3.50	.1518
		3	124	80	7.00	1.50	.0565
		4	44	56	5.50	1.00	.1250
	400	1	67	37	3.25	0.0	.0485
		2	44	23	6.25	3.00	.1420
		3	48	31	4.00	1.75	.0833
		4	23	18	3.50	1.75	.1522
10% R	400	1	50	37	6.25	1.25	.1250
		2	39	11	4.75	.25	.1281
		3	39	26	4.00	1.25	.1026
		4	48	19	3.25	.25	.0677
50% R	400	1	33	15	3.75	1.00	.1136
		2	67	29	3.25	1.50	.0485
		3	26	34	3.75	.50	.1442
		4	72	45	3.75	2.00	.0521
90% R	400	1	42	10	4.50	0.0	.1071
		2	62	18	4.25	.75	.0685
		3	30	11	5.25	.25	.1750
		4	40	13	8.50	.25	.2125
100% R	400	1	7	7	1.25	0.0	.1786
		2	8	10	.50	1.00	.0625
		3	7	8	1.00	1.25	.1429
		4	8	24	2.25	1.75	.2813
	800	1	62	17	1.50	.50	.0242
		2	19	6	1.25	.75	.0658
		3	39	10	2.50	.50	.0641
		4	49	13	4.50	2.00	.0918

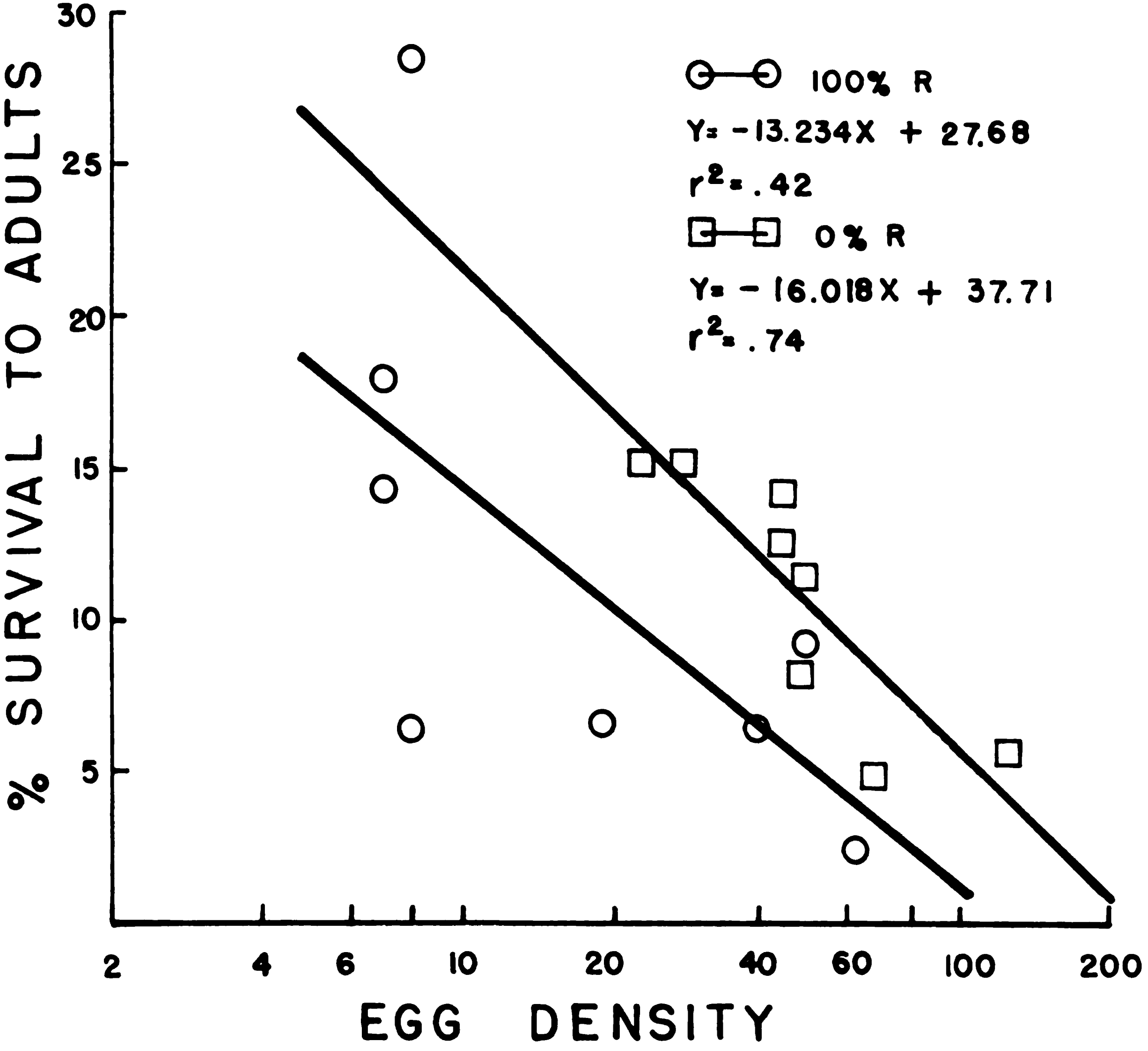


Fig. C4. Within generation survival of CLB's in pure resistant and pure susceptible wheat as a function of initial egg density. (Neg. 752244-7) similar. Since these survival rates are measured in populations of unnatural age distribution (i.e., resulting from a pulse of eggs), their utility is limited to comparisons and not pr jections for natural populations.

Tetrastichus julis. In Table C8, it can be seen that there are large differences in the number of pupal cells in the 100% R and 0% R plots ($\overline{X}$ = 13.38 and 27.75, respectively). However, there is no apparent relationship between density and percent parasitism at this range of densities, so it is possible to directly compare the % parasitism in the different plots. This comparison reveals an average parasitism of 49, 41, 48, 49, and 46% in the 100% R, 90% R, 50% R, 10% R, and 0% R plots, respectively. None of these differences are significant, so apparently the attack rate of T. julis is not affected by either pure resistant wheat or mixtures of R and S.

T. julis is a gregarious parasite with a facultative diapause (in contrast with L. curtus and Diaparsis n. sp. which are solitary with a facultative and an obligatory diapause, respectively). A comparison of the percent diapause of of T. julis reveals no significant difference between the 100% R and 0% R plots. Also, there is no difference in the number of parasite larvae per pupal cell in the 2 plots. Thus, it appears that R wheat has no direct effect on T. julis.

Other parasites. The densities of Lemophagus curtus and Diaparsis n. sp. were very low in 1973 and as a result there was a low parasitism rate by these species, as shown in Table C8. These small numbers preclude any significant analysis; however, they give no indication of an effect of R wheat on either parasite. There was an average parasitism by L. curtus

Table C8. Evaluation of cereal leaf beetle pupal cells in the 4-ft^2 subplots.

Plot	CLB's Introduced	Subplot	Total Cells	Emerged CLB's	Dead CLB's	Cells with Emerged *T. j.*	Cells with Diapausing *T. j.*	Diapausing *T. j.* Larvae	Total Cells with *T. j.*	Cells with *L. curtus*	Cells with *Diaparsis* n. sp.
0% R	800	1	28	14	2	6	5	22	11	1	0
		2	24	10	2	4	7	27	11	1	0
		3	38	27	5	11	14	47	25	2	0
		4	28	15	1	4	8	38	12	0	0
	400	1	32	17	3	4	8	29	12	0	0
		2	31	14	1	4	10	31	14	2	0
		3	21	11	3	3	3	14	6	1	0
		4	20	6	2	3	8	30	11	0	1
10% R	400	1	22	7	0	4	10	32	14	0	0
		2	29	12	1	5	11	37	16	0	0
		3	18	6	2	3	5	24	8	0	1
		4	27	17	1	5	4	24	9	0	0
50% R	400	1	16	6	1	2	4	22	6	1	0
		2	19	8	1	1	9	34	10	0	0
		3	23	11	0	4	7	27	11	1	0
		4	11	4	0	1	5	23	6	0	0
90% R	400	1	22	4	0	5	10	38	15	3	0
		2	23	16	0	2	5	23	7	0	0
		3	23	9	2	3	4	13	7	2	0
		4	26	13	1	4	6	20	10	2	0
100% R	400	1	8	4	0	0	4	18	4	0	0
		2	16	8	0	2	5	21	7	0	1
		3	7	3	1	0	3	16	3	0	0
		4	8	4	1	0	1	5	1	2	0
	800	1	18	8	1	4	3	9	7	0	2
		2	10	4	0	2	4	15	6	0	0
		3	24	7	2	8	6	26	14	1	0
		4	16	5	0	2	8	27	10	1	0

of 3.7% in the 100% R plot vs. 3.2% in the 0% R plot which is remarkably close considering the small sample size. *Diaparsis* was found to parasitize larvae in both plots--however, in numbers too small to compare.

Discussion

The results of this study on the impact of R wheat can be summarized as follows:

1. CLB's oviposit less on R wheat than S, in both pure stands and in mixtures.
2. CLB's exhibit density dependence in within-generation survival in both R and S wheat.
3. When this density dependence is taken into account, there is no difference in within-generation survival on pure R or S, or mixtures of R and S wheat.
4. The parasite *T. julis* is not directly affected by R wheat, although by reducing CLB oviposition, the R wheat seems to reduce the number of potential parasite hosts in wheat.

Thus, the primary effect of R wheat is to reduce oviposition; however, since densities are lower, within-generation survival can be higher in R than S wheat. Because the relationship between density and within-generation survival is not affected by R wheat, the model of Helgesen and Haynes (1973) can be used to predict within-generation survival in S, R, and mixtures of wheat. Also, since R wheat causes no direct effects on *T. julis*, the model of Tummala *et al.* (1975) can be applied to R, S, and mixtures of wheat.

The R wheat apparently retained as many adult CLB's as S wheat until oviposition began (Fig. C2). It appears that the beetles which left the R wheat during peak oviposition did not move to native grasses or susceptible wheat, but to nearby oats, the preferred host, which was up at that time. However, as seen in C2, the R wheat maintained a significant CLB population even during oviposition, so in a future management program, the impact of beetles moving to oats from R wheat may not be too severe. Also, a slight adjustment of the planting date could result in oats not yet being out of the ground at the time of this movement and more beetles might move to native grasses.

A large-scale release and utilization of R wheat would apparently result in a large reduction in oviposition in wheat. As a result, CLB damage in R wheat would not be significant, and numbers of CLB's produced in the wheat would be greatly reduced. During the first season, some of those beetles which would normally oviposit in S wheat might be expected to move to nearby oat fields. However, in subsequent years, the small number of CLB's produced in R wheat should not significantly affect the oat populations.

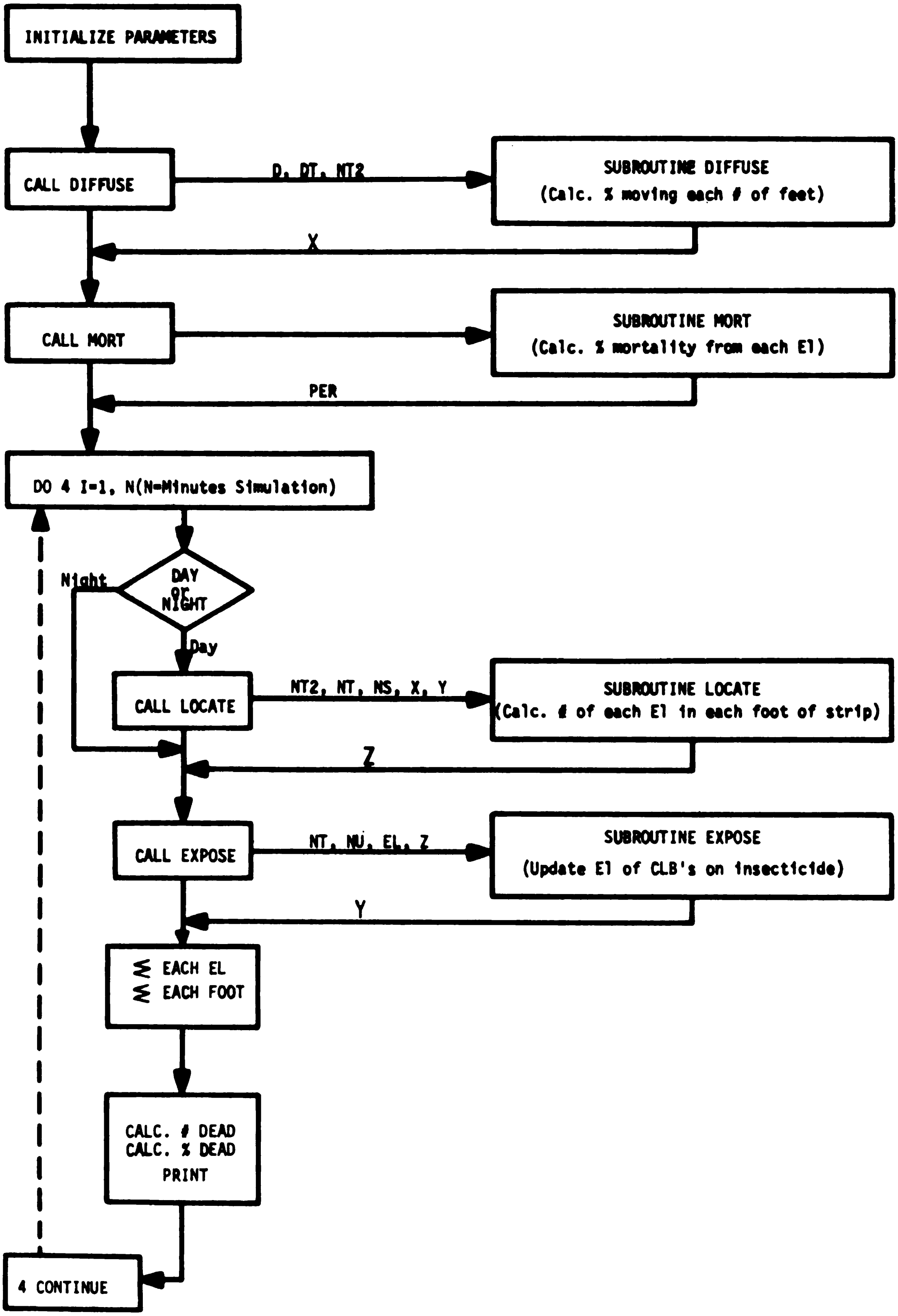
Under current planting practices the three larval parasites would be adversely affected by a large scale release of R wheat. The ovipositing beetles, denied access to the wheat, might be expected to move to native grasses, however if oats are available, as they were in this study, the beetles will most readily move to the young and highly preferred oats. As a result, a greater proportion of a regional population of CLB larvae and parasites would be found in oats where they would be subject to insecticide application. Although the temporal and spatial availability of oats is an important factor in a particular year, it appears that in general the loss of the CLB and parasite refuge in wheat would result in a greater degree of instability in the system and an increased reliance on chemical control.

The resistant wheat variety Vel probably has a higher level of resistance than would be required in most areas in a CLB management program. For a regional program, several R wheats with a range of resistance levels would be desirable, or the resistant seed can be mixed with its susceptible counterpart to achieve the optimum level of resistance.

APPENDIX D

THE STRIP SPRAY COMPUTER SIMULATION USED IN APPENDIX A

Program Strip



```
      PROGRAM STRIP (INPUT=128,OUTPUT=128)
      DIMENSION X(240),PER(200),Z(200,40),Y(200,40),TOT2(40),
     +TOT3(200)
      MIN=5000
C*****MIN=MINUTES SIMULATED
      CO=1.5
C*****CO=INITIAL CONCENTRATION
      AK=.1555
C*****AK=DECAY CONSTANT OF INSECTICIDE
      D=207.46
C*****D=DIFFUSION COEFFICIENT (IN**/MIN)
      DENS=100.
C*****DENS=INITIAL DENSITY IN UNSPRAYED PART
      DT=5.
      NS=8
C*****NS=WIDTH OF SPRAYED PART
      NU=12
C*****NU=WIDTH OF UNSPRAYED PART
      NT=NS+NU
      NT2=NT/2
      N=(MIN+.0001*MIN)/DT
      TD=DENS*NU
      DO 1 I=1,200
      DO 2 J=1,NT
      Y(I,J)=0.
 2    CONTINUE
 1    CONTINUE
      DO 3 J=1,NU
      Y(1,J)=DENS
 3    CONTINUE
      CALL DIFFUSE (D,DT,NT2,X)
      CALL MORT(PER)
      TM=0.
      TMD=0.
      DO 4 II=1,N
      TM=TM+DT
      T=TM/60.
      IF(T.GT.10..AND.T.LT.19.) GO TO 70
      IF(T.GT.34..AND.T.LT.43.) GO TO 70
      TMD=TMD+DT
      TD1=TMD/60.
      T1=TD1-(.5*(DT/60.))
      C=CO*(EXP(-AK*T1))
      EL=C*DT
      CALL LOCATE(NT2,NT,X,Y,Z)
 70   CONTINUE
      DO 5 I=1,200
      TOT3(I)=0.
 5    CONTINUE
      DO 6 J=1,NT
      TOT2(J)=0.
 6    CONTINUE
```

```
      DO 77 I=1,200
      DO 88 J=NS,NT
      Y(I,J)=0.
88     CONTINUE
77     CONTINUE
      CALL EXPOSE(NT,NU,EL,Y,Z)
      DEAD=0.
      DO 7 I=1,200
      DO 8 J=1,NT
      TOT3(I)=TOT3(I)+Y(I,J)
      TOT2(J)=TOT2(J)+Y(I,J)
8     CONTINUE
      DEAD=TOT3(I)*PER(I)+DEAD
7     CONTINUE
      PD=DEAD/TD
      A=100.-PD
      PRINT10, T,TD1,C,A,PD
4     CONTINUE
10    FORMAT(1X,2F10.5,10F10.3)
      END
      SUBROUTINE DIFFUSE(D,DT,NT2,X)
C*****COMPUTES THE NUMBER OF CLBS MOVING 1 TO NT2 FT.
C*****INITIALLY THE NO./IN. IS COMPUTED AND THEN THE NO./FT.
      DIMENSION X(240)
      S=SQRT(2*D*DT)
      M=NT2*12
      DO 1 I=1,M
      Y=I-1
      X(I)=(1/(S*2.506627))*2.71828**(-(Y*Y)/(S*S*2))
1     CONTINUE
      R=0.
      DO 2 I=1,6
      R=R+X(I)
2     CONTINUE
      X(1)=2*R-X(1)
      TOT=X(1)
      M=7
      DO 3 I=2,NT2
      R=0.
      DO 4 J=1,12
      R=R+X(M)
      M=M+1
 4     CONTINUE
       X(I)=R
       TOT=TOT+2*X(I)
 3     CONTINUE
       DO 5 I=1,NT2
       PRINT 11,I,X(I)
 11    FORMAT(1X,I5,F10.6)
 C*****C CORRECTION FOR INDIVIDUALS WHICH MOVE OUT OF THE STRIP
       X(I)=X(I)*(1./TOT)
 5     CONTINUE
```

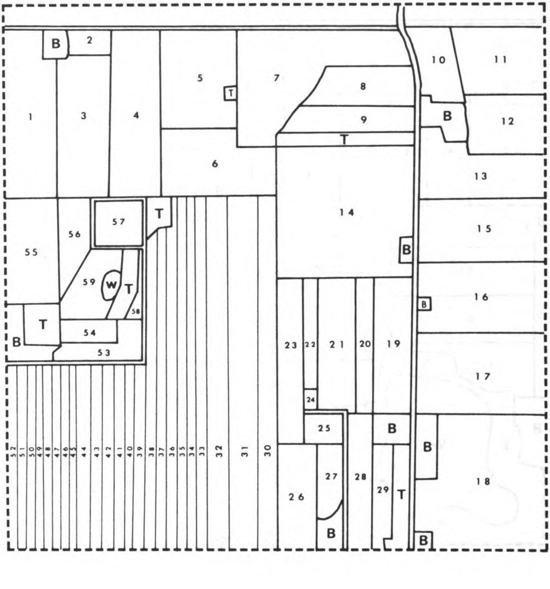
```
      RETURN
      END
      SUBROUTINE MORT(PER)
C*****CALCULATES PERCENT MORTALITY ASSOCIATED WITH EACH EL
      DIMENSION PER(200)
      DO 1 I=1,200
      EL=I
      ALEL=ALOG(EL)
      EX=(,9412*ALEL)+1.8713
C*****THIS SEQUENCE CONVERTS PROBITS TO PERCENTS
      EX=EX-5.
      AX=ABS(EX)
      TT=1./(1.+.2316419*AX)
      D=0.3989423*EXP(-EX*EX/2.)
      P=1.-D*TT*((((1.330274*TT-1.821256)*TT+1.781478)
     +*TT-0.3565638)*TT+0.3193815)
      IF(EX)3,2,2
3     P=1.-P
2     PER(I)=P*100.
1     CONTINUE
      RETURN
      END
      SUBROUTINE LOCATE(NT2,NT,X,Y,Z)
      DIMENSION Z(200,40),Y(200,40),X(240)
C*****CALCULATES THE NUMBER OF EACH EL IN EACH FT. OF THE STRIP
      DO 1 L=1,200
      DO 4 I=1,NT
      Z(L,I)=Y(L,I)*X(1)
      M=I+1
      IF(M.GT.NT)M=1
      DO 5 J=2,NT2
      Z(L,I)=Z(L,I)+X(J)*Y(L,M)
      M=M+1
      IF(M.GT.NT)M=1
5     CONTINUE
      M=I-1
      IF(M.LT.1)M=NT
      DO 6 J=2,NT2
      Z(L,I)=Z(L,I)+X(J)*Y(L,M)
      M=M-1
      IF(M.LT.1) M=NT
6     CONTINUE
4     CONTINUE
1     CONTINUE
      RETURN
      END
      SUBROUTINE EXPOSE(NT,NU,EL,Y,Z)
      DIMENSION Z(200,40),Y(200,40)
C*****UPDATES EXPOSURE LEVEL (EL) OF INSECTS ON THE INSECTICIDE
      N1=NU+1
      KL=EL+.5
      DO 1 I=1,200
```

```
       DO 4 K=1,NU
       Y(I,K)=Z(I,K)
 4     CONTINUE
       KK=I+KL
       DO 2 J=N1,NT
       IF(KK.LT.200) GO TO 3
       Y(200,J)=Y(200,J)+Z(I,J)
       GO TO 2
 3     Y(KK,J)=Z(I,J)
 2     CONTINUE
 1     CONTINUE
       DO 5 I=1,200
       DO 6 J=1,NT
       Z(I,J)=Y(I,J)
 6     CONTINUE
 5     CONTINUE
       RETURN
       END
```

APPENDIX E

STUDY AREA SURROUNDING THE KELLOGG GULL LAKE RESEARCH FARM, KALAMAZOO COUNTY, ROSS TOWNSHIP

SECTION 4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2
1
3
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5
T
6
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8
9
T
10
11
B
12
13
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15
B
16
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17
18
B
B
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55
56
57
T
59
W
T
58
T
54
B
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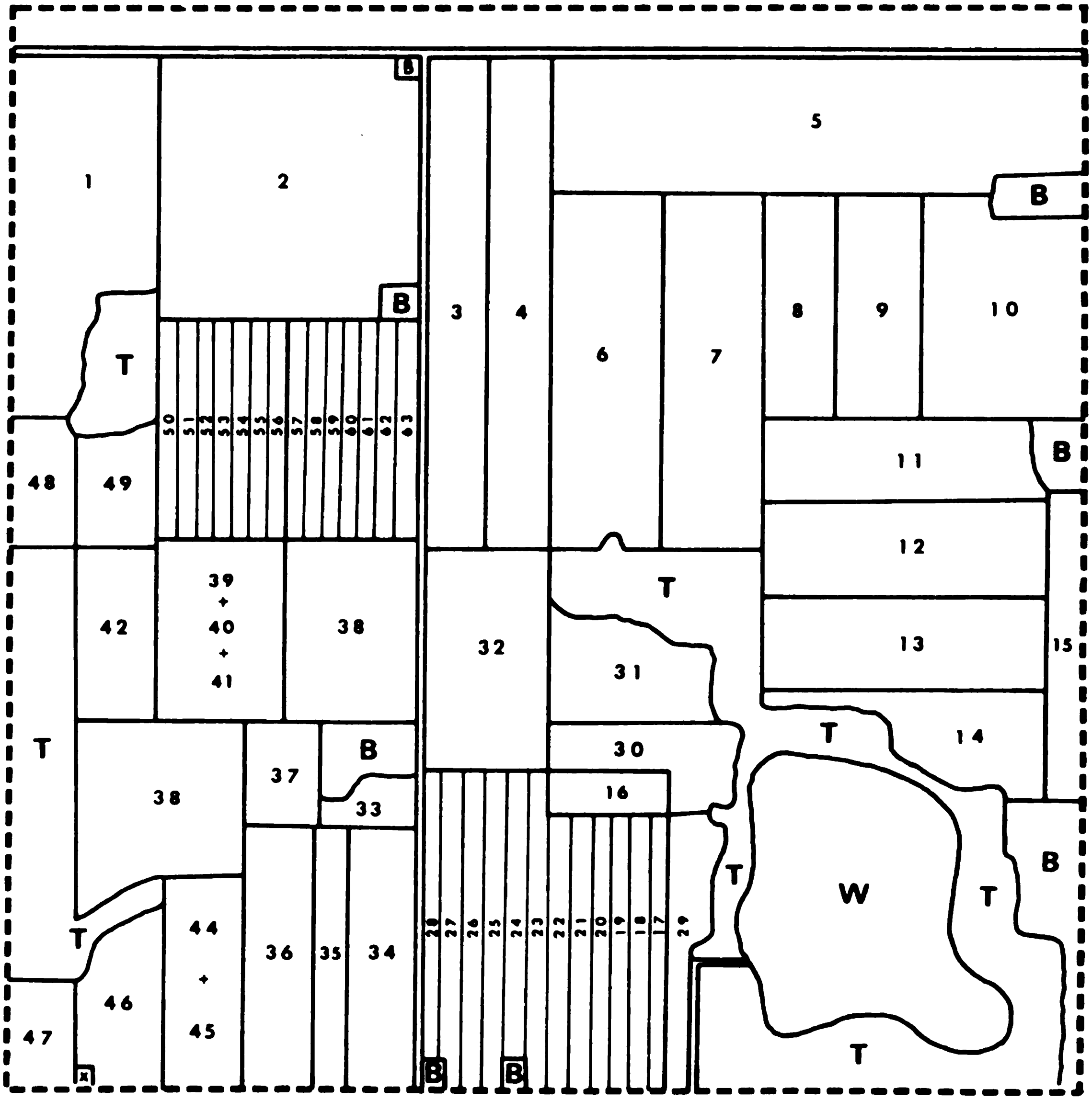
29
T

SECTION 5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3
4
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10
T
50 51 52 53 54 55 56 57 58 59 60 61 62 63
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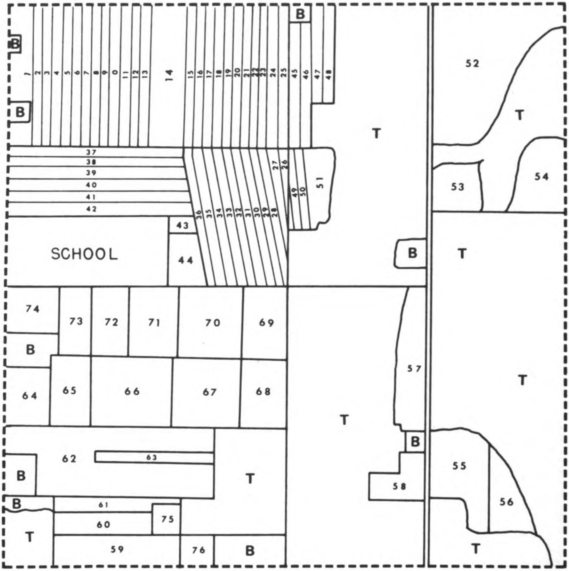
49
12
T
39 + 40 + 41
42
38
32
31
13
15
T
B
30
T
14
38
37
33
16
T
W
B
T
44 + 45
36
35
34
28 27 26 25 24 23 22 21 20 19 18 17 29
46
47
x
B
B
T

SECTION 8

SECTION 9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43
SCHOOL
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B
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60
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76
B
T