SOME EXPERIMENTS ON THE ROLE OF OLFACTORY STIMULANTS IN INSECT BEHAVIOUR

A Thesis

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Master of Science

by

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ABSTRACT

A series of essential oil chemicals was tested outdoors to determine their merits as baits for distinct insect groups. Methyl disulphide and ethyl disulphide were attractants for some Calliphoridae, Sarcophagidae and Muscidae. Several other chemicals had some attraction for certain insect groups.

Investigations employing several isothiocyanates revealed them to be ovipositional stimulants for _Plutella maculipennis_ (Curt.) at concentrations of less than 500 p.p.m. Optimal stimulation occurred between 10 and 100 p.p.m. A chain of "preference" among the isothiocyanates was demonstrated for oviposition as follows:

phenylethyl- = phenyl- > allyl- = butyl- > ethyl isothiocyanate.

ACKNOWLEDGEMENTS

I wish to thank my advisor, Dr. A. J. Thorsteinson, for his guidance and encouragement throughout these investigations. The Entomology Research Institute in Ottawa kindly identified some Dipterous specimens for me. Dr. C. Ellenby of King's College, Newcastle on Tyne, England provided a sample of chemical that was used in this work.

CHAPTER I

A PRELIMINARY SURVEY OF OLFACTORY ATTRACTIVENESS OF SOME ESSENTIAL OILS TO INSECTS

INTRODUCTION

The basis of this investigation is laid in the time honored assumption that odors in nature affect the behaviour of insects (Minnich, 1929). Odors are not the exclusive agents that affect insects in this way but they are important. This influence of odor has been confirmed for many insects as for example, the honey bee and its reactions to floral scents (von Frisch, 1950). Additionally, the locations of some olfactory receptors have been demonstrated (Frings, 1941).

Food and oviposition substrates are often peculiarly specific to given insect species. The question arises as to how these insects fulfill their frequently obligatory requirements. It is not unusual that oviposition sites and food sources have characteristic odorous chemical substances that seem to serve as guides to the foraging insects. Whenever a behaviour pattern of this nature is clearly recognized or established, the possibility arises for taking advantage of it either to investigate the population dynamics of such insects or to increase our ability for direct or indirect control of such populations.

The essential oils are a group of odorous chemicals of plant origin. Their function in living plants has not been satisfactorily explained. Essential oils are quite varied as to their chemical constitution. Most of them are terpenes but the group also includes alcohols, phenols, aldehydes and isothiocyanates.

This investigation of the influence of essential oils on insects was initiated in the spring of 1960 when field studies were undertaken to test their value as baits. The objective was to discover any possible specificity of attractiveness to taxonomic groups of insects. Additionally, the relation of certain weather factors to this method of trapping was studied.

The literature on baits and traps has been extensively reviewed by Dethier (1947) in several chapters of his book on insect attractants and repellents. A few of the more pertinent findings of other investigators will be included in this brief introductory statement to illustrate points of discussion.

The uses of bait traps in Entomology are several. A few of the more important applications were mentioned by Dethier (1947) as follows:

1. to sample insect populations
2. to lure insects into traps or poisons to decimate the population
3. to lure insects away from crops
4. to offset the repellent properties of certain sprays, and

5. to act as counteragents against which to test the efficiency of repellents.

Certainly, not all of these uses could be encompassed in this project. However, the first three possibilities were studied to some degree.

The effectiveness of bait traps as control measures has not proven spectacular when compared to such other alternatives as chemical sprays and dusts. Some noteworthy exceptions where baits have proven to be of practical value might be noted as follows:

Bait Employed	Insect Attracted
geraniol	Japanese beetle
anethole	Codling moth
linalool	Oriental fruit moth
sex attractant	Gypsy moth
amino acids	Fruit flies
ammonia	Mediterranean fruit fly
carbon dioxide	Mosquitoes

These bait traps were not so successful as to constitute complete controls but they were useful additions to the more common methods.

The use of baits for insect population sampling has had some success. Frost and Dietrich (1929) used bait traps to sample beetle populations. Their data suggested selectivity in captures from the insect fauna according to the different odors and locations of the traps. Eyer and Medler (1940) found bait traps to give an accurate account of Oriental fruit moth populations in orchards. Mosquito populations have been sampled and rechecked to test the effectiveness of control measures (Dethier and Whitley, 1944). Obarski (1960) has made mention of successful chemical baiting for pests of rape in Poland. His trapping procedure was similar to ours in that baited, colored containers were used.

The tendency of investigations on baits for insects has been to try artificial substances in preference to natural chemicals. This was a logical choice as artificial chemicals have certain advantages for the scientific study of baits as compared with naturally occurring substances. The vast number of synthetic compounds provides us with a greater possibility of discovering useful baits. Of course, their numbers are a disadvantage in that much chance is involved in isolating the useful from the useless. One approach to this latter problem would be to investigate some of the elements and related compounds known to occur in essential oils. This was the basis for our choice of test compounds. The selected test chemicals were present in some of the plants native to Manitoba. Another point to be noted is that artificial bait substances could be superior to the natural chemical substances. The synthetic baits could be composed either of the more attractive of the natural compounds in their most effective concentrations or of attractive compounds not found in nature. The bait formulations would be simpler due to the synthetic rather than analytic approach to the problem. It might be more economical for potential users of such baits if only proven attractants were to be included in the lures. This would be preferable to haphazardly composing them of all the chemicals in the naturally attractive source of food or oviposition substrate. This additive process of bait formulation might be examined for blow flies. Various substances such as indole, skatole and allyl sulphide were found to be attractive for these insects but their effects were enhanced by the inclusion of such substances as calcium carbonate. The additional materials were not of great use as lures in themselves but they seemed to interact to increase the value of the primary bait components.

It was postulated by McIndoo (1919) that the different odors of plants were due either to a summated effect of all the plant chemical odors or to one plant chemical odor that masked all the others. The possibility of luring insects with single chemical baits has been proven in many instances as earlier noted in successful baits used as control measures. This being so it was logical that we chose to use single chemical baits in our testing of some essential oils.

MATERIALS AND METHODS

The test chemicals were selected on the bases of two factors. Firstly, certain of the chemicals were chosen as representative of some of the essential oils found in native plants of Manitoba. Most of the first seven groups listed below fitted this category. Secondly, some of the chemicals were selected to test the relative attractiveness of compounds with similar chemical structure. The sulphides, disulphides and isothiocyanates were in this latter category.

The test chemicals were grouped as follows:

1. terpenes - limonene, cymene, pinene, camphene
2. alcohols - geraniol, linalool, citronellol, cineol, menthol, terpineol, benzyl alcohol, octyl alcohol
3. phenols - thymol, eugenol, carvacrol
4. aldehydes - vanillin
5. ketones - carvone
6. esters - methyl salicylate, methyl anthranilate
7. various substances - coumarin, indole, putrescine
8. sulphides - allyl-, butyl-, ethyl-, methyl-, phenyl-
9. disulphides - butyl-, ethyl-, methyl-, phenyl-
10. isothiocyanates - allyl-, butyl-, ethyl-, methyl-, phenyl-

The chemicals were obtained from Matheson Coleman and Bell, Mann Research Laboratories Inc. and Eastman Organic Chemicals.

The essential oils were used without dilution in order to avoid introducing odors of diluents such as ether or alcohol.

The traps were bright yellow plastic trays twelve inches in diameter and four inches deep essentially conforming to the design described by Moericke (1952). Yellow is the standard color used for Moericke traps in studies of aphids and leaf hoppers. Each tray was secured to the ground by wire retainers inserted about their periphery (Figure 1).

A layer of odorless mineral oil one half inch deep in the bottom of the plastic trays served to trap insects that visited them. Because of its low rate of evaporation it was required to change the oil only after heavy rains.

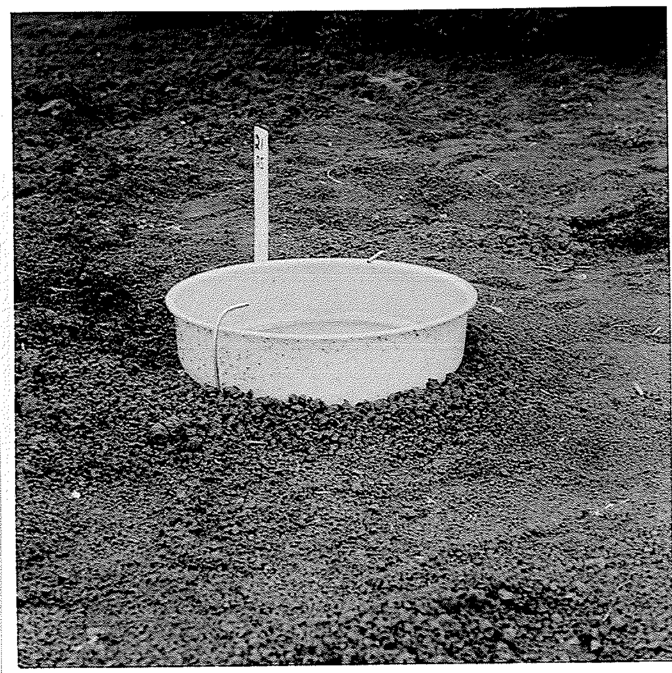
Odor substance was applied to blotting paper discs which were inserted in clear glass dishes and placed in the center of each plastic tray. Each application of odor substance consisted of four or five drops applied early each day.

Twenty-five traps were located in a plot twelve feet square. The test chemicals were allocated at random one to each trap.

Two plot locations were chosen on the University of Manitoba campus. The eastern location was on a grassy slope at the edge of a group of elm trees. This open site was

FIGURE 1

MODIFIED MOERICKE TRAP
(DISH OF BAIT IN CENTER NOT VISIBLE)



exposed to wind. The western plot location was on summer fallow land beside a willow shelter belt (Figure 2). Winds were excluded to a greater extent than at the eastern plot.

Collections from the traps were made every second day if weather conditions permitted. The insects were recovered from the mineral oil and placed in three-dram vials. The catches were preserved in seventy per cent ethyl alcohol until classified.

Data on weather conditions were obtained from the local weather office. This was not wholly satisfactory as local topography probably altered the temperature, wind and precipitation to some extent. However, these more general weather data were applied to the trap catches with reasonably good results (Table I).

FIGURE 2

PLOT LAYOUT OF BAITED MOERICKE TRAPS
(WESTERN LOCATION, 1960)

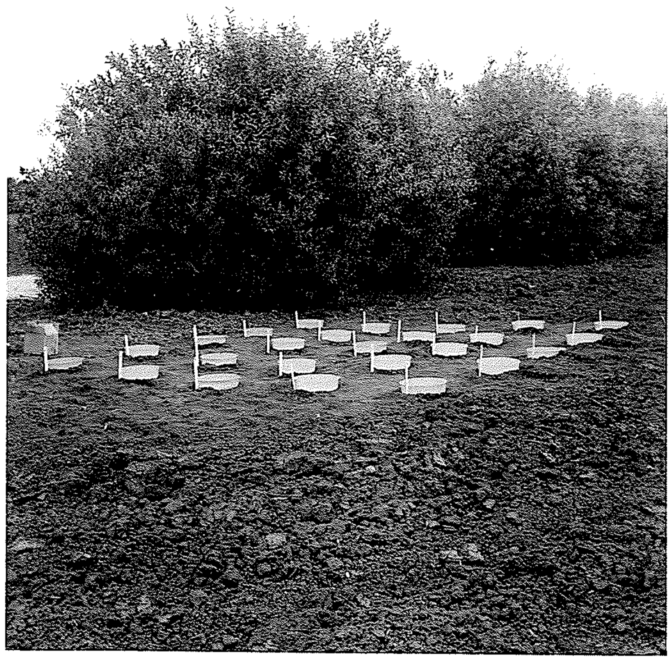


TABLE I

WEATHER DATA FOR TRAPS BAITED WITH ESSENTIAL OILS

	Weather Conditions of Collection Period		
Collection date	Average temperature °F	Average wind velocity m.p.h.	Prevailing wind direction
May 30	63	10	NW
May 31	67	9	SE
June 3	60	17	SW
June 6	53	16	NW
June 7	54	5	S
June 10	63	10	S
June 11	63	11	NE
June 13	67	7	E
June 15	65	11	W
June 16	55	14	SE
June 24	62	11	E
June 25	66	9	S
June 28	73	18	NW
July 6	61	7	N
July 13	70	9	N

RESULTS AND DISCUSSION

The results of the trap catches were analyzed in two ways. Firstly, the potential attractiveness of the bait compounds was evaluated. Each chemical was compared with the unbaited control trap for possible attraction for various insect groups. Secondly, the trap data were averaged to a one-day basis. In this way, it was hoped to be able to show any differences in average catches due to weather factors.

The captured insects were classified to families except where otherwise indicated. The data while incomplete in this way are at least suggestive as to the attraction for orders and families and as to the influence of certain weather factors on insect behaviour.

Essential oils as baits

Insects were captured in thirteen orders and seventy families. This might seem to suggest a general attraction for a wide number of forms due to the many baits. This was not the case as most of the traps regardless of bait or of position captured most of the commonly represented groups at some time during the summer. A few families (e.g. Chrysomelidae) were captured regularly but most families were taken more sporadically. However, there appeared to be some specificity of attractancy of some of the chemicals for particular insect groups.

Some of the orders were captured in consistently high numbers. Others were apparently accidental captures as they were not taken often. A rough analysis of this aspect of the captured groups in shown in Table II.

The number of families captured at the two plot locations differed slightly. Sixty families were detected by the traps at the western location. In the east, ten more families were captured in addition to those in the western plot although the eastern location was less intensively trapped. This discrepancy might be explained by the more diverse plant cover and the proximity to a river at the eastern plot. Some of the additional captures were Formicidae, Andrenidae, Mymaridae, Drosophilidae, Buprestidae and Reduviidae.

Complete uniformity of data between the two plot locations was not to be expected as they were not in exactly similar habitats. The relative numbers of insect forms represented at the two locations were similar but differed significantly as to absolute numbers of species and individuals. For instance, the grassy slopes around the eastern plot yielded many grasshoppers whereas few were found on the bare soil around the western plot. Conversely, the western plot had large numbers of thrips and springtails while the eastern plot had lesser numbers of these insects.

TABLE II

A COMPARISON OF THE NUMBERS OF INDIVIDUALS OF VARIOUS CAPTURED ORDERS

Low Numbers	Moderate Numbers	High Numbers
Ephemeroptera	Orthoptera	Collembola
Odonata	Homoptera	Thysanoptera
Psocoptera	Hemiptera	Diptera
Neuroptera	Coleoptera	Hymenoptera
	Lepidoptera	

The seventy families were analyzed as to consistency of capture throughout the season among the twenty-five traps in the plots (Figure 3). The resulting picture showed twenty-five families captured in five or less traps and eighteen families in twenty-one or more traps. This distribution seems to suggest a twofold grouping of the captured families into consistent and rare captures. Some of these consistently captured groups that might lend themselves more economically to further investigation are listed in Table III. These insects may have been captured in part by the operation of random factors related to high population density. Another interpretation to this situation is that these are groups whose behaviour is adapted to capture in the traps used in this study. The rarely captured groups are not as readily interpreted as they might have been relatively uncommon or were not adapted to response to the trap locations or to the chemical baits that were used. The intermediate groups including six to twenty traps could not receive an explanation here without considerable detailed inquiry.

A few observations might be made as to insect types that either were not attracted or were not captured if attracted to the traps. Table IV shows a few of the more prominent families that one would expect to be present but in fact were captured in low numbers or were absent during the two months of bait trapping. This exclusion from capture may have been

FIGURE 3

INSECT FAMILIES RELATED TO THE TOTAL NUMBER

OF TRAPS IN WHICH EACH WAS CAPTURED

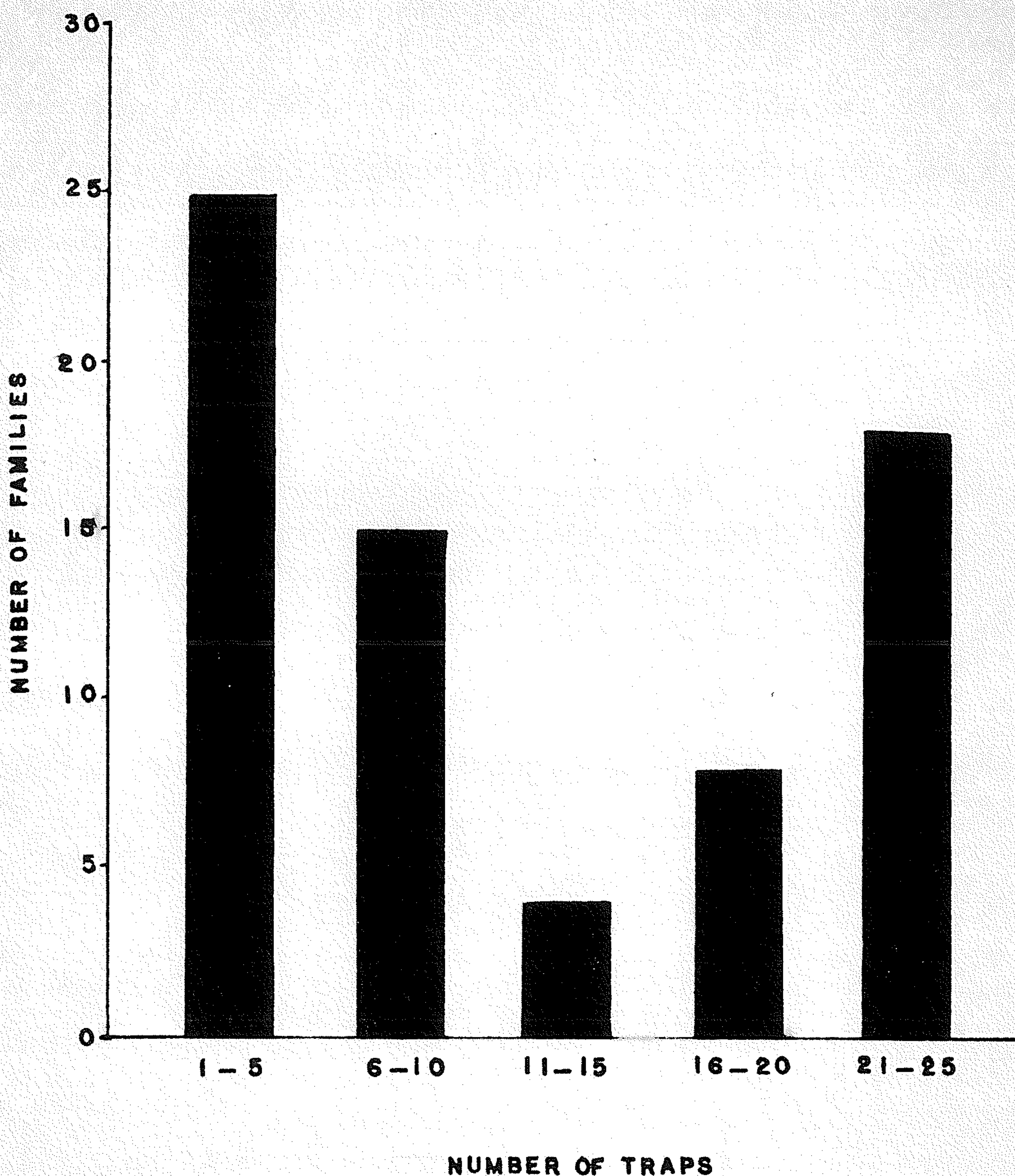

30
25
20
15
10
5
0
NUMBER OF FAMILIES
1 – 5
6 – 10
11 – 15
16 – 20
21 – 25
NUMBER OF TRAPS

TABLE III

GROUPS OF CONSISTENTLY CAPTURED INSECTS

Order	Family	No. of sample dates represented (maximum =15)	No. of baits represented (maximum =25)
Hymenoptera	Chalcidoidea	15	25
	Cynipidae	15	20
	Halictidae	14	17
	Ichneumonidae	13	21
	Braconidae	11	23
	Tenthredinidae	13	16
Diptera	Bibionidae	13	19
	Mycetophilidae	15	25
	Sciaridae	15	25
	Cecidomyidae	14	15
	Dolichopodidae	15	25
	Phoridae	15	25
	Ephydridae	12	25
	Chloropidae	15	25
	Tipulidae	13	23
	Chironomidae	15	25
	Tachinidae	13	18
	Calliphoridae	8	23
	Muscidae	11	25
	Syrphidae	10	25
Coleoptera	Chrysomelidae (mainly flea beetles)	15	25
	Staphylinidae	13	21
Homoptera	Cicadellidae	15	25
	Aphididae	14	25
	Psyllidae	15	19
Orthoptera	-	9	16
Thysanoptera	-	15	25
Collembola	-	15	25

TABLE IV

COMMON FAMILIES THAT WERE ABSENT OR CAPTURED IN LOW NUMBERS

Order	Family: Rarely captured	Family: Absent
Hymenoptera	Colletidae Platygasteridae Vespidae Sphecidae	Andrenidae Cephidae
Diptera	Ceratopogonidae Simuliidae Culicidae Anthomyiidae	Tabanidae Bombyliidae Asilidae Gasterophilidae Oestridae Hypodermatidae Cuterebridae
Coleoptera	Chrysomelidae Curculionidae Coccinellidae	Tenebrionidae Scarabaeidae
Hemiptera	Lygaeidae Membracidae	Scutelleridae Pentatomidae

due to evasive behaviour similar to that described for Calliphoridae (p.23), to a lack of attraction to the traps or to low population levels in the plot areas.

From the work done, certain chemicals would appear to be attractants for some of the insect families (Table V). The data did not lend themselves to any simple formal or precisely quantitative analysis. Thus, the chemicals were ranked under the more prominently captured families on the basis of the mean captures for ten consecutive collection periods in June. In cases where more than one chemical has been listed under one numeral, the chemicals were judged to be about equal in attraction. Otherwise, the numerical listing indicates the apparent magnitudes of attraction shown by the various compounds for the insect families. Frost's work on baiting for the Oriental fruit moth suggested that certain factors were likely to alter responses to baits from year to year. Thus, the effective baiting for these insects by the listed chemicals may not hold exactly another year. A priori, one would expect that insects that are most specific in their food plant preferences would be captured consistently by the same bait chemicals. In any further studies, the Chrysomelidae would lend themselves best to such an inquiry because many of the flea beetles are specific for plants containing isothiocyanates. More complete data for this family are shown in Figure 4.

TABLE V

POTENTIAL BAIT CHEMICALS

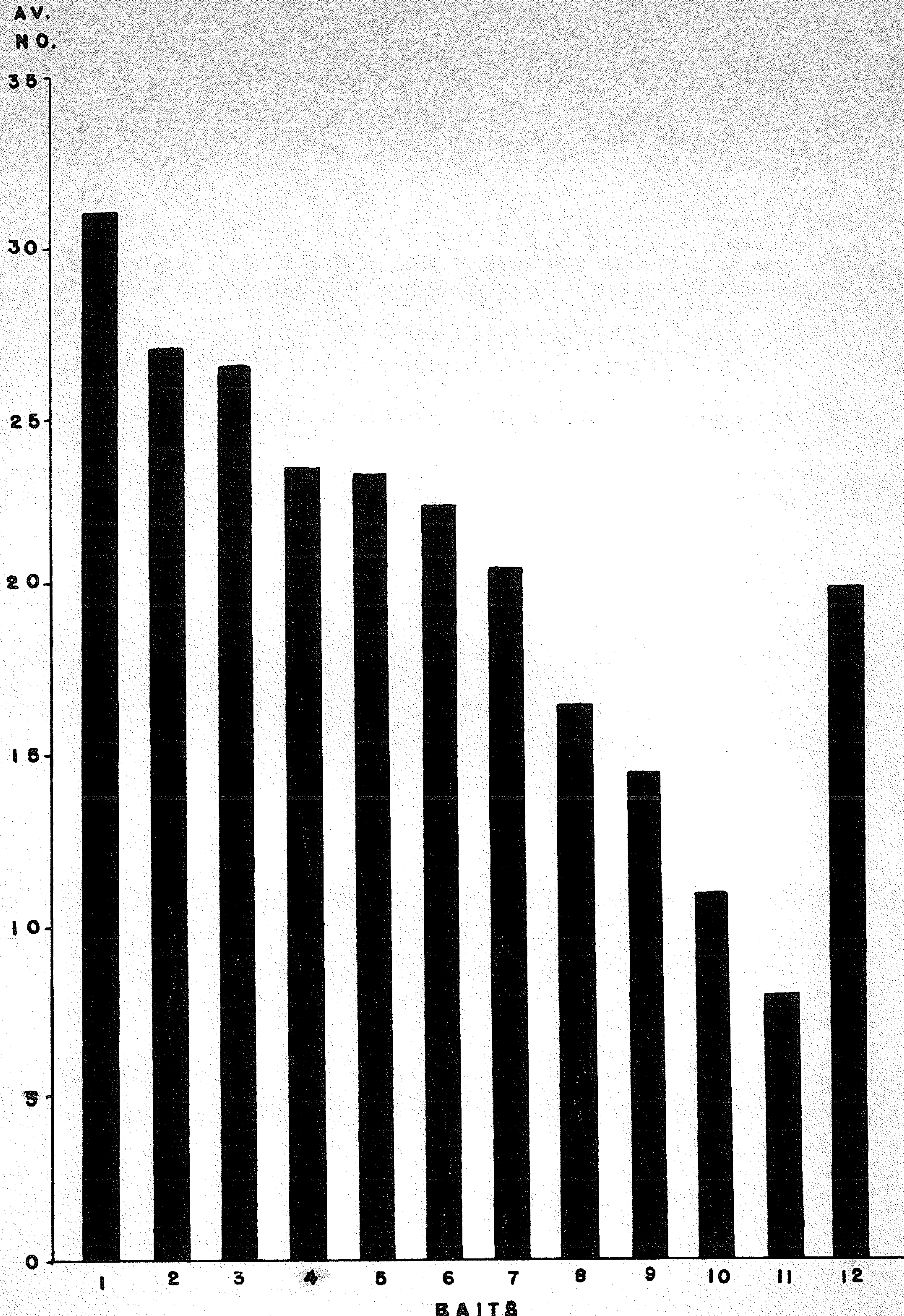
Insect Group	Rank	Chemical Bait	Mean no. captured	Control mean
Chalcidoidea	1	eugenol	17.5	6.1
	2	methyl salicylate	15.1	
	3	benzyl alcohol	11.0	
		allyl sulphide	10.0	
Mycetophilidae	1	octyl alcohol	14.5	7.3
		benzyl alcohol	14.3	
		butyl disulphide	14.1	
	2	methyl salicylate	12.3	
		ethyl sulphide	12.1	
	3	phenyl isothiocyanate	10.8	
		eugenol	10.3	
Sciaridae	1	phenyl isothiocyanate	32.0	13.7
	2	pinene	28.0	
	3	benzyl alcohol	22.8	
Chloropidae	1	allyl sulphide	18.1	7.7
	2	allyl isothiocyanate	14.7	
		phenyl isothiocyanate	14.6	
		ethyl sulphide	14.5	
Calliphoridae	1	methyl disulphide	12.6	1.4
	2	ethyl disulphide	4.6	
Chrysomelidae	1	benzyl alcohol	31.0	20.0
	2	pinene	27.0	
		allyl isothiocyanate	26.5	
	3	methyl salicylate	23.5	
		phenyl isothiocyanate	23.3	
Cicadellidae	1	benzyl alcohol	8.7	3.1
	2	methyl salicylate	6.7	
		pinene	6.3	
Aphididae	1	ethyl sulphide	5.7	3.2
		benzyl alcohol	5.4	
		methyl salicylate	5.4	

FIGURE 4

AVERAGE NUMBER OF CHRYSOMELIDAE CAPTURED IN VARIOUS BAITS

(Means for ten collection periods in June, 1960)

1. benzyl alcohol
2. pinene
3. allyl isothiocyanate
4. methyl salicylate
5. phenyl isothiocyanate
6. eugenol
7. allyl sulphide
8. geraniol
9. ethyl sulphide
10. octyl alcohol
11. butyl disulphide
12. unbaited control


AV.
NO.
35
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
11
12
BAITS

It was not possible due to limitations of time to analyze the captures taxonomically beyond the family level. It seems likely that such analyses might throw more light on the specificity of baits for particular taxonomic groups of insects. With reference to blow flies that were captured, many of them appeared to be similar but exact specific determination revealed more variety than was apparent at the family level (p.42). However, even at the family level some definite trends or correlations did emerge.

No attempt was made to compensate for unequal persistence of odors in outdoor tests except to replenish each trap fresh each morning. Separate investigations showed that some chemicals had high persistence lasting for two or more days at a detectable level to the human sense (e.g. eugenol) whereas others lasted but a few hours (e.g. methyl isothiocyanate). This factor of unequal persistence could affect the total numbers caught in any one bait. Highly persistent chemicals would have higher relative totals when compared to chemicals of possibly equal or superior attractiveness but of lesser persistence.

The early warning potentialities of this type of trap were proven to be promising. The population trends of certain groups of insects could be followed by the trap catches despite some daily variations caused by weather conditions (Figs. 5-8). This population information could prove of value in locating new infestations, in determining emergence times and in co-ordinating control programs with peak population periods. Emergence times were noted for several insect types (e.g. flea beetles) and a flight period on June 25 for aphids was detected (Figure 9). Such information was always slightly ahead of the peak infestations as the emergences and the periods of flight occurred over several days. The data obtained provided a basis for plotting such trends for several families listed in Table VI.

In this type of trapping where odor and perhaps color were involved, one of many pictures was revealed of the insect fauna present in the trap locations. The taxonomic composition of the insect fauna reflected by the trap captures is presumably a function of the bait chemicals used. A different set of baits quite probably would yield a somewhat different picture.

Chemicals have been noted as attracting or repelling insects. The odorous chemicals found in nature tend to act as sign posts that prompt many insects to channel their behaviour reactions into certain patterns. The token nature of attractants was attested by the revelation that attractants alone do not always elicit feeding or oviposition behaviour when the odorous source has been reached (Dethier, 1947). This latter observation was confirmed by the reaction of blow flies to methyl disulphide. The blow flies were

TABLE VI

INSECT GROUPS WITH DETECTABLE POPULATION TRENDS

Order	Family
Hymenoptera	Chalcidoidea Platygasteridae Cynipidae
Diptera	Cecidomyidae Bibionidae Chironomidae Ceratopogonidae Dolichopodidae Phoridae Ephydridae
Coleoptera	Chrysomelidae
Homoptera	Cicadellidae Aphididae Psyllidae
Lepidoptera	Phalaenidae Hesperiidae Olethreutidae
Orthoptera	(not classified)
Thysanoptera	(not classified)
Collembola	(not classified)

readily attracted by the odor of this chemical but rarely could be caught in a baited Moericke trap as they would fly near to the trap but would rarely alight and be captured. If they were not immediately captured, they flew away despite earlier attraction to the bait. This behaviour for blow flies might have been true for other unobserved types of insects that were attracted but not captured by the trapping methods employed in this project.

Weather factors and population sampling

Weather factors were of some importance in determining the total number of insects captured and what groups were caught in any one sampling period. The mean daily temperature, the prevailing wind direction and the average wind velocity were considered in this regard. The data for each of these weather factors for the sampling periods was reduced to a one-day basis.

Wind seems to have been of prime importance in influencing our trap collections. Its velocity determined the total number of insects caught on a warm day. As is suggested by Table VII, some insects at least are caught more frequently on the windward side of the plot regardless of bait chemical.

Temperature was important in that it regulated the activity of the insects during each sampling period. This influenced the population trend data but did not appear to interfere with the determination of relative attractiveness among the chemical baits.

The averaged figures for temperature and for wind velocity were compared to the average catch per trap for some consistently captured families. In this comparison, Dolichopodidae appeared to be captured in low numbers if the temperature for the period dropped below 60°F. The catch varied only slightly with changing temperatures above this level. At the same time, wind velocities greater than 10 m.p.h. seemed to be related to low catches of these flies. Below 10 m.p.h., variations in wind velocity were of slight significance (Figure 5). The captures of Chloropidae were not affected by temperature to any noteworthy extent. Average wind velocities greater than 10 m.p.h. lowered the catch of this family (Figure 6). Chrysomelidae were influenced in captures by temperature and by wind velocity. Most of the captured members of this family were flea beetles and comments on behaviour have been confined to flea beetles. Higher temperatures were correlated with increased captures of these insects. Lowered wind velocity brought about higher catches of flea beetles (Figure 7). Many families such as Cicadellidae did not seem to be greatly affected either by temperature or by wind velocity (Figure 8). This latter observation may not be absolutely valid as the samples for such families may have been too low to show any significant effects when related to these two weather factors.

The average captures of three additional families that

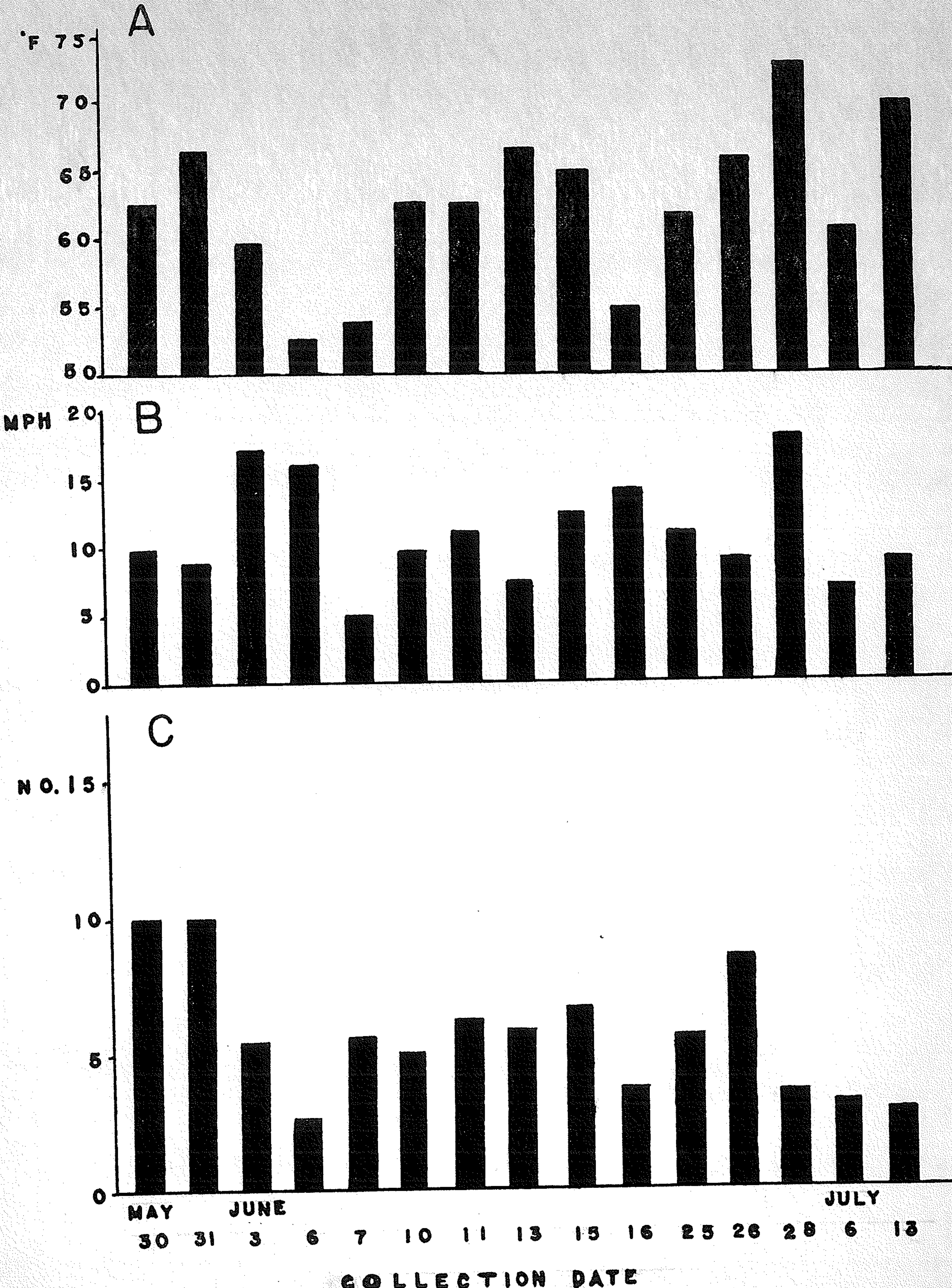
FIGURE 5

CAPTURES OF DOLICHOPODIDAE RELATED TO TEMPERATURE AND TO WIND VELOCITY

A. Temperature

B. Wind velocity

C. Captures



A
°F 75
70
65
60
55
50
B
MPH 20
15
10
5
0
C
NO. 15
10
5
0
MAY
JUNE
JULY
30 31 3 6 7 10 11 13 15 16 25 26 28 6 13
COLLECTION DATE

FIGURE 6

CAPTURES OF CHLOROPIDAE RELATED TO TEMPERATURE AND TO WIND VELOCITY

A. Temperature

B. Wind velocity

C. Captures

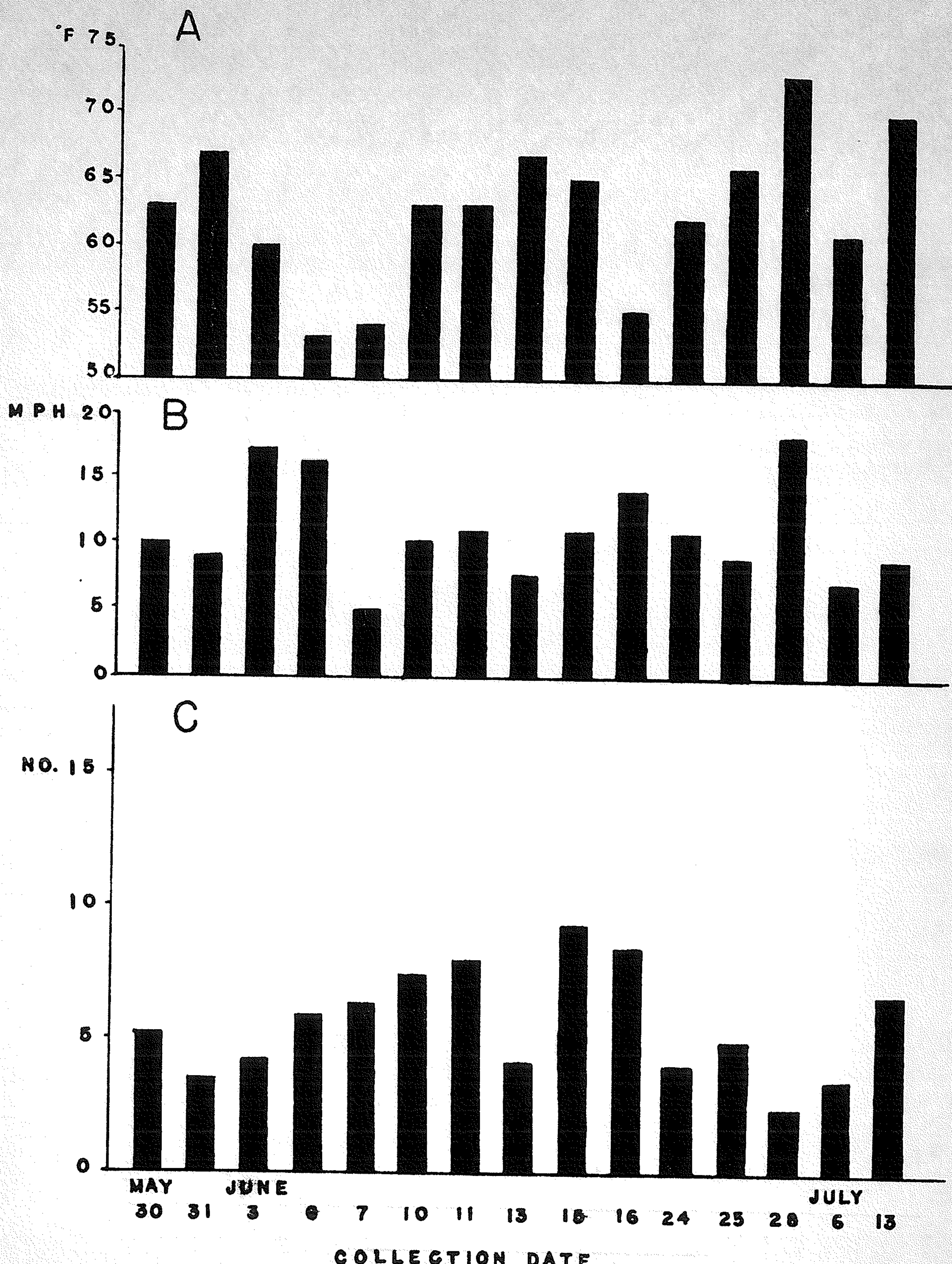

A
°F 75
70
65
60
55
50
B
MPH 20
15
10
5
0
C
NO. 15
10
5
0
MAY
30
31
JUNE
3
6
7
10
11
13
15
16
24
25
28
JULY
6
13
COLLECTION DATE

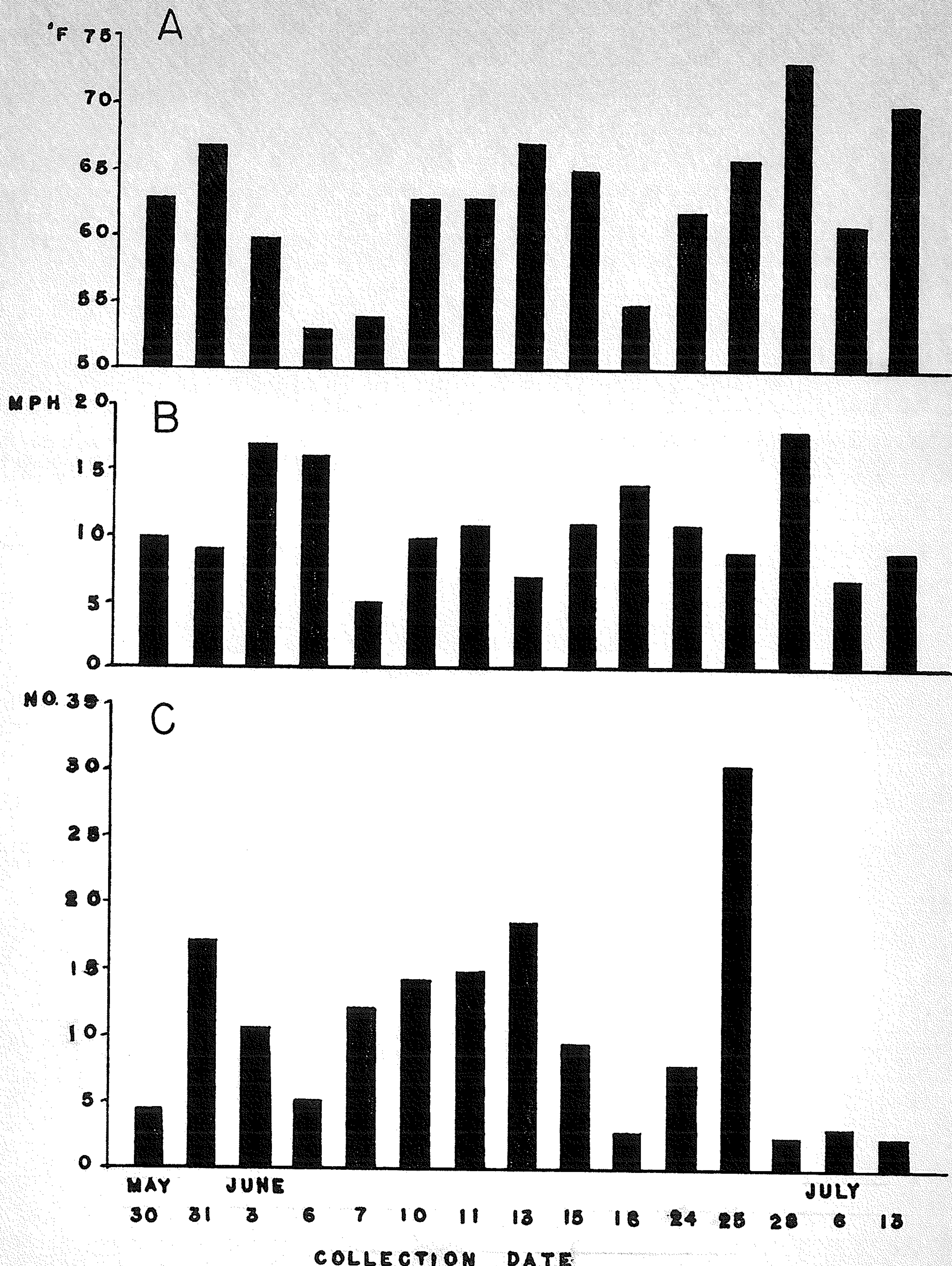
FIGURE 7

CAPTURES OF CHRYSOMELIDAE RELATED TO TEMPERATURE AND TO WIND VELOCITY

A. Temperature

B. Wind velocity

C. Captures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°F
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70
65
60
55
50
B
MPH
20
15
10
5
0
C
NO.
30
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15
10
5
0
MAY
JUNE
JULY
30
31
3
6
7
10
11
13
15
18
24
25
28
6
13
COLLECTION DATE

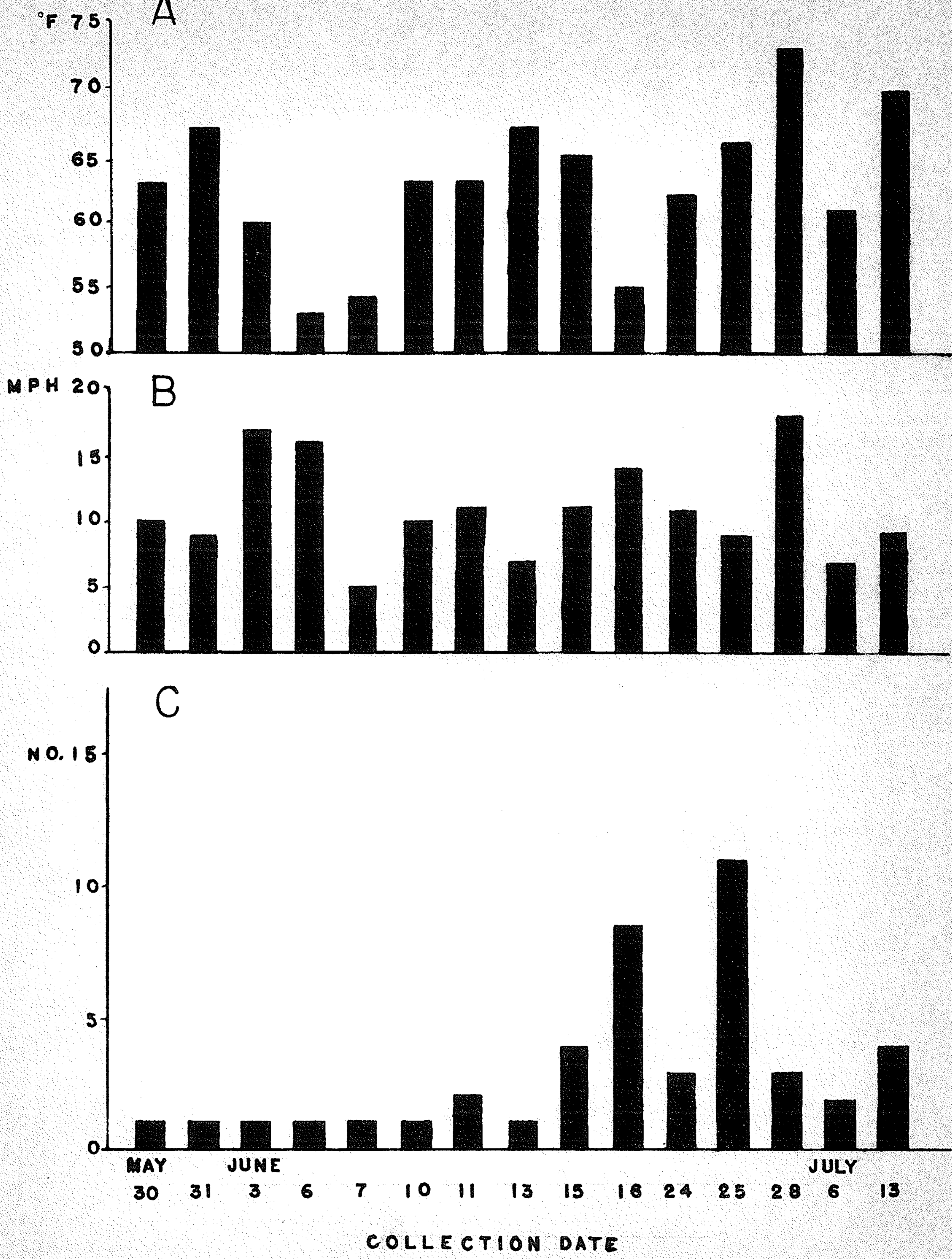
FIGURE 8

CAPTURES OF CICADELLIDAE RELATED TO TEMPERATURE AND TO WIND VELOCITY

A. Temperature

B. Wind velocity

C. Captures



A
°F 75
70
65
60
55
50
B
MPH 20
15
10
5
0
C
NO. 15
10
5
0
MAY
JUNE
JULY
30 31 3 6 7 10 11 13 15 16 24 25 28 6 13
COLLECTION DATE

were caught in some numbers has been illustrated (Figure 9). Some rather interesting features may be noted at a glance. Sciaridae showed two high peaks that might have corresponded to the emergence of two generations. Aphids were captured in very low numbers all season except in late June when a flight of adults was detected. Mycetophilidae were trapped at an almost constant rate throughout the period of investigation.

From these few observations, it might be concluded that different insect families respond differently to temperature and to wind velocity. This differing response affected the total number of insects caught in the various collection periods.

Wind direction was a factor that influenced the maximum catches within the plots for various samples. The effect of this factor was analyzed for three families (Table VII). Dolichopodidae, Chloropidae and Chrysomelidae appeared to have had at least two thirds of their maximum catches on the windward side of the plots. This seems to suggest an entry into the plots directed by the prevailing wind direction. Once within the plots, odor or color factors of the traps probably arrested the insects at the traps located along the periphery of the plots. This situation interfered with the definite determination of the relative attractiveness among some of the tested compounds.

Various observations might be made as to peaks, trends

FIGURE 9

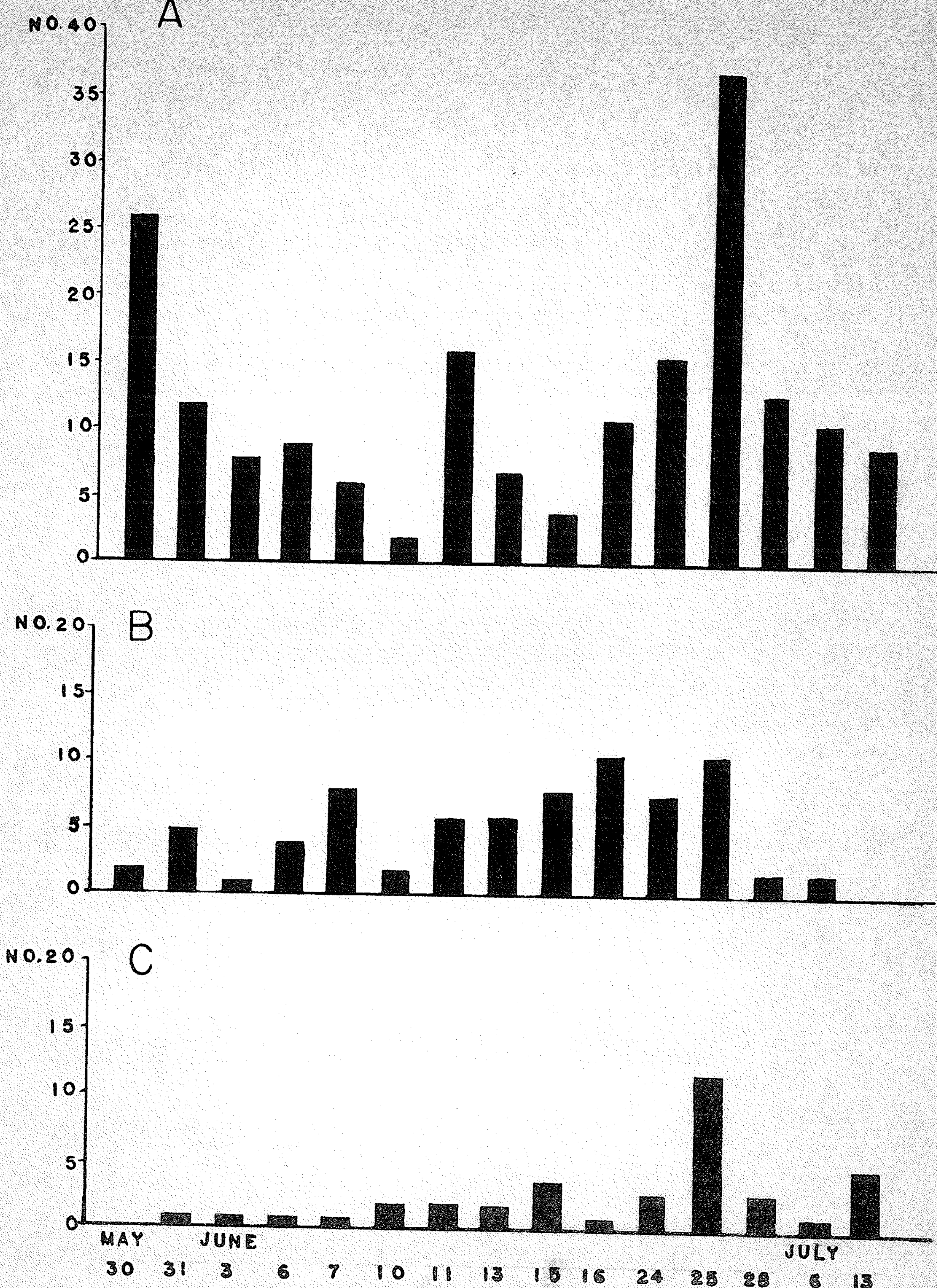
POPULATION TRENDS IN SOME INSECT FAMILIES

(Average number captured per trap)

A. Sciaridae

B. Mycetophilidae

C. Aphididae



A
NO. 40
35
30
25
20
15
10
5
0
B
NO. 20
15
10
5
0
C
NO. 20
15
10
5
0
MAY
JUNE
JULY
30 31 3 6 7 10 11 13 15 16 24 25 28 6 13

TABLE VII

MAXIMUM CATCHES WITHIN THE PLOTS RELATIVE TO THE PREVAILING WIND DIRECTION

Sample date	Dolichopodidae With wind	Dolichopodidae Against wind	Chloropidae With wind	Chloropidae Against wind	Chrysomelidae With wind	Chrysomelidae Against wind
May 30	x			x	x	
May 31		x	x		x	
June 3	x		x		x	
June 6	x		x		x	
June 7		x	x			x
June 10	x		x			x
June 11	x		x		x	
June 13	x		x		x	
June 15	x		x		x	
June 16		x		x		x
June 24		x	x		x	
June 25		x		x		x
June 28	x			x	x	
July 6		x	x		x	
July 13	x			x		x
Totals	9	6	10	5	10	5

and relative abundance of the populations of some of the detected insect families. Several of the more consistently captured groups were analyzed as to these characteristics of population (Table VIII). From this table, it might be seen that June was the month when most insect populations reached a peak. Most groups were noted as building to peaks then declining slowly as the season progressed. As mentioned before, the trap captures may not reflect absolute population densities judging from the samples. Chalcidoidea, Sciaridae, Bibionidae, Thysanoptera and Collembola were captured in relatively high numbers but only at certain times that might have corresponded to population peaks due to emergence (e.g. flea beetles) or to maturation to more active adult forms (e.g. aphids). Some types such as Mycetophilidae seemed to vary little with time or with weather factors. They were attracted to the traps in such constant numbers that no apparent peaks in population could be detected.

TABLE VIII

POPULATION CHARACTERISTICS OF SOME CONSISTENTLY CAPTURED INSECT FAMILIES

Order	Family	Peak	Abundance	Trend
Hymenoptera	Chalcidoidea	June	Moderate	Rapid decline
	Cynipidae	June	Low	Slow decline
Diptera	Bibionidae	June	Low	Slow decline
	Sciaridae	June	High	Steady
	Mycetophilidae	June	Moderate	Steady
	Cecidomyidae	May	Low	Slow decline
	Dolichopodidae	June	High	Rapid decline
	Phoridae	June	Low	Slow decline
	Ephydridae	June	Low	Slow decline
	Chloropidae	June	Moderate	Steady
Coleoptera	Chrysomelidae	June	Moderate	Rapid decline
Homoptera	Cicadellidae	June	Low	Slow decline
	Aphididae	July	Low	Steady
	Psyllidae	June	Low	Slow decline

CHAPTER II

DISULPHIDE BAITS FOR DIPTERA

INTRODUCTION

A second phase of chemical bait testing arose in midsummer of 1960 through the observation of several groups of Diptera being attracted to the disulphides mentioned in Chapter I. Separate tests were started to investigate this clue to as great an extent as time would allow. The late start on this aspect of chemical baiting necessarily limited its results.

According to Dethier (1947), work by various investigators has revealed several attractants for blow flies. Amyl mercaptan, methylallyl thiocyanate, skatole, indole, ammonium carbonate and ethyl mercaptan were determined as attractive in low concentrations. Others with attractive properties were allyl sulphide, ethyl sulphide and ethyl disulphide.

The insects that were baited in this work were rather nonspecific in their feeding habits. They were forms that were attracted to the products of fat and protein decomposition. Several insect families such as Staphylinidae, Silphidae, Histeridae, Scarabaeidae, Muscidae, Sarcophagidae and Calliphoridae have been noted as dung and carrion feeders. Staphylinidae, Muscidae, Sarcophagidae and Calliphoridae were captured in the baited Moericke traps. In the special project on Diptera, another trap design was adopted, as described later, which did not capture beetles.

The research on baiting for coprophagous and necrophagous insects has been active for some years. Dethier (1947) listed many workers and their various contributions to the research on odorous chemicals attractive to blow flies.

Freney (1937) tried to isolate various attractive materials extracted from carrion baits but had slight success. This author did not report on the attraction of muscids and sarcophagids which were captured in the present study.

Vanskaya was mentioned by Dethier (1947) as carrying on experiments in Russia using manure with the addition of ammonium carbonate to produce a useful bait for the control of the house fly. In the United States, *Muscina* spp. and *Musca domestica* were captured with similar baits.

MATERIALS AND METHODS

This chemical bait testing was carried on near the University of Manitoba during July and August of 1960. The procedure was somewhat different from the work on essential oils described in Chapter I.

Wire screen traps of several designs were employed to determine an adequate means of trapping the flies attracted by the baits. One design was found to be more effective and was used throughout most of this work. The design was such as to trap the flies after their resumption of flight from the bait container (Figure 10). Once inside the trap, the flies were killed by a coating of residual mixed insecticides applied with an aerosol bomb. A second trap design that was used in some of the preliminary tests is illustrated in Figure 11.

The odor source consisted of a screen-topped dish which contained water-soaked cotton. A small amount of the undiluted chemical baits i.e. methyl disulphide, ethyl disulphide, indole or putrescine, was applied to the cotton on each day of testing. The bait containers were located either beneath (trap I) or inside(trap 2) the trap.

The baited traps were exposed daily. The collections were separated grossly to families and counted.

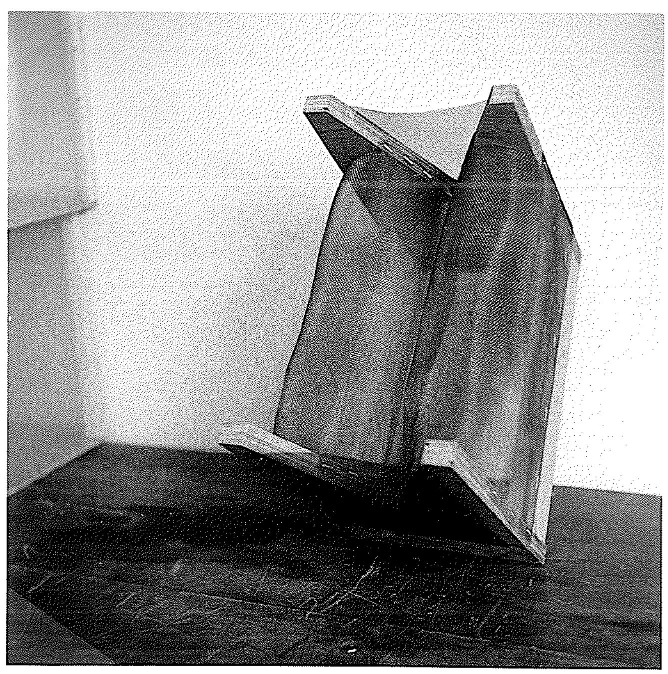
FIGURE 10

BLOW FLY TRAP DESIGN NUMBER I

A. Side view

B. Bottom view

A



B

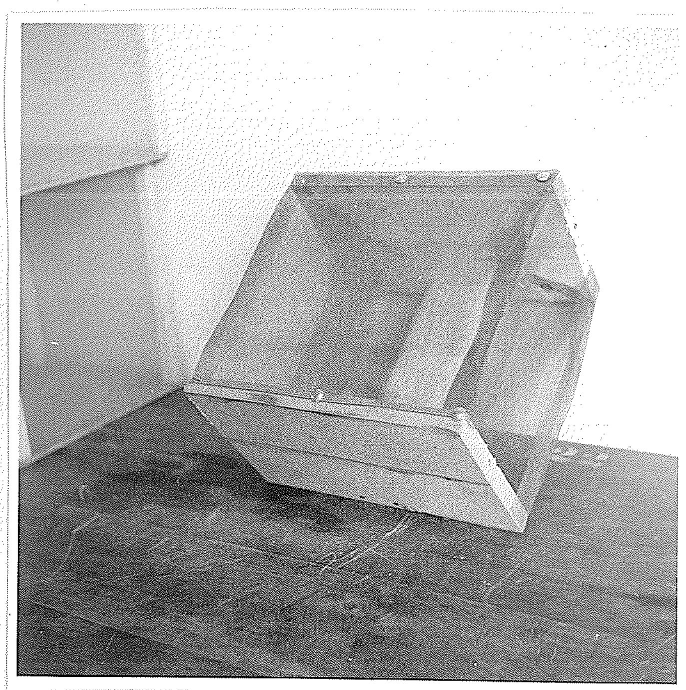
FIGURE 11

BLOW FLY TRAP DESIGN NUMBER 2

A. Side view

B. Top view

A



B

RESULTS AND DISCUSSION

Methyl disulphide proved to be the best attractant and ethyl disulphide was nearly as good. Putrescine and indole were much less attractive in early trials and all of the experiments thereafter concerned the two disulphides.

In this study, the largest captures (80-250 flies per day) were obtained on hot, calm days. On cooler and windier days, the captures usually numbered 40-60 flies.

The captures were largely composed of calliphorids, a moderate number of muscids and a few sarcophagids.

It would seem from the literature that attempts to trap blow flies with attractive compounds extracted from carrion baits have not been attended by marked success. Freney was quoted by Dethier (1947) as stating that the only chemical attractive in the pure state for blow flies is methyl mercaptan. However, indole, skatole and ammonium carbonate were judged to exert some attraction as well. Sodium sulphite, acetic acid, alkyl sulphides and calcium carbonate have been shown to increase the attractiveness of baits. Methyl disulphide was not mentioned in the literature as an attractive compound. Certainly, its action in these tests must lead to its inclusion as a potentially important bait for blow flies. At least, this is true for the common species in our locality.

Some of the captured flies were sent to the Entomology Research Institute, Ottawa for determination to species. The identified flies were grouped in three families as follows:

Calliphoridae - *Phormia regina* (Mg.)
Protophormia terraenovae (R.D.)
Calliphora vomatoria (L.)
Calliphora coloradensis Hgh.
Lucilia illustris (Mg.)
Phaenicia sericata (Mg.)(Formerly called *Lucilia sericata*(Mg.))

Sarcophagidae - *Sarcophaga* (*Sapromyia*) *bullata* Park.
Sarcophaga (*Ravinia*) *l'herminieri* R.D.
Sarcophaga (*Ravinia*) *planifrons* Ald.

Muscidae - *Ophyra leucostoma* Wied.
Muscina stabulans Fall.
Muscina assimilis Fall.
Musca domestica L.

Of the calliphorids, *Phormia regina* (Mg.) and *Phaenicia sericata* (Mg.) were captured consistently. Some of the blow flies such as *Calliphora coloradensis* Hgh. were much rarer. In Sarcophagidae, only *Sarcophaga bullata* Park. appeared to be consistently captured in appreciable numbers. Most of the muscids captures were composed of *Musca domestica* L. and *Muscina stabulans* Fall.

It was noted that the disulphides attracted both sexes of *Phaenicia sericata* (Mg.), *Muscina assimilis* Fall., *Muscina* *stabulans* Fall. and *Musca domestica* L. The captures of other species were not sexed. The capturing of both sexes of these insects would seem to indicate that the disulphide lures are food-type attractants.

CHAPTER III

MUSTARD OILS AND THEIR EFFECTS ON THE REGULATION OF OVIPOSITION BY *PLUTELLA MACULIPENNIS* (CURT.)

INTRODUCTION

The aim of this project was to investigate the effects of several isothiocyanates on the ovipositional behaviour of diamondback moth adults *Plutella maculipennis* (Curt.). Optimal concentrations of the chemicals affecting ovipositional stimulation were to be determined.

Thorsteinson (1955) from his investigations on phytophagous insects indicated that allyl mustard oil was likely to be an ovipositional stimulant for diamondback moth adults rather than a feeding stimulant for the larvae.

In further investigations using *Plutella*, Gupta and Thorsteinson (1960) indicated that tactile stimuli caused oviposition to occur regardless of whether or not an attractive oviposition-stimulating odor was present for the test moth. The odor of allyl mustard oil increased the effects on oviposition caused by a pitted or rugose surface.

This phase of behavioural investigation extended the work conducted by Gupta and Thorsteinson (1960) to other mustard oils of the isothiocyanate group.

MATERIALS AND METHODS

Adult moths were taken from a pure culture of *Plutella maculipennis* (Curt.) that was maintained in the laboratory. The adults were housed in wooden cages covered with plastic screening. Sugar solution was fed to the adults. Eggs were recovered from cabbage leaves placed in the cage. The larval stages were reared on cabbage leaves in paper cartons. The temperature for rearing and for experimentation was 70°-75°F. and the relative humidity was 10%-25%.

The series of chemicals tested for ovipositional affects were isothiocyanates. Allyl-, butyl-, ethyl-, and phenyl isothiocyanates were obtained from Eastman Organic Chemicals Ltd. The other tested chemical was phenylethyl isothiocyanate which was supplied by Dr. Ellenby of King's College in England. This sample was old and slightly discolored but still had a very characteristic mustard oil odor. The chemicals were used as various dilutions in distilled water.

Glass vials of ten-dram capacity with rugose plastic tops were used for oviposition substrates (Figure 12). Each cap had an opening of five millimeters cut out of the center. Plastic screening was placed on the inside of each cap to prevent the moths from entering the vials.

Some of the earlier tests were carried on in quart paper cartons with plastic screen tops (Figure 12). This

FIGURE 12

PAPER CARTON CAGE FOR OVIPOSITION TESTS

arrangement was not satisfactory and was abandoned. Plastic screen cages of one cubic foot volume were employed in most of this work. These cages had screen on four sides with an opening on top to allow the test vials to be placed in the cages (Figure 13).

In early tests, the diluted chemicals were applied directly to the vial caps. Better results were obtained when the chemicals were placed inside the vials and this method was used in most of the work. This arrangement provided a more lasting source of chemical attractant.

In testing the chemicals, one vial was placed in each of the four corners of each cage. The treatments were randomized among the various positions in the cages. Testing was carried out in a darkened room overnight.

The number of eggs deposited on each vial cap was counted. The average number for six replicates was taken as indicative of the relative acceptability among the various dilutions or among the chemical compounds. Each experiment was repeated at least once to prevent undue conclusions based on one day's testing.

Each chemical was tested in several dilutions to determine the more effective concentrations. These optimal dilutions were tested in turn against the optimal concentration of allyl isothiocyanate (50 parts per million). The chemicals were rated as more favorable, less favorable, or equal to allyl isothiocyanate as oviposition stimulants.

FIGURE 13

PLASTIC SCREEN CAGE FOR OVIPOSITION TESTS

RESULTS AND DISCUSSION

All the tested chemicals elicited oviposition responses from the moths at concentrations of less than 500 parts per million. Above 500 p.p.m., the moths appeared to be repelled. Further, the best oviposition responses were those obtained at less than 100 p.p.m. Much of the experimental work was confined to these lower concentrations to determine the optimal dilution (Table IX).

From the experiments, the concentration optima appeared to differ only slightly among the compounds. Usually, the moths responded best to allyl mustard oil at concentrations between 50 and 100 p.p.m. but sometimes 50 p.p.m. was optimal. Butyl and phenylethyl mustard oils had a similar range to allyl but were consistently better at 100 p.p.m. Ethyl isothiocyanate was similar in range and in optimal concentration to allyl mustard oil. A lower range of 10 to 50 p.p.m. with an optimum at 50 p.p.m. was determined for phenyl mustard oil.

Phenylethyl and phenyl mustard oils were about equal and superior to allyl mustard oil (Table X). Allyl and butyl isothiocyanates were nearly equal as oviposition stimulants. Ethyl mustard oil elicited the poorest response in the series of test chemicals.

Because of variability of data, it seems rather doubtful that definite optimal concentrations for any of the chemicals could be established without more precise control of certain factors. In all of this work, the moths were present in

TABLE IX

COMPARISON OF OPTIMAL CONCENTRATIONS OF ISOTHIOCYANATES FOR

PLUTELLA MACULIPENNIS (CURT.)

Percentages of Total Oviposition in Representative Experiments

Concentration	Allyl	Butyl	Ethyl	Phenyl	Phenylethyl
Control	15.0%	22.6%	13.3%	10.9%	14.1%
10 parts	12.5%	16.0%	25.7%	27.0%	23.9%
50 parts	38.6%	23.5%	33.1%	37.8%	29.0%
100 parts	34.1%	37.8%	27.7%	24.0%	32.9%
Total eggs	200	600	847	574	389

Note: Total eggs are not subject to comparison.

TABLE X

"PREFERENCES" OF *PLUTELLA MACULIPENNIS* (CURT.) AMONG THE ISO-THIOCYANATES

Percentages of Total Oviposition in Representative Experiments

Concentration	Phenyl	Phenylethyl	Butyl	Ethyl
50 parts	46.1%	51.9%	32.9%	20.0%
100 parts	-	-	18.5%	32.5%
	Allyl	Allyl	Allyl	Allyl
50 parts	33.9%	37.2%	27.8%	36.4%
	Control	Control	Control	Control
-	19.8%	10.8%	20.6%	11.0%
Total eggs	312	468	470	703

Note: Data provide for comparisons only with columns, not rows.

numbers of less than 100 per cage. These populations would vary as to sex ratio, age and physiological condition. More rigid regulation of such factors might enable a more exact determination of optimal concentrations of the chemicals affecting oviposition. In spite of this uncontrolled variability, some statistically significant differences at the 5% level emerged.

It might be noted that with but rare exceptions the chemicals at the concentrations tested proved superior to the water controls. In these rare exceptions, it is possible that thirst arrested the movement of some moths on the control vials containing water. The rough surface of the test vial caps would have elicited some eggs and the water vapor might have contributed to the ovipositional response. Such an explanation is consistent with the findings of Gupta and Thorsteinson (1960).

CHAPTER IV

SUMMARY AND CONCLUSIONS

Several chemicals found in essential oils were tested as baits for taxonomic groups of insects using a form of Moericke trap. Methyl disulphide and ethyl disulphide were definite attractants for some Diptera. Benzyl alcohol, octyl alcohol, eugenol, allyl isothiocyanate, phenyl isothiocyanate, allyl sulphide and ethyl sulphide were shown to be potentially attractive for some insect families.

Weather factors proved to be of some importance in our trapping procedure by influencing the total daily captures and the location of the daily maximum catches within the plots. Temperature, wind velocity and wind direction will have to be more accurately known at the plot locations to enable more exact estimation of attraction by baits.

A separate series of disulphide tests using a different type of trap than for the essential oils confirmed the attraction by these chemicals for several families of Diptera. Certain species of Calliphoridae, Sarcophagidae and Muscidae were trapped consistently.

Several concentrations of some isothiocyanates were tested as oviposition stimulants for the diamondback moth. All these chemicals were ovipositional stimulants at concentrations below 500 p.p.m. Optimal stimulation occurred at concentrations between 10 and 100 p.p.m. A chain of "preference" among the chemicals was revealed as follows:

phenylethyl- = phenyl- > allyl- = butyl- > ethyl isothiocyanate.

REFERENCES

1. Bedoukian, P. Z., 1951. Perfumery Synthetics and Isolates. D. Van Nostrand Company Ltd.

2. Borror, D. L., D. M. Delong, 1957. Introduction to the Study of Insects. Rinehart and Company.

3. Brown, F. W., 1928. Odors and Insects. Amer. Museum Novitates No. 299.

4. Budd, A., 1952. Plants of the Farming and Ranching Areas of the Canadian Prairies. Queen's Printer (Ottawa).

5. Dethier, V. G., 1937. Gustation and Olfaction of Lepidopterous Larvae. Biol. Bull. 72: 7-23.

6. Dethier, V. G., F. H. Whitley, 1944. Population Studies of Florida Mosquitoes. J. Ec. Ent. 37(4): 480-484.

7. Dethier, V. G., 1947. Chemical Insect Attractants and Repellents. The Blakiston Company.

8. Eyer, J. R., J. T. Medler, 1940. Attractiveness to Codling Moth of Substances Related to Those Elaborated by Heterofermentative Bacteria in Baits. J. Ec. Ent. 33 (6): 933-940.

9. Freney, M. R., 1937. Studies on the Chemotropic Behaviour of Sheep Blowflies. Australian Council Sci. Ind. Research, Pamphlet 74.

10. Frings, H., 1941. The Loci of Olfactory End-organs in the Blow Fly, Cynomyia cadaverina Desvoidy. J. Exp. Zool. 88(1): 65-93.

11. Frisch, K. von, 1950. Bees - Their Vision, Chemical Senses and Language. Cornell University Press.

12. Frost, S. W., H. Dietrich, 1929. Coleoptera Taken From Bait Traps. Ann. Ent. Soc. Am. 22(3): 427-437.

13. Frost, S. W., 1936. A Summary of Insects Attracted to Liquid Baits. Ent. News 47(3): 64-68, 89-92.

14. ______, 1937. Tests on Baits for Oriental Fruit Moth. J. Ec. Ent. 30(5): 693-695.

15. Gildmeister, E., F. Hoffman, 1913, 1916, 1922. The Volatile Oils. Volumes I-III. John Wiley and Sons Inc.

16. Goulden, C. H., 1956. Methods of Statistical Analysis. John Wiley and Sons Inc.

17. Guenther, E., 1948. The Essential Oils. D. Van Nostrand Company Ltd.

18. Gupta, P. D., A. J. Thorsteinson, 1960. The Food Plant Relationships of the Diamondback Moth (Plutella maculipennis (Curt.)). Ent. Exp. and Appl. 3: 305-314.

19. McIndoo, N. E., 1919. The Olfactory Sense of Lepidopterous Larvae. Ann. Ent. Soc. Am. 12(2): 65-84.

20. Metcalf, Z. P., C. L. Metcalf, 1928. Key to Orders and Families of Insects. North Caroline State College

21. Minnich, D. E., 1929. The Chemical Senses of Insects. Quart. Rev. Biol. 4: 100-112.

22. Moericke, V., 1952. Farben als Landereize fuer gefluegelte Blattlaeuse (Aphidoidea). Z. Naturforsch 7b: 304-309.

23. Obarski, J., 1960. Appearance of Pests on Rape During Spring. Biuletyn Instytutu Ochrony Roslin pp. 69-90.

24. Parry, E. J., 1918. The Chemistry of Essential Oils and Artificial Perfumes. Vol. I. Scott, Greenwood and Son.

25. Roeder, K. D., 1953. Insect Physiology. John Wiley and Sons, Inc.

26. Schwinck, I., 1956. A Study of Olfactory Stimuli in the Orientation of Moths. Proc. of Tenth Int. Cong. Ent. Vol. 2 pp. 577-582.

27. Swain, R. B., 1957. The Insect Guide. Doubleday and Co. Ltd.

28. Thorsteinson, A. J., 1955. The Experimental Study of the Chemotactic Basis of Host Specificity in Phytophagous Insects. Can. Ent. LXXXVII (2): 49-56.

29. Thorsteinson, A. J., 1960. Host Selection in Phytophagous Insects. An. Rev. of Ent. Vol. 5 pp. 193-218.