The Effects of High Voltage Transmission Lines on Honey Bees

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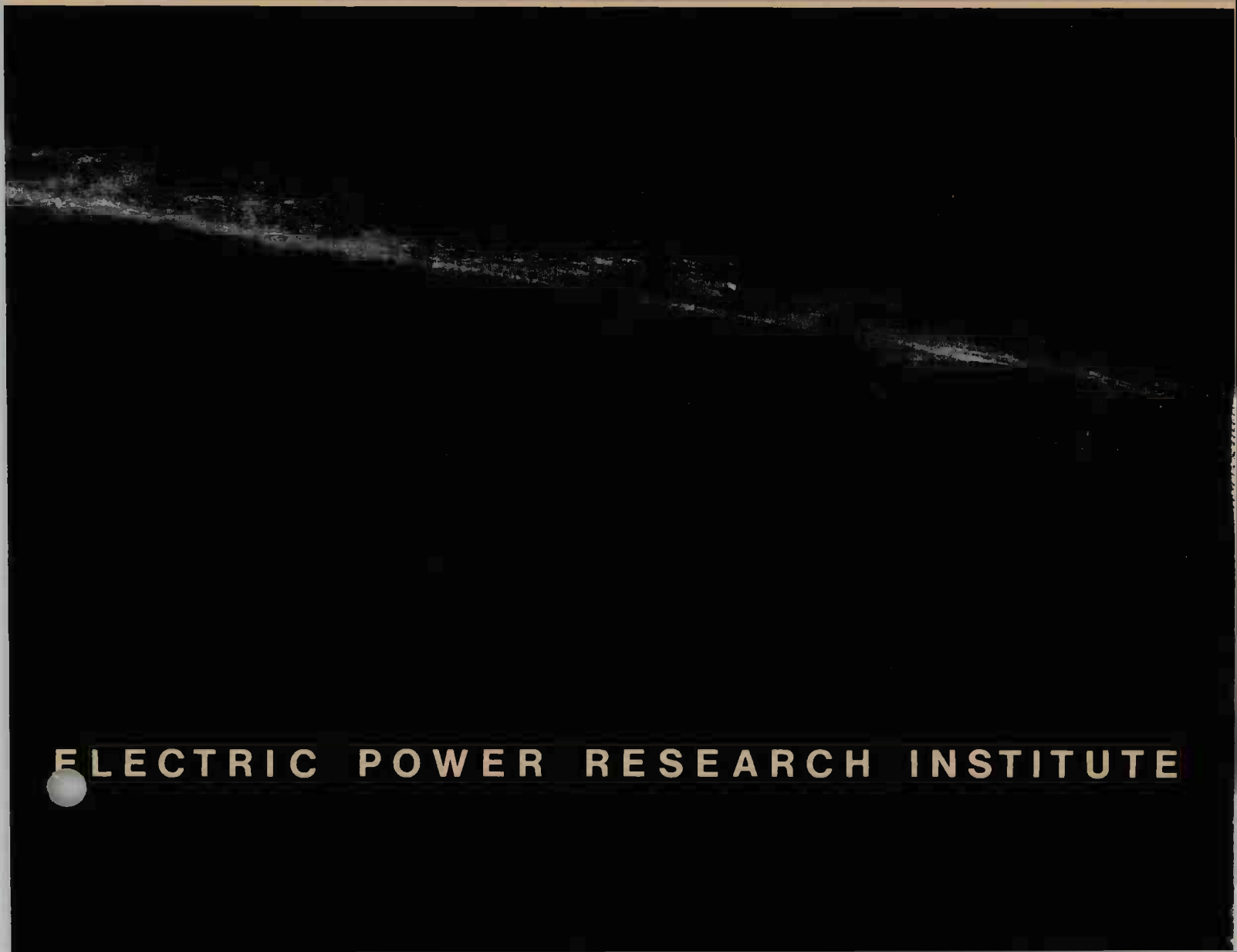

ELECTRIC POWER RESEARCH INSTITUTE

The Effects of High Voltage Transmission Lines on Honey Bees

EA-841
Research Project 934-1

Interim Report, October 1978

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ABSTRACT

Results of the first year's field study of possible effects on honey bees of a 765 kV transmission line are reported. Conventional hives and metal-free hives, shielded and unshielded, were placed under the line (E-field, ca. 7 kV/m) and in a control area (E-field, ca. 10 V/m) about 400 m away. Bees in unshielded conventional hives under the line weighed less, stored little honey whose moisture content was subnormal (hive weight gain was essentially zero), propolyzed hive entrances excessively but not completely, produced fewer pupae but normal numbers of eggs and larvae, and failed to survive the winter. Unshielded metal-free hives under the line had the following normal features: bee weight; hive weight gain; honey moisture content; and numbers of eggs, larvae, and pupae. Their abnormal features were: propolization of hive entrances, but at a slower rate and to a lesser extent than conventional hives; aggressive clusters of bees at lower front hive corners; poor overwintering survival; and possibly higher hemocyte counts.

EPRI PERSPECTIVE

PROJECT DESCRIPTION

The first annual report, The Effects of High Voltage Transmission Lines on Honey Bees, describes preliminary results obtained during the initial year of Research Project 934-1. This report was preceded by a feasibility study, described in EPRI report EA-489.

In this field study, honey-bee hives were placed under a 765 kV transmission line; control hives were situated 400 m away. Both conventional metal-containing and specially constructed polyethylene hives were used. Half of each type were shielded (in a wire mesh faraday cage), producing four exposure conditions.

This project is part of an extensive EPRI program on the biological effects of electromagnetic fields.

PROJECT OBJECTIVES

If there are biological effects from high voltage transmission lines, they will probably be extremely subtle. The complexity of the social behavior of bees and the opportunity they afford for observation of statistically valid numbers of individuals over several generations increase the probability of identifying such subtle effects. Results of research performed elsewhere on honey bees are highly ambiguous (ranging from increased honey production to abnormal and hostile behavior). The identification of any such effects and the clarification of conflicting findings are the primary objectives of this project.

CONCLUSIONS AND RECOMMENDATIONS

Noticeable effects could be seen in the responses of bees in the unshielded metal-containing hives under the line, whereas other colonies did not show such effects. It appears that many of the previously reported effects on bees were due to shocks

from the metal parts in the hives. Additional evidence from this project that this effect is a result of shocks is the more aggressive behavior (stinging and biting) observed among bees clustered at the lower front corners of the unshielded non-metal hives under the line. A tentative explanation is that the unshielded non-metal polyethylene hives carry a buildup of charge because they lack wooden bottoms. Winter survival rate for the colonies under the line appears to be lower than that for colonies at the control site.

Is this outcome a change result, or do even the polyethylene hives cause shocks, as suggested above? At this point we do not know the answer. We think it important to replicate the results and to determine the reason for the low survival rate.

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ACKNOWLEDGMENTS

We thank the personnel of the Electronics Division of IIT Research Institute, Chicago, Illinois, for the engineering support which they provided. We are grateful to William Fern, Commonwealth Edison Technical Center, Maywood, Illinois, for much useful information regarding the 765 kV line used in our experiments. And we are deeply indebted to the administration of the U. S. Army Ammunition Plant at Joliet, Illinois, and particularly to Frank Forsythe, who have generously provided a site and a lab for our studies. We thank Dr. Elbert R. Jaycox, Department of Horticulture, University of Illinois at Urbana-Champaign, for critical reading of the manuscript.

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SUMMARY

Honey bees provide large and sensitive populations for the study of possible transmission line effects on behavior, fecundity, development, and stress. We wished to determine whether metal hive parts are responsible for some of the effects reported in the literature, possibly caused by field enhancement, corona, and collateral phenomena, and to ultimately separate these factors from the ambient electric field alone.

In Part 1, we report a field study of honey bees placed under a 765 kV transmission line in shielded and unshielded metal-free hives and conventional hives. Corresponding control hives were placed in the same area 400 m from the line. Ambient electric fields were approximately 7 kV/m under the line and 10 V/m in the control area. Brood and queen production, hive weight, weights of individual bees, brood chamber temperature, moisture content of honey, in-hive acoustical response to disturbance, propolization of hive entrances, aggression, and overwintering survival were monitored as indicators of the general condition of each hive. The results are as follows:

1. There were no statistically significant differences in numbers of eggs, larvae, pupae, or queen cells among the four categories of metal-free* hive: shielded or unshielded under the line, and shielded or unshielded in the control area. Among the metal-containing hives, there was no difference for eggs, larvae, or queen cells; there was, however, a decrease ($p=.01$) in the number of pupae in unshielded metal hives under the line. This shows that all of the highly complex processes and interactions involved in egg production and brood rearing were not demonstrably affected by the 765 kV line, in the absence of metal hive parts. Another season of sampling with a larger number of hives is underway, in order to obtain additional data.

*The terms metal-free and non-metal are used interchangeably; likewise the terms metal-containing and metal.

2. The metal, unshielded hives under the line showed a significant failure to gain weight ($p=.005$). All other hives, including the unshielded non-metal ones under the line, doubled or tripled their weights in seven weeks.
3. Bees from metal unshielded hives under the line also weighed significantly less than their shielded metal counterparts ($p=.01$).
4. Analysis of brood chamber temperatures revealed no significant differences among any experimental group.
5. Moisture content of honey from metal unshielded hives under the line was significantly less ($.05 > p. > .025$) than that of corresponding shielded hives. The small amount of honey available in these hives limited the sample size, however. The degree of consistency in honey moisture data of all the other groups and the scarcity of honey in this one group are noteworthy.
6. Progressive propolization of hive entrances began in unshielded metal hives about two weeks after placement under the line but complete sealing did not occur, even after several months. Unshielded non-metal hives under the line were propolized at a slower rate and to a lesser extent. No other group exhibited abnormal propolization.
7. Stinging and biting occurred among bees clustered at the lower front corners of unshielded non-metal hives under the line. Data are presented to support a tentative explanation of this phenomenon.
8. Acoustical analyses of bee sound in each hive following jarring of the hive showed overall similarities at all frequencies except 125 Hz. At this frequency, post-disturbance sound level was markedly depressed in unshielded metal-containing test hives while it was elevated in their shielded counterparts.
9. Fifteen of 26 hives (58%) under the line failed to survive the winter, compared to only 2 of 16 (13%) at the control site. Among the colonies under the line, casualties included all 5 of the unshielded metal-containing hives, 2 of 5 shielded metal containing hives, 7 of 9 unshielded metal-free hives, and 1 of 7 shielded metal-free hives. At the control site, casualties included 1 of 3 shielded metal-containing hives, and 1 of 5 shielded metal-free hives. The death of all the unshielded metal-containing hives under the line during the course of the winter was inevitable, given their failure to accumulate adequate honey stores in fall. However, the unshielded metal-free test hives had collected normal amounts of honey and pollen by fall, and yet their overwintering losses were extensive (78%) compared to their shielded counterparts under the line

(14%) and both groups of controls (9%). In many cases, it seemed that their food stores had been depleted, resulting in starvation. Among the survivors as well, food stores generally seemed to be lower in hives at the test area than in hives at the control area.

In Part 2, we compare the results of a small preliminary study of blood cell counts in honey bees hived under the 765 kV transmission line with blood cell counts in honey bees in a control hive about 400 m from the line (electric field 10 V/m). Pre-exposure mean hemocyte counts from bees of both hives were very close ($P \sim 0.5$). Honey bees began to have higher counts about one week after placement under the transmission line and continued to have higher counts for the next 5 weeks when sampling was terminated with the onset of cold weather. In addition to a possible transmission line effect, differences in blood cell counts appear to be related to foraging conditions with significantly higher counts occurring in bees of the control hive when active foraging is going on than when there is no foraging ($P=0.001$).

Trial disc gel electrophoresis of worker blood has separated 21-23 protein bands. Frozen samples of hemolymph from exposed and control bees await analysis in the coming months.

The studies detailed in Parts 1 and 2, with the exception of the acoustic analyses, are continuing on an expanded scale in 1978. This includes a larger number of metal-containing hives assembled by us to match, in all other respects, the metal-free hives, and instrumentally inseminated queens of the same age for greater uniformity in fertility and performance.

PART 1

I. INTRODUCTION

As the number of miles spanned by extreme high voltage transmission lines continues to increase, there is a growing need for accurate research in the area of biological effects of electric fields. Russian studies (1, 2) have indicated possible hazards to switchyard workers. On the other hand, work by R. Hauf (3) and G. Hauf (4), among others, has shown no harmful effects due to exposure.

Altmann (5) reported that frogs, mice, fish, and locusts exposed to E-fields in the laboratory showed increased activity under static fields and AC fields of 10 Hz; oxygen consumption increased parallel to the activity level. AC fields of 1.75 and 5 Hz had a negative effect. However, Knickerbocker et al. (6) with mice, Meda et al. (7) with guinea pigs, and other workers found no effects due to E-field exposure.

Sharks and other elasmobranchs are known to be sensitive to extremely weak AC fields of 1×10^{-6} V/m (8). In monkeys, E-fields of 10 to 56 V/m induce brain potentials of 10^{-5} V/m and shorten their response times (9). This similarity in sensitivity to electric fields becomes even more interesting when one considers that these vertebrates are separated by more than 100 million years of evolution in different elements. Clearly, the convincing demonstration of bio-effects requires selection of appropriately sensitive species, tissues, and behaviors.

The honey bee (Apis mellifera) has been a useful experimental subject in this area because of its highly complex, integrated society and its known sensitivity to earth's magnetic field (10, 11). Warnke and Paul (12) found that bees exposed to E-fields of 7-11 kV/m died after completely sealing hive entrances with propolis. Wellenstein (13) reported that during a mild summer, bees under a 110 kV line collected twice as much honey as controls while suffering a loss of gross weight, and during a cool rainy summer, bees under a 220 kV line displayed increased irritability and a marked tendency to swarm. Numerous laboratory studies have also shown that electric fields may have effects on honey bee behavior and physiology. Altmann (14) found that an electrostatic field of 1.4 kV/m in a Faraday cage caused an increase in O_2 consumption and food intake, and a decrease in life-span. Altmann and Warnke (15) reported that bees in 50-Hz high voltage fields showed an increase in the rate of metabolism following an increase in motor activity: caged

bees in fields of 10 kV/m showed differing metabolic rates; in 20-40 kV/m fields, the metabolic rate increased relative to the field strength; and when fields strengths of 50 kV/m were used, the bees stung one another.

Questions as to dosimetry and experimental control of past studies, and the need for detailed field studies, have led to this investigation. We proposed to conduct a field study using a 765 kV line with shielded and unshielded metal-free hives, as well as a group of standard hives. We wished to determine whether metal hive parts are responsible for some of the effects reported in the literature, possibly caused by field enhancement, corona, and collateral phenomena, and to separate these factors from the ambient electric field alone. Brood and queen production, hive weight, weights of individual bees, in-hive temperature, moisture content of honey, in-hive acoustical response to disturbance, propolization of hive entrances, aggression, and overwintering survival were monitored as indicators of the general condition of each hive. This report presents the results of the first year's study in 1977. At this writing, in spring 1978, an expanded field study is underway.

II. MATERIALS AND METHODS

The site for the establishment of the hives is the U. S. Army Arsenal and Ammunition Plant, Will County, Illinois. The area is a mosaic of woods, pastures, old fields, and cultivated land (mostly corn). Streams are near control and test areas; Commonwealth Edison's transmission line 11216 runs east-west through the latter. From June 17, 1977 to November 20, 1977, the line's nominal voltage of 765 kV ranged from 682 kV to 746 kV, changing hour by hour and day by day; the line was in service 97% of the time (W. Fern, personal communication). Electric fields at hive placements under the line were approximately 7 kV/m, and 10-12 V/m in the control area, about 400 meters away. For a detailed characterization of the electromagnetic environment, see Frazier (16).

Metal-free supers and frames (Dadant) were assembled using Weldwood Plastic Resin and wooden dowels for the supers and Borden's resinous glue, WB-732, (mixed 9 to 1 with PR-205, a urea-based compound which increases water resistance) for the frames. Wax foundation (Dadant), without crimp wire, was used for the frames. Outer and inner covers and bottom boards were polyethylene (Kelly), with no metal parts. The bees were Dadant's Italian honey bees of the "Starline" variety for this initial phase of the study. Fifteen conventional, metal-containing hives were acquired from a local beekeeper to go with our 29 non-metal hives. Pre-treatment baseline data on population and brood size, and hive weight were collected, and then hives were matched with equivalent counterparts. Where hives could not be closely matched with any others, they were placed in those groups for which a larger sample size was most desirable, i.e., unshielded metal-free tests and controls. See Table 1 for a summary of the hive categories.

Ten of the metal-containing hives were placed at pre-determined positions of known E-field strengths under the 765 kV line on June 15, 1977, and 5 at the control site in order to maximize the number of "exposed" hives using our limited supply. Grounded wire-screen E-field shields were placed over all of the hives under the line to reduce in-hive exposure until the desired time; in-hive E-fields were reduced approximately 300-fold to levels around 10 V/m or less. On June 17, shields were removed from 5 of the 10 metal-containing hives under the line while the 5 hives at the control site had their shields installed. While the shields provide

Table 1

Code for individual hive types and treatment groups.
Numbers are used to identify individual hives.

METAL-CONTAINING HIVES

EXPERIMENTAL GROUP		CONTROL GROUP
SHIELDED (SMT)	UNSHIELDED (UMT)	ALL SHIELDED (SMC)
40	39	41
38	37	42
35	36	43
33	34	44
31	32	45

METAL-FREE HIVES

EXPERIMENTAL GROUP		CONTROL GROUP	
SHIELDED (SNT)	UNSHIELDED (UNT)	SHIELDED (SNC)	UNSHIELDED (UNC)
18	8	28	6
0	11	20	24
13	5	21	2
7	29	10	1
4	12	9	27
14	23		15
25	19		17
	16		30
	3		

about a 50-db reduction in E-fields, their large spacing maintains other environmental conditions unchanged (16). For added assurance that the shields produced no side effects of their own, all metal-containing control hives were provided with shields for this phase of the experiment.

On June 20 all metal-free hives were moved from the control area where they had been started in April; before moving they were assigned to shielded/unshielded, test/control groups as described earlier. Uniform treatment required that controls be moved to an adjacent control area (with the conventional hives) while test hives were moved under the line. Grounded shields were immediately placed over appropriate test control hives. E-fields in unshielded hives under the line were 0.6 to 4.5 kV/m in metal-free hives and 1 to 8.3 kV/m in metal-containing hives (16). Figure 1 shows the various hive types; Figures 2 and 3 show the test and control areas, respectively.

Standard beekeeping procedures were used in maintaining the hives, including springtime feeding with 1:1 sugar solution, administration of antibiotics in spring and fall, weekly inspection of all frames in each hive, addition of supers as needed, and removal of excess burr and brace comb. Nothing was done to differentially alter any of the experimental results due to beekeeping procedures.

A. BROOD ESTIMATION

A measure of population size of a colony was obtained by estimating amount of brood. Our method makes use of the regular nature of honeycomb and the systematic egg-laying pattern of the typical queen. Most brood appears as solid areas on the frame, and by counting the number of cells on two sides of a rectangular or square patch of brood, an estimate of the total number of occupied cells in the patch was obtained by multiplying the two figures. Other, more irregular or scattered patterns were counted directly. Each category of immature bee - egg, larva, and capped pupa - was estimated separately. As a check on the accuracy of the method, 27 frames (54 sides of brood) were first estimated, then actually counted. The result was a percent error of -3.2. Periodically we re-checked our accuracy by actually re-counting each type of brood for randomly selected frames. The error remained ca. 3% or lower.

Each frame in every hive was marked with a number, with each side designated A or B. To estimate a given hive, frames were removed and examined, one by one, the results recorded, and the frames replaced. A cover was placed on the hive during

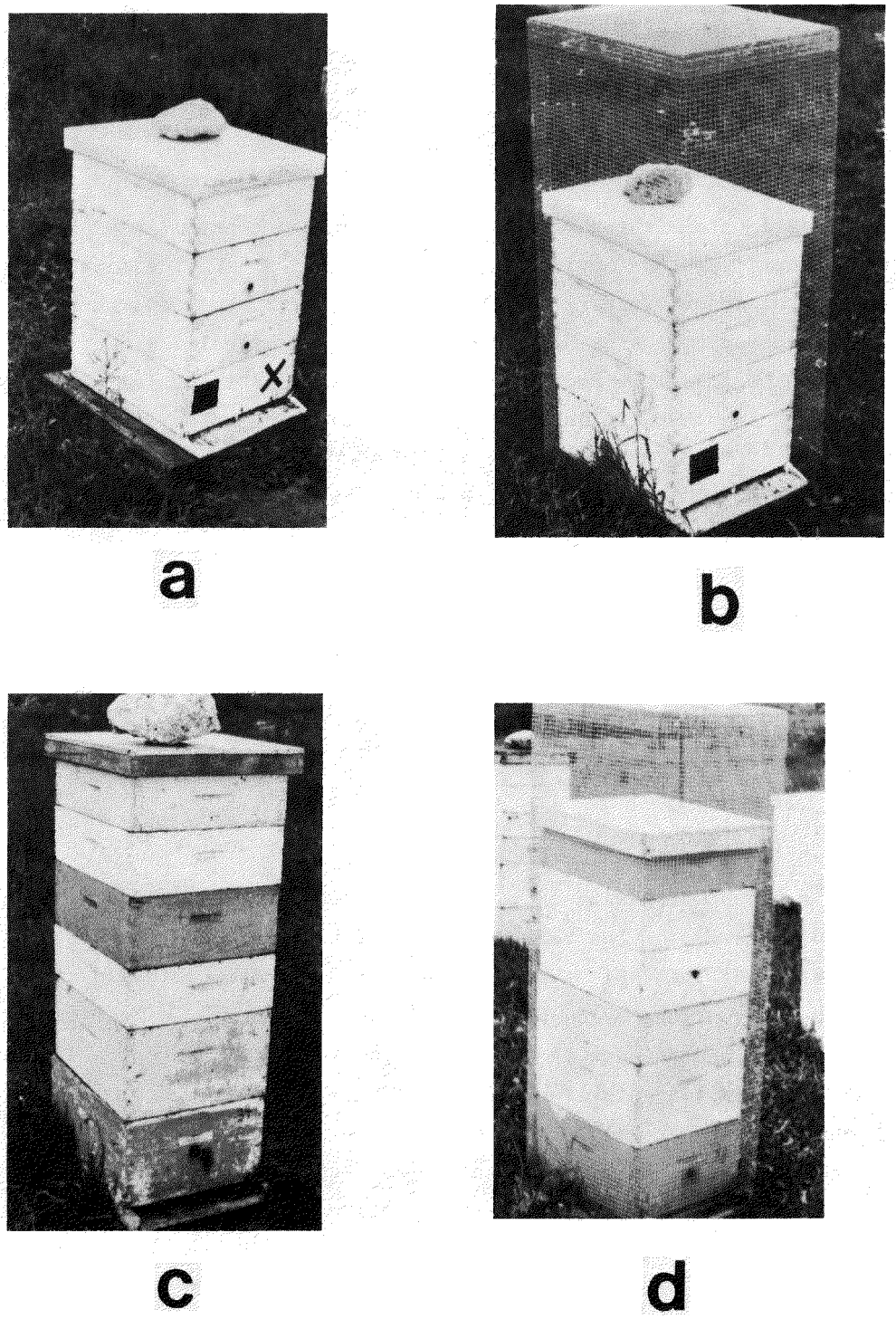

Figure 1. Hive Types: a. Metal-free, unshielded; b. Metal-free, shielded; c. Metal-containing, unshielded; d. Metal-containing, shielded.

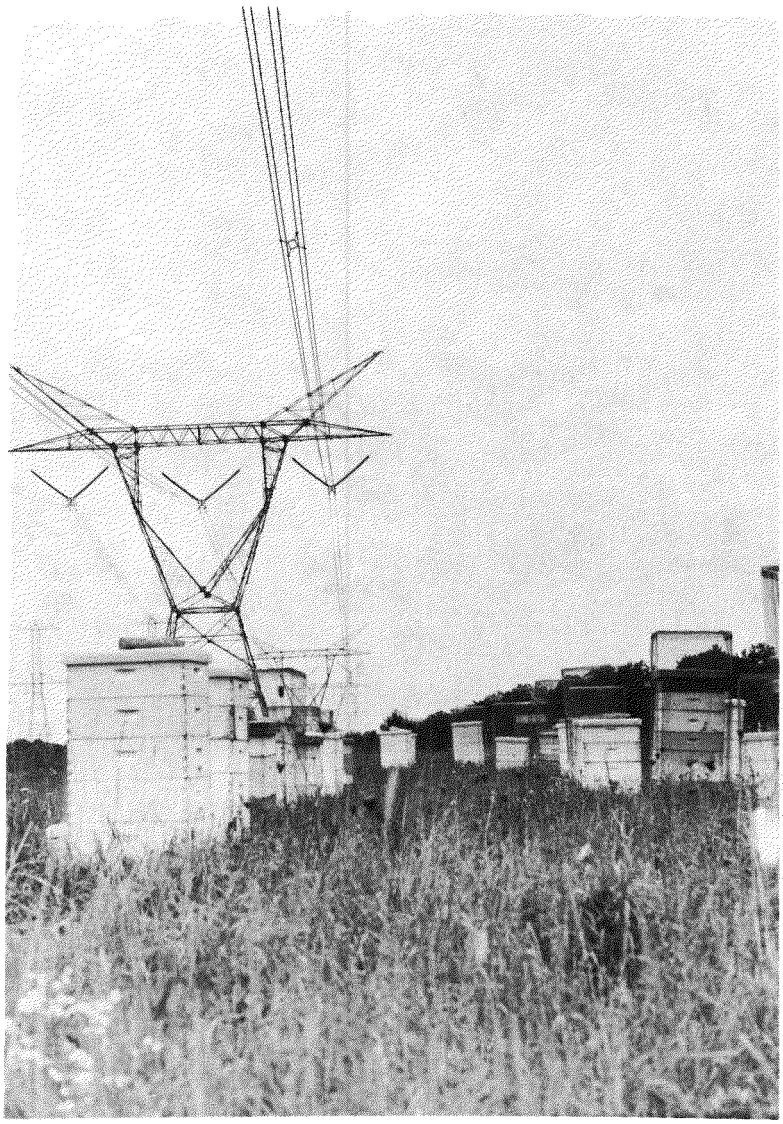

Figure 2. Test Hive Emplacement with 765 kV Transmission Line Overhead

Figure 3. Control Hive Emplacement Approximately 400 m from Transmission Line.

this operation to reduce disturbance. The shielded hives had their shields replaced each time frames were removed, to minimize exposure. Also, frames were analysed far enough from the line to provide minimal exposure; average counting time per frame was 10 min. Queen cells were counted and destroyed to forestall swarming.

The initial brood count was taken just prior to the start of the experiment, and three subsequent counts were made, at four-week intervals to avoid overlap of generations being counted. Each sampling took 5 days to complete, but the hives were always analysed in the same order to maintain the same 28-day interval for each hive.

B. HIVE WEIGHT

Each hive was weighed at weekly intervals and approximate net weights of metal-free hives were obtained by subtracting the weights of the empty supers and frames, along with top and bottom boards and inner covers. For the metal-containing hives, the boxes were not of uniform construction, and all hives had the same number of hive bodies, so only the gross weight is presented here.

The scale was an American platform balance model 104 (error ± 1.5%).

C. BEE WEIGHT

Worker bees were collected at random from the uppermost honey supers of each colony (17), then placed in jars and cold-immobilized within an hour after capture and weighed in groups of 25 using an Ainsworth balance (error ± 0.1 mg). Four such samples were taken from each hive at the same time, in late August.

D. IN-HIVE TEMPERATURE

Three-quarter inch holes were drilled at three positions in the rear of each super: near the left side wall, the middle, and near the right side wall. These holes were normally plugged with tight-fitting corks (Fig. 4). Readings were taken by removing the corks and inserting Daigger thermometers (76 mm X 1 mm) to a uniform depth between the frames. The thermometers were fitted through rubber stoppers which effectively sealed the holes, eliminating exposure and reducing disturbance while providing an easy way to insert the thermometers a uniform distance of 15 cm. Nine thermometers were inserted into the lowest three boxes of each hive, three per super, and readings were made at 5, 10, and 15 min. As a corollary, several

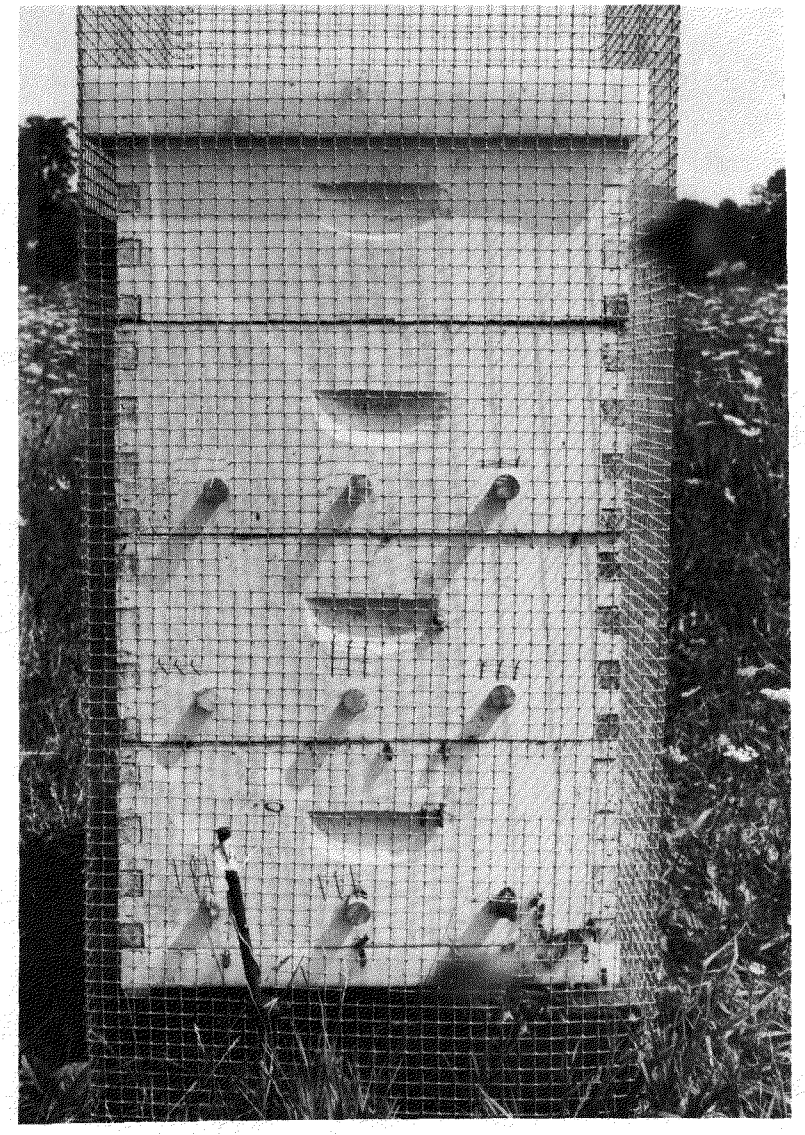

Figure 4. Rear of Shielded Metal-free Hive under Transmission Line with Grounding Rod and Corked Holes for Thermometers.

hives were sampled, and subsequently inspected to determine the approximate numbers of bees as well as the composition of the surrounding frames at each sampling position.

For the metal-containing hives, we were not at liberty to drill holes in the supers, so we sampled between supers instead of between frames, and therefore these data can only be compared with other metal-containing hives, not with metal-free hives.

E. MOISTURE CONTENT OF HONEY

Moisture content of honey in each hive was measured with an Atago honey refractometer (error ± 0.1%). Capped honey cells were analysed from the center of frames taken from the uppermost honey chamber of each hive.

F. IN-HIVE ACOUSTICAL LEVEL

Instrumentation for acoustical measurements and methods of analysis are described by Schechter (18). From our brood inventory we knew which supers contained the greatest amounts of brood, and therefore, adults to care for it. We monitored acoustical levels by prying open the hive between the two most populous boxes and inserting the portable acoustical probe, inside a protective plastic sheath. The probe was calibrated at each hive, inserted, and a sample recording was made. Then, the hive was disturbed by dropping one side from a height of one inch, and the bee response was recorded immediately, and at 5, 10, and 15 min.

G. STATISTICAL ANALYSIS

Following procedures described by Sokal and Rohlf (19), one-way analysis of variance was performed on the data for temperature, bee weight, and moisture content of honey. Hive weights were analysed with a two-way ANOVA, using the increment in weight from one sample to another, and also with a one-way using the final weights only. Model I regression analysis was carried out on the brood data of each life stage to compare the population development of the various experimental types. Analyses were made among metal-free hive types or metal-containing types, not between them.

III. RESULTS

A. BROOD

Table 2 shows mean values for each type of brood for each experimental group, exclusive of values* which were abnormally low due to loss of queen; the data are graphed in Figures 5-7 (see also Table 9). Table 3 summarizes number of queen cells observed (and subsequently destroyed) in each type of hive for the entire observation period. Statistically there is no difference in numbers of eggs, larvae, or pupae among the four categories of metal-free hive, viz. shielded and unshielded under the line, and shielded and unshielded in the control area. Also there is no difference among the metal-containing hives for eggs or larvae throughout the sampling period. There was, however, a significant decrease ($p=.01$) in the number of pupae in unshielded metal-containing hives under the line following exposure, in comparison to the other metal-containing groups. The same 1% level of significance was obtained using each of the following three treatments of the data for pupae: exclude data for queenless hives for the periods in which they were queenless; exclude data for queenless hives for the entire study; or include all data, including zero values. There was no statistically significant difference in queen cell production for any group.

B. HIVE WEIGHT

These data were statistically analysed in two ways: using only the final weight; and using the increment in weight gain between each sampling period. Each method revealed no significant differences among any groups except for the unshielded metal-containing test hives. As can be seen from Figures 8 and 9 (see also Table 10) this group of hives failed to show any increase in weight for the duration of the exposure period whereas all other groups exhibited normal, significant gains. The difference between unshielded metal tests and shielded metal tests was highly significant ($p=.005$). The consistently lower weight gains recorded for the metal-free hives (shielded and unshielded) under the line, although statistically not significant, warrant further study.

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*Excluded hive numbers and periods, respectively: 35, 3-4; 33, 1-2; 32, 3; 43, 3-4; and 45, 3.

Table 2

Summary of mean brood production for each hive type and treatment. Samples taken at -2, 26, 54, and 82 days following the start of the experiment. Hives with queen loss or failure not included. See Table 1 for explanation of hive code.

HIVE TYPE	EGGS				LARVAE				PUPAE			
	1	2	3	4	1	2	3	4	1	2	3	4
SMT	3369	3243	2420	3306	5842	5876	4870	4205	10084	12369	11201	13022
UMT	2808	2860	2750	2510	5866	4368	3581	2582	12870	4795	7028	4544
SNT	2169	2788	2279	3012	4067	4223	4285	5985	9822	10326	11869	12834
UNT	3287	3074	2883	3435	4631	5190	4246	5566	10402	12966	11883	11455
SMC	2530	2430	2448	1215	4829	4836	4691	4325	10315	8733	11013	6155
SNC	2187	2250	2224	3106	3525	4708	4944	6257	10337	10592	12401	13705
UNC	2574	2523	3207	3698	4405	5173	5140	6284	10493	13966	14361	13813

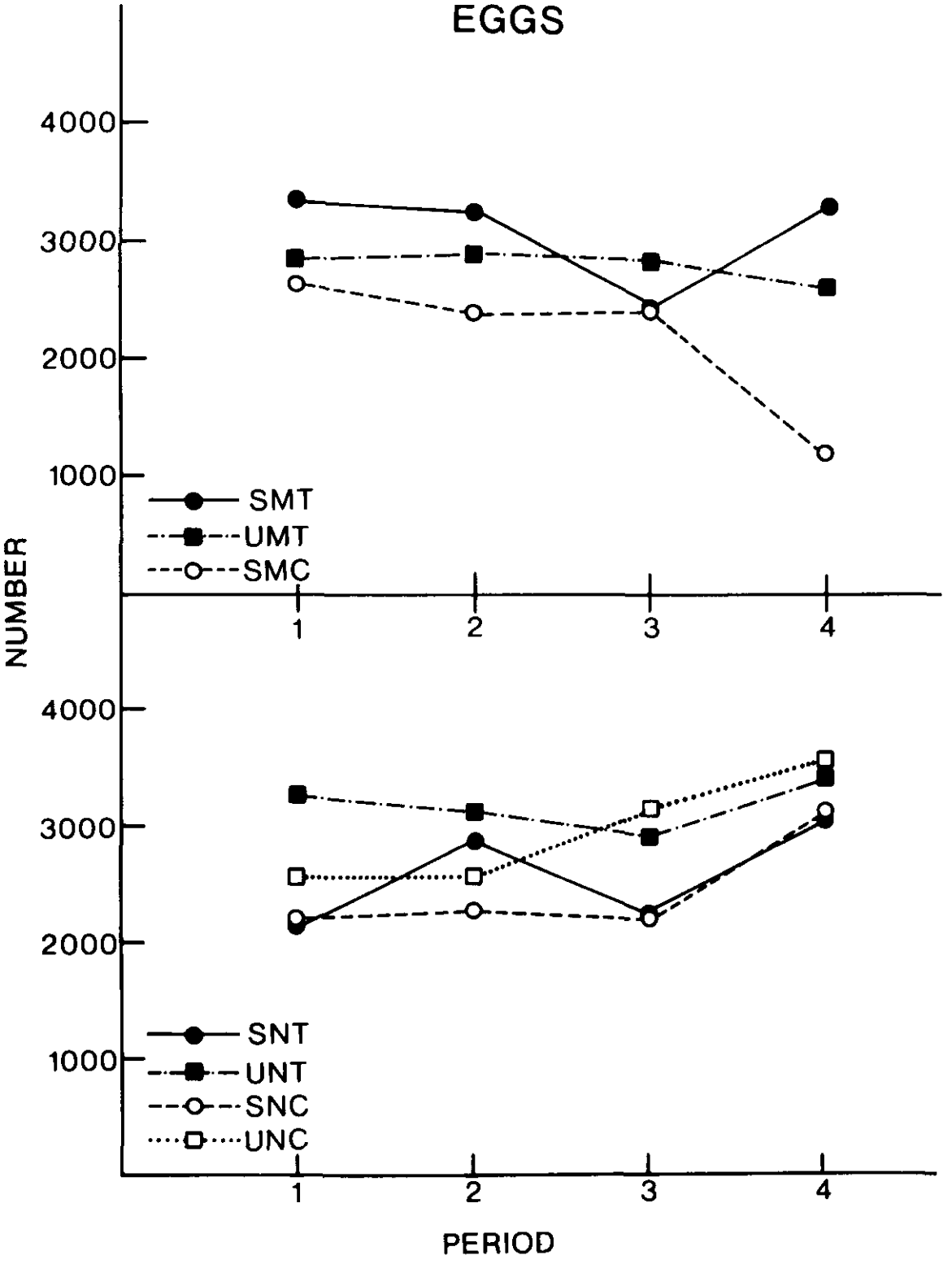


Figure 5. Average Egg Production, per 28-day Interval, in Hives Grouped According to Type and Treatment. S, Shielded; U, Unshielded; M, Metal-containing; N, Metal-free; T, Test; and C, Control.

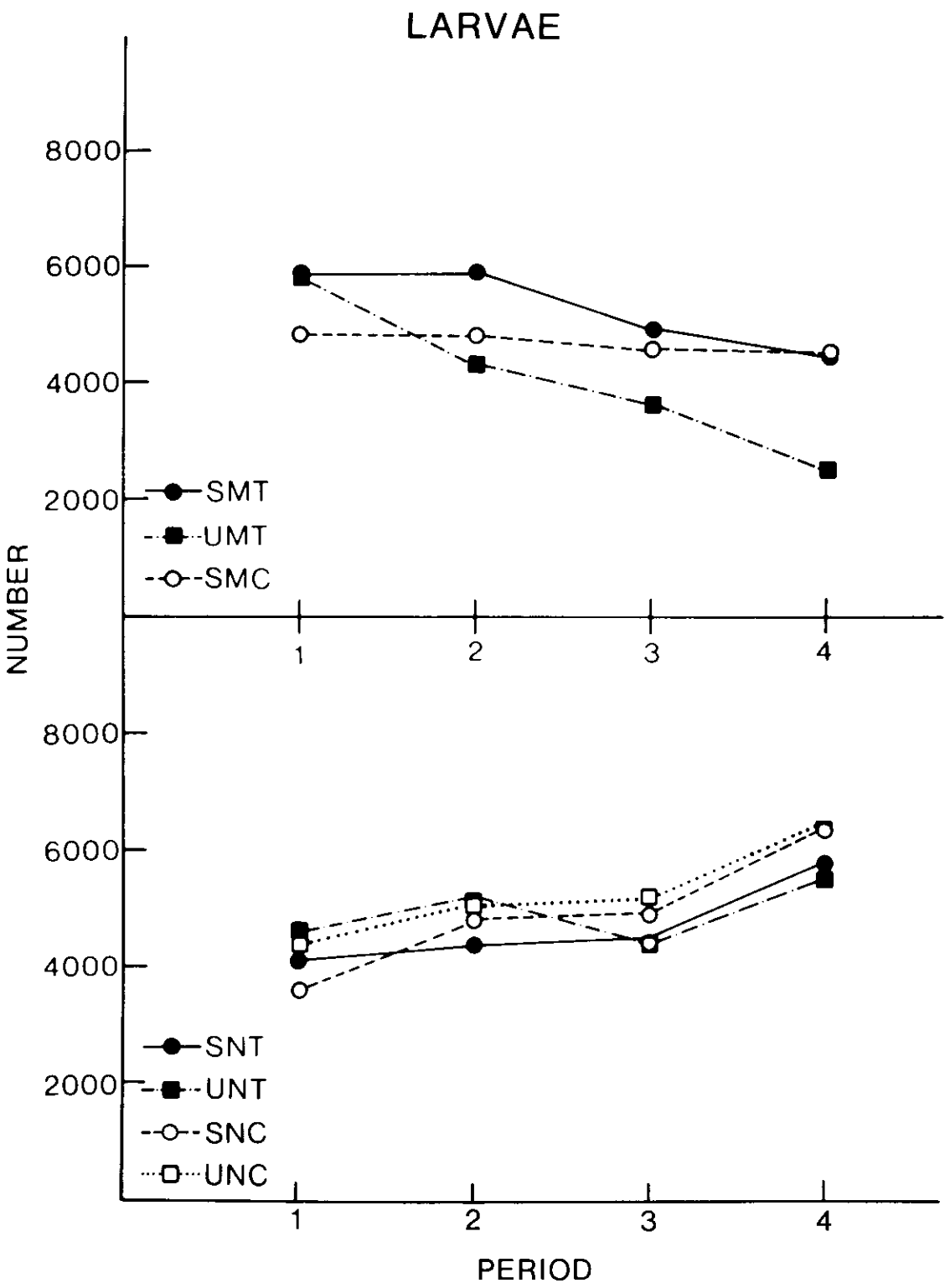


Figure 6. Average Larval Production, per 28-day Interval, in Hives Grouped According to Type and Treatment. See Figure 5 for Explanation of Legend.

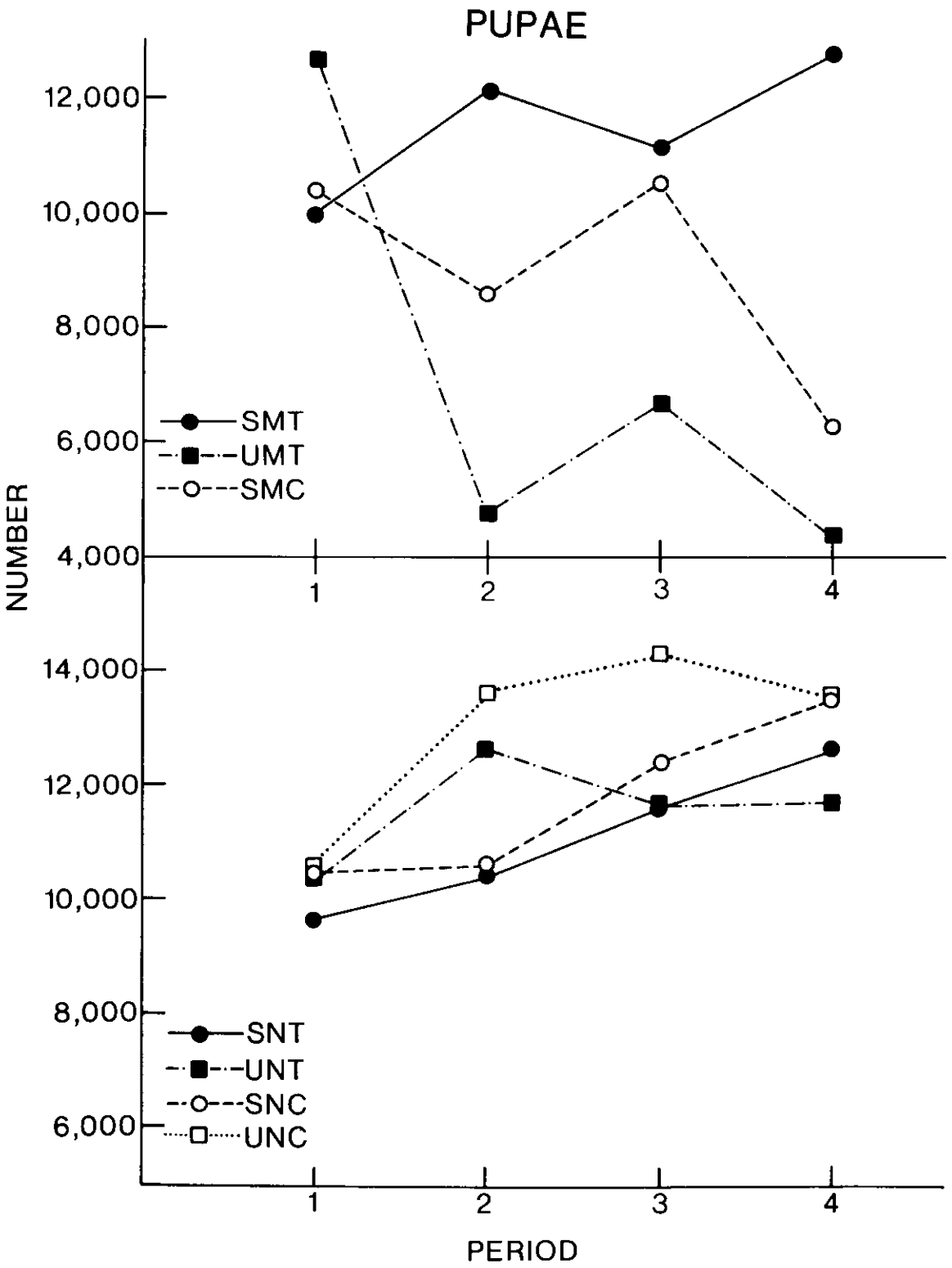


Figure 7. Average Pupal Production, per 28-day Interval, in Hives Grouped According to Type and Treatment. See Figure 5 for Explanation of Legend.

Table 3

Queen cell production: mean number (and standard deviation) per hive group per observation period.

HIVE TYPE	1	2	3	4
SMT	5.0 (4.69)	4.8 (2.86)	5.3 (10.26)	2.8 (3.27)
UMT	4.8 (4.56)	4.0 (2.92)	3.2 (2.17)	2.0 (1.73)
SNT	5.0 (4.73)	3.86 (2.79)	5.43 (3.99)	9.86 (8.71)
UNT	5.33 (5.43)	6.11 (6.97)	5.0 (5.29)	7.44 (5.41)
SMC	7.8 (9.65)	14.2 (12.48)	7.6 (10.45)	7.8 (9.26)
SNC	3.6 (3.78)	12.2 (15.1)	8.2 (8.07)	11.4 (11.99)
UNC	9.88 (7.12)	7.63 (6.70)	10.5 (8.12)	13.38 (7.03)

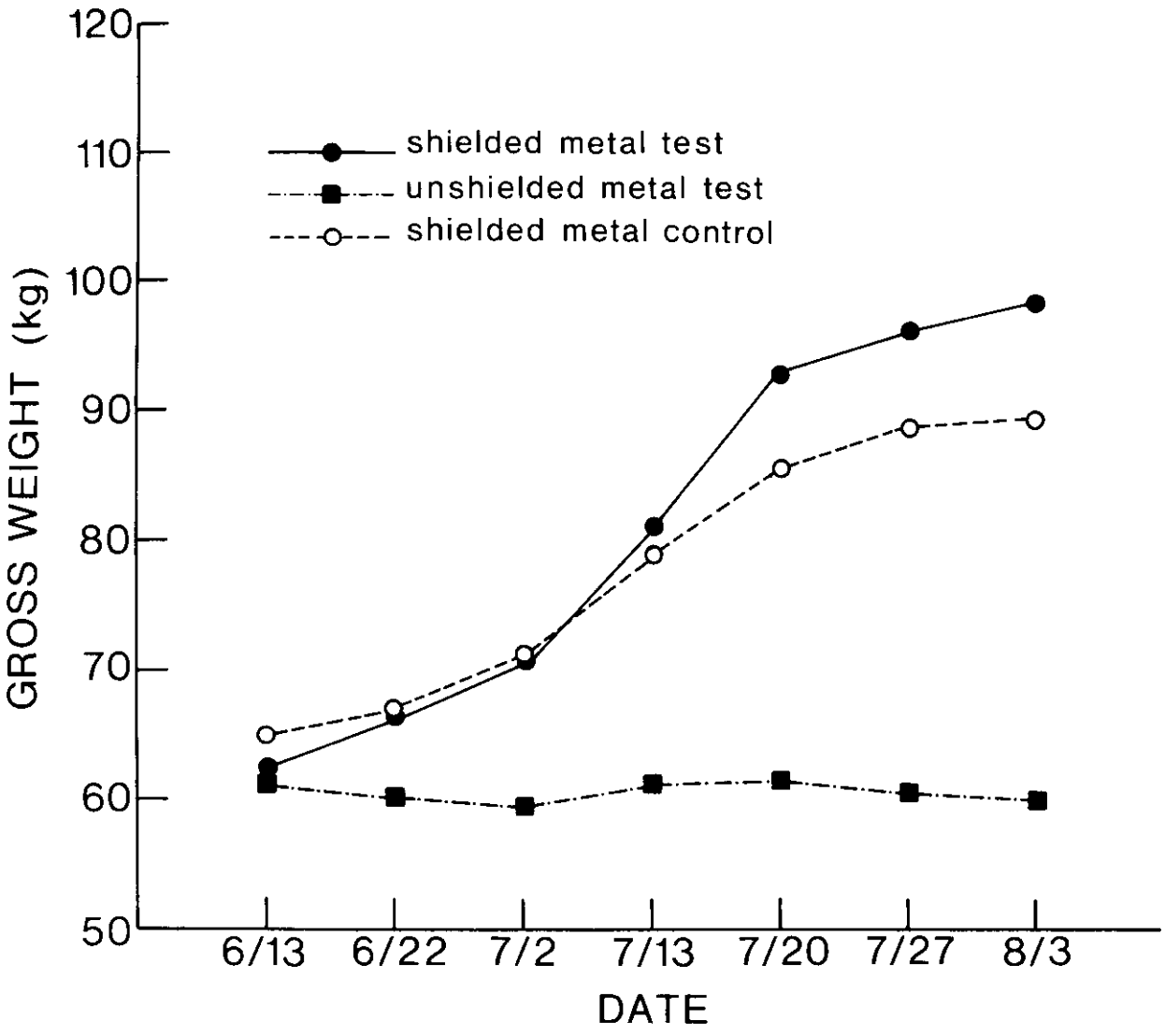


Figure 8. Gross Weight Gain of Metal-containing Hives before Start of Experiment (6/13) and after Emplacement (6/17).

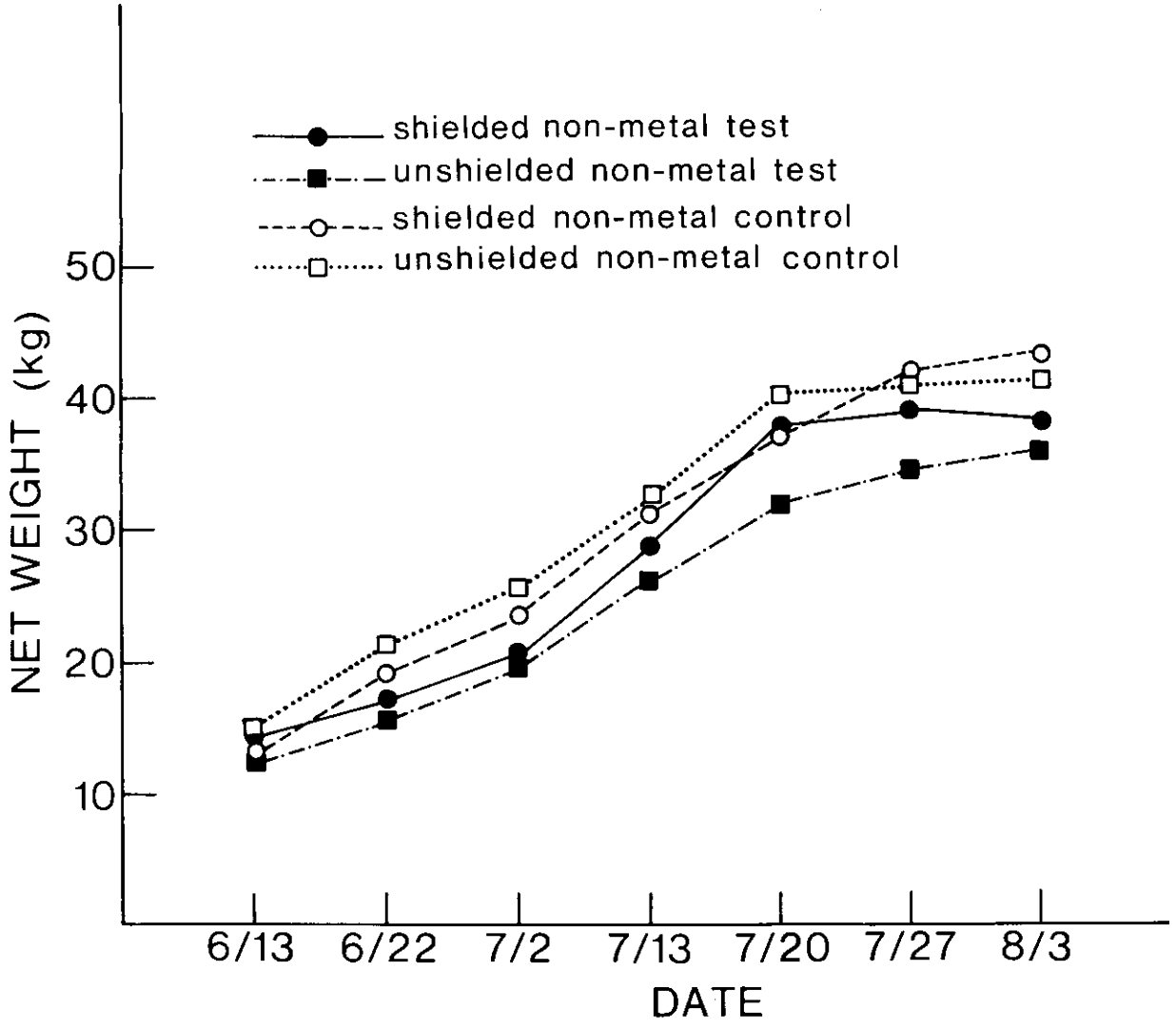


Figure 9. Net Weight Gain of Metal-free Hives before Start of Experiment (6/13) and after Emplacement (6/20).

C. BEE WEIGHT

Table 4 summarizes mean bee weights based on 4 samples, 25 workers per sample (see also Table 11). Normal weight is about 127 mgm and the only group which showed a significant difference from the rest was the unshielded metal-containing test hives, with a mean weight of 116 mgm, significantly lower ($p=.01$) than their shielded counterparts under the line and in the control area.

D. IN-HIVE TEMPERATURE

Temperature data are summarized in Table 5. Because of the variable composition of the frames and differing numbers of adults, we chose to use only the highest reading in comparing the hives, since this would correspond to the brood chamber, with the maximum number of bees present. As our corollary study indicated, the areas surrounded by brood frames, with the largest number of workers present, gave the highest temperature readings whereas frames containing honey or empty foundation had highly variable temperature readings, depending on the numbers of bees present at the moment.

Analysis of our data for brood chamber temperatures revealed no significant differences among any of the experimental groups.

E. MOISTURE CONTENT OF HONEY

Table 6 summarizes these results. No significant differences were found among any of the experimental groups with the exception of the unshielded metal-containing test hives. This group had a lower moisture content (16.87%) than any of the others ($0.5 > p > .025$).

F. GENERAL BEHAVIOR

On July 1, about 2 weeks after they were placed under the line, we noticed abnormal propolis build-up in the unshielded metal-containing hives, principally at the entrances. All of these hives exhibited progressive, heavy propolization, whereas none of the shielded hives did. Among the metal-free hives, the build-up was much less and at a slower rate, but again all of the unshielded tests showed some abnormal propolis deposition while none of the shielded or control hives had abnormal build-up. The extent of closure of hive entrances gradually increased but never became complete. Figures 10-18 show examples of each experimental type; these photographs were taken in mid-August. In most of the affected hives there was

Table 4

Mean weight per bee per hive group.

HIVE TYPE	MEAN BEE WEIGHT (gm)
SMT	0.1269
UMT	0.1157[1]
SNT	0.1270
UNT	0.1270
SMC	0.1266
SNC	0.1273
UNC	0.1279

[1]Significant difference (p = .005) in unshielded metal test hives only.

Table 5

In-hive temperatures: mean highest recorded values per hive group (^{o}C).

HIVE TYPE	MEAN HIGHEST TEMPERATURE
SMT	36.4
UMT	36.8
SMC	37.0
SNT	37.6
UNT	37.6
SNC	37.8
UNC	37.8

Table 6

Moisture content of honey: mean value per hive group.

HIVE TYPE	MEAN VALUE
SMT	18.37
UMT	16.87
SNT	17.92
UNT	17.95
SMC	18.09
SNC	17.84
UNC	18.01

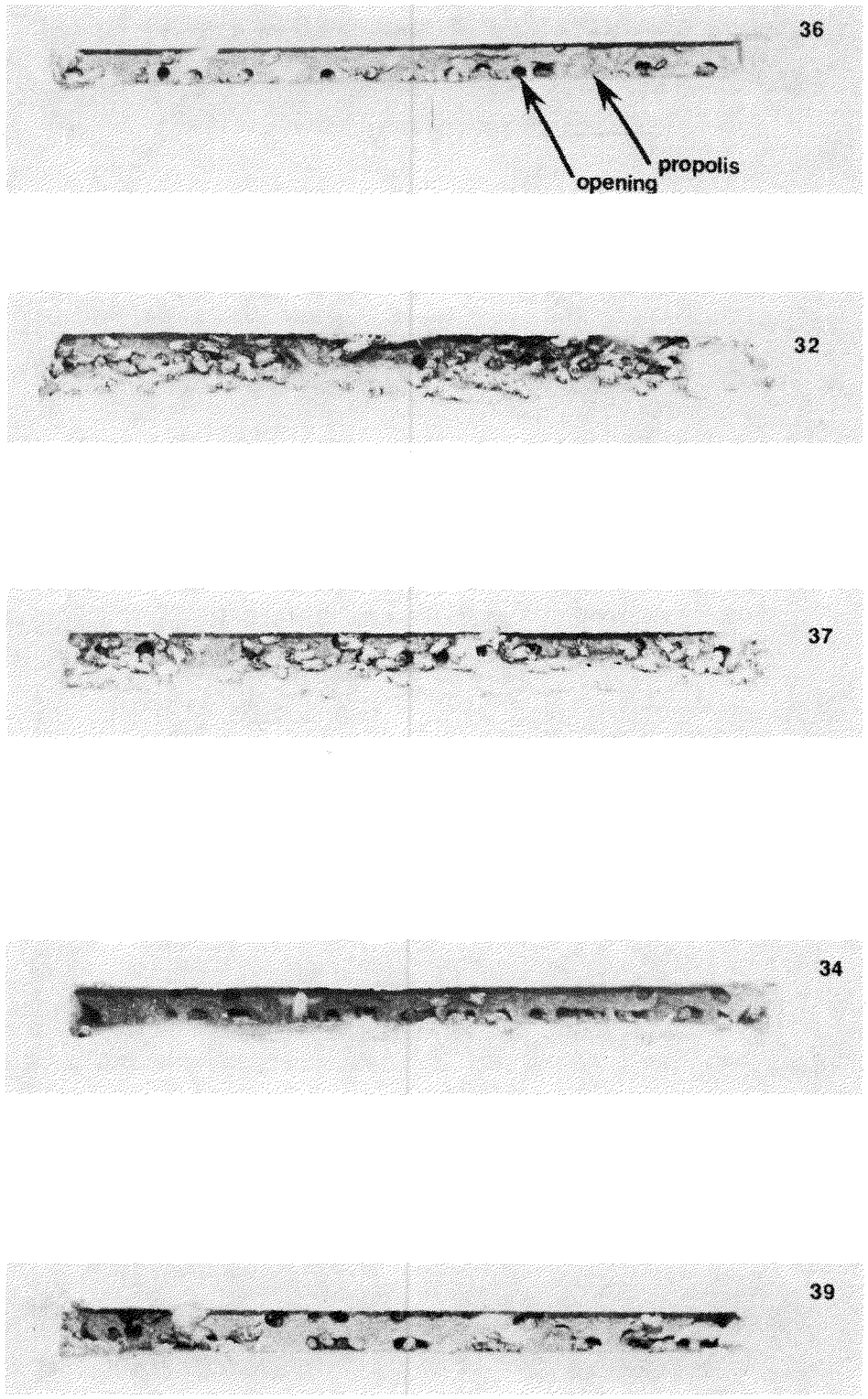


Figure 10. Unshielded Metal-containing Test Hives Showing Heavy Propolization of Hive Entrances About 8 Weeks after Emplacement under the Line.

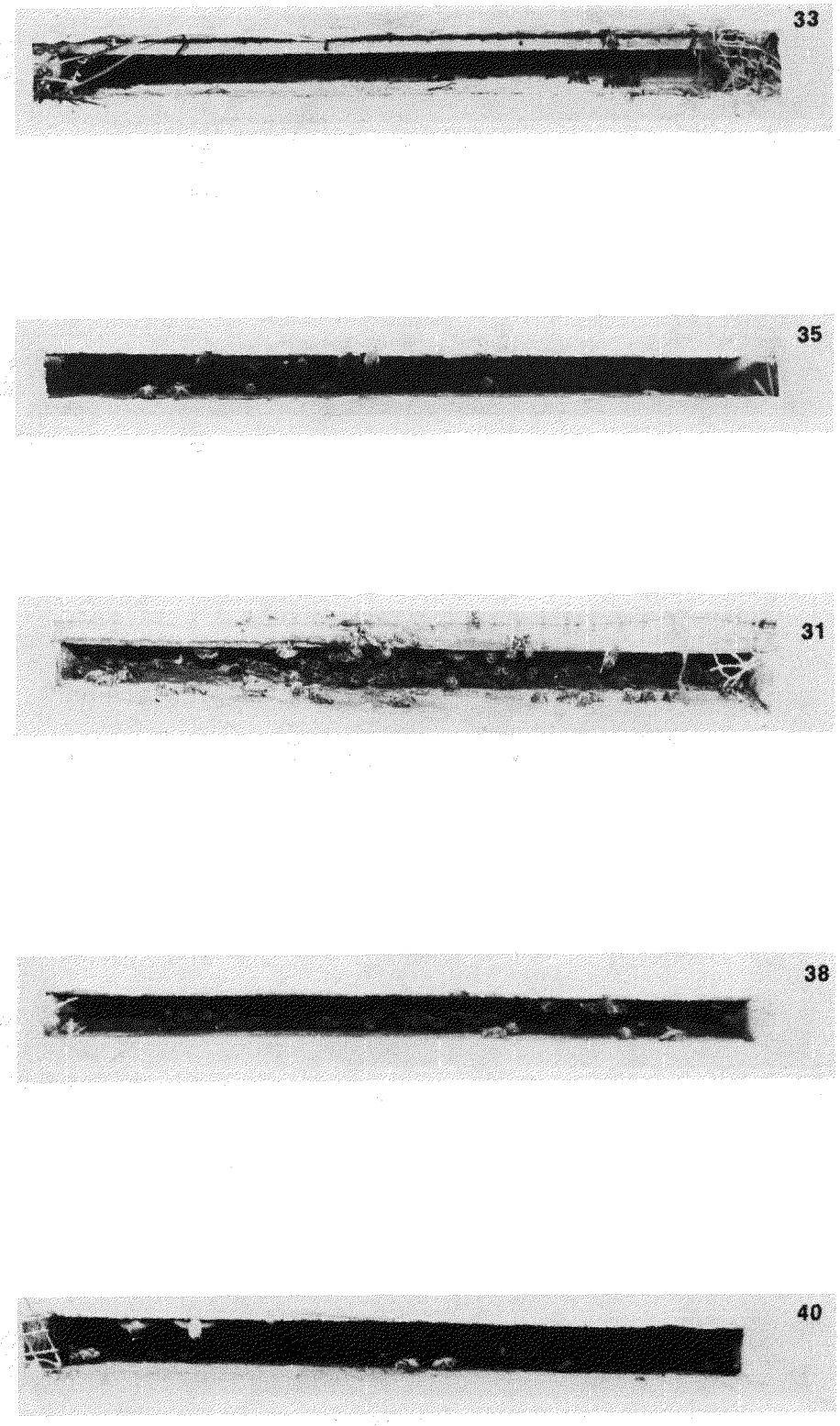


Figure 11. Shielded Metal-containing Test Hives Showing No Propolization of Hive Entrances After 8 Weeks under the Line.

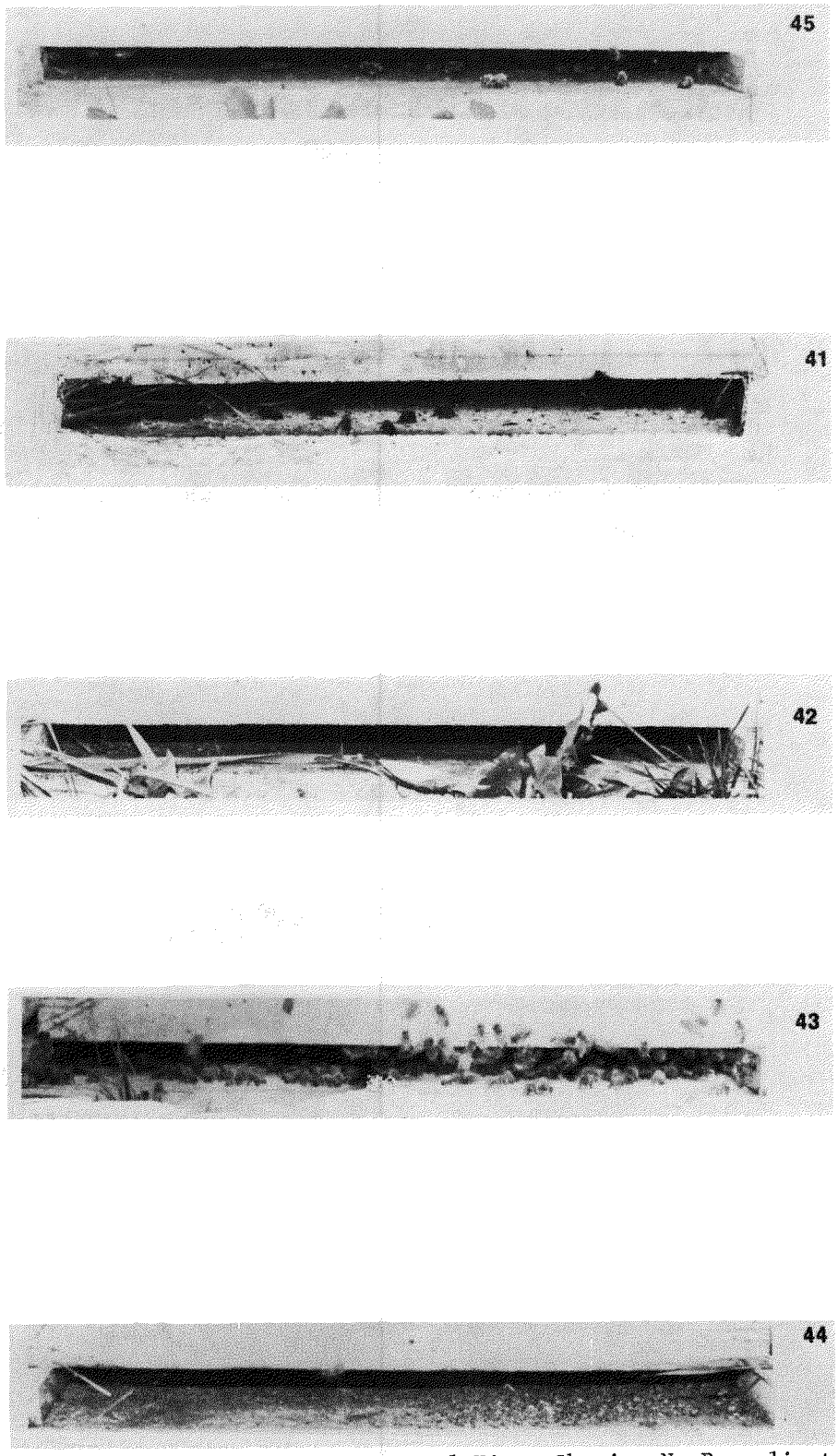


Figure 12. Shielded Metal-containing Control Hives Showing No Propolization of Hive Entrances After 8 Weeks.

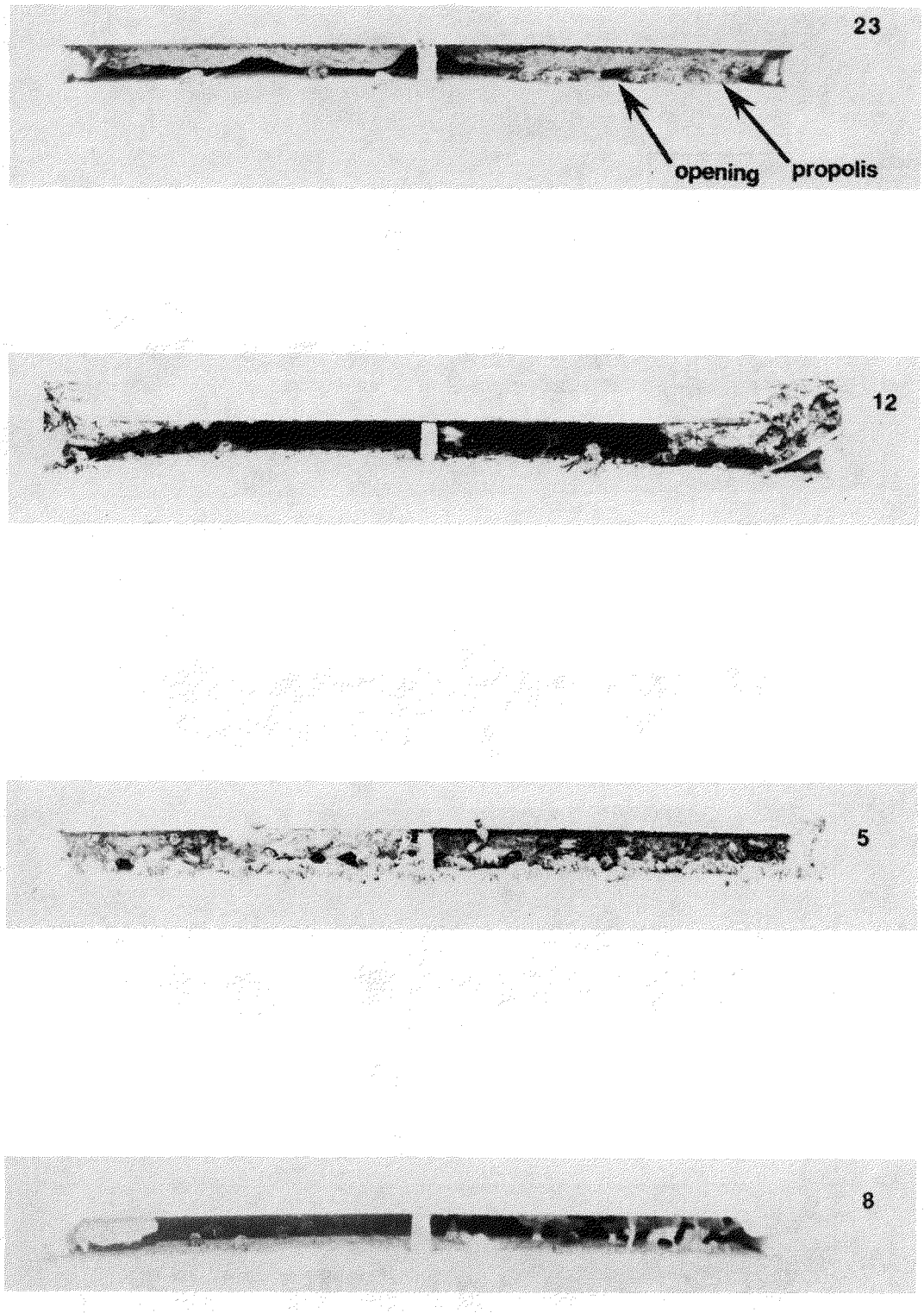
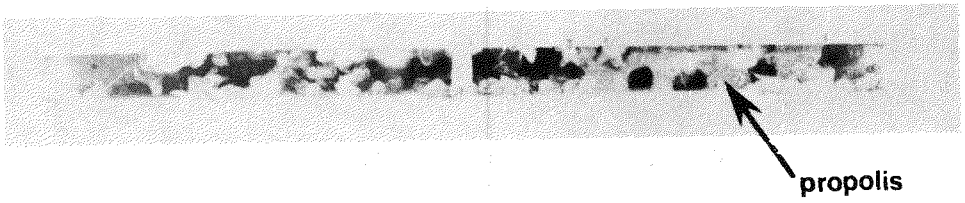
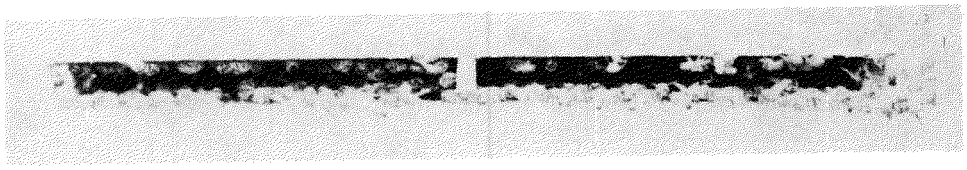


Figure 13. Unshielded Metal-free Test Hives Showing Partial Propolization of Hive Entrances After 8 Weeks under the Line.





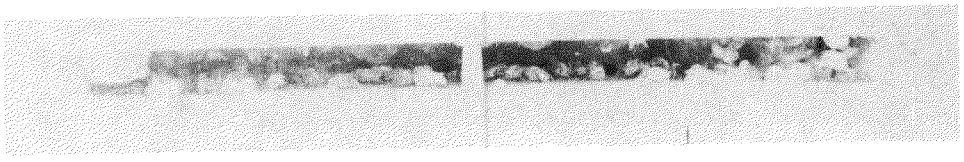


Figure 14. Same as Figure 13.

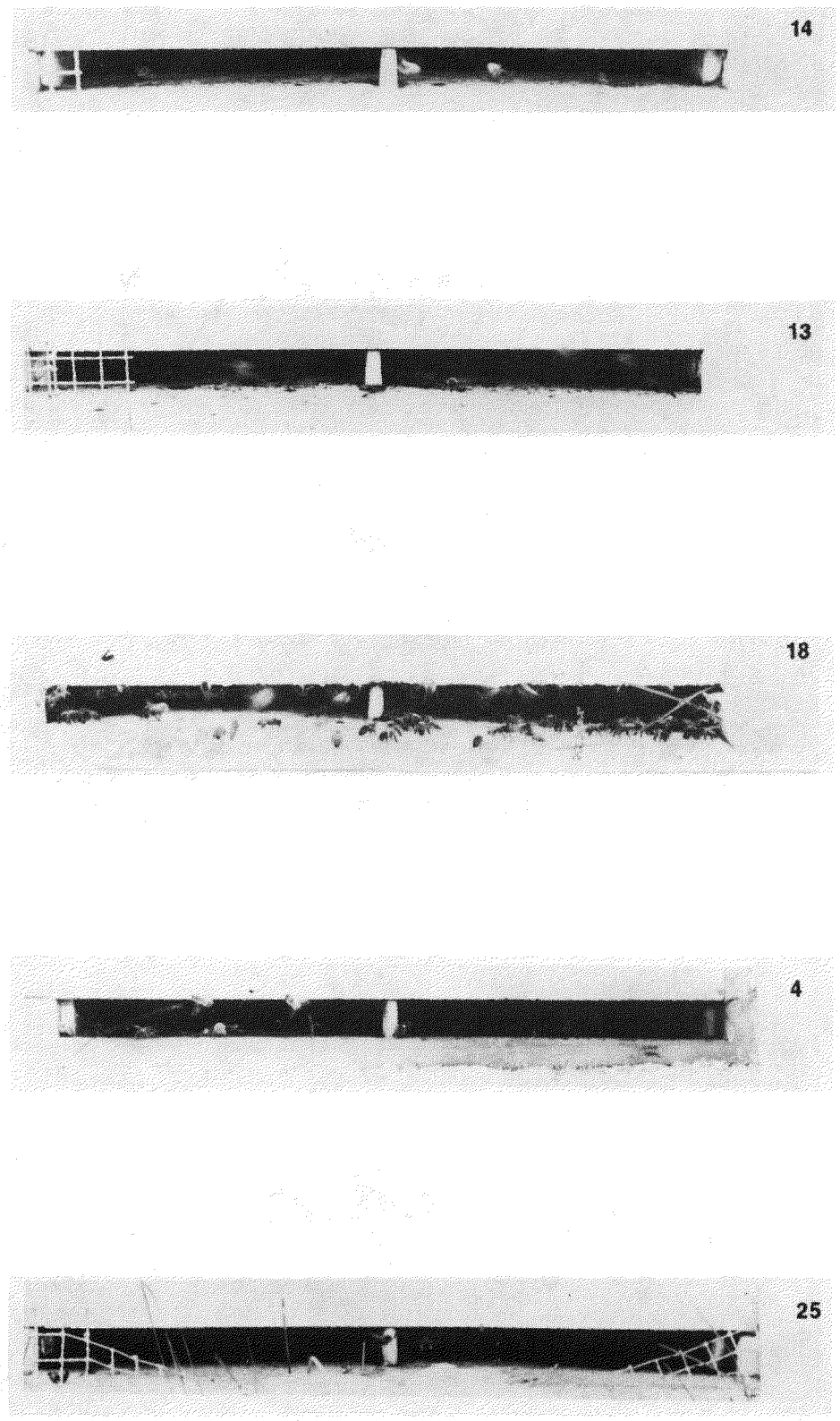
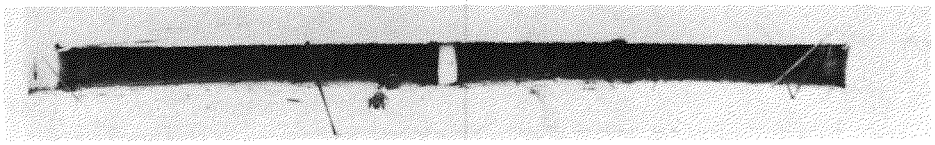
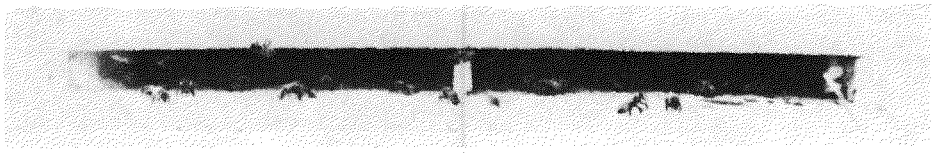
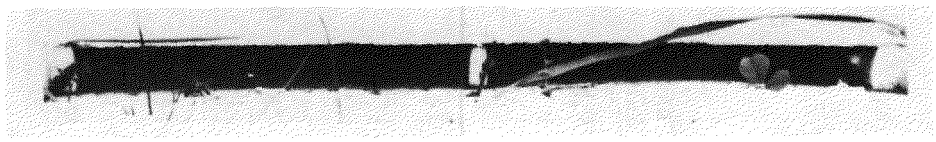


Figure 15. Shielded Metal-free Test Hives Showing No Propolization of Hive Entrances After 8 Weeks under the Line.







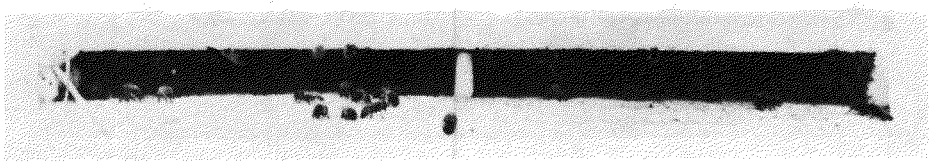
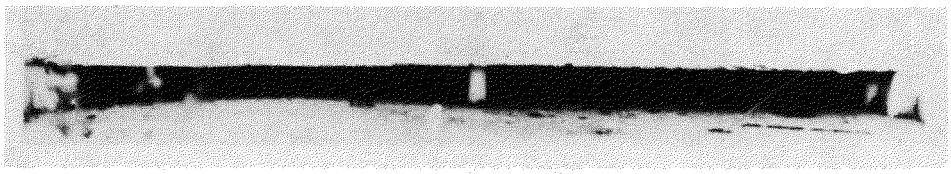
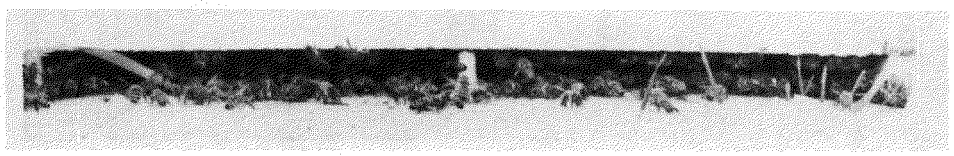


Figure 16. Unshielded Metal-free Control Hives Showing No Propolization of Hive Entrance After 8 Weeks.





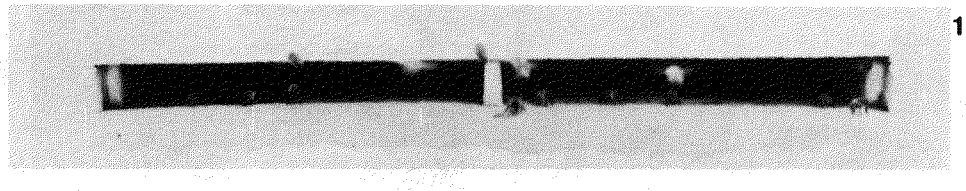


Figure 17. Same as Figure 16.

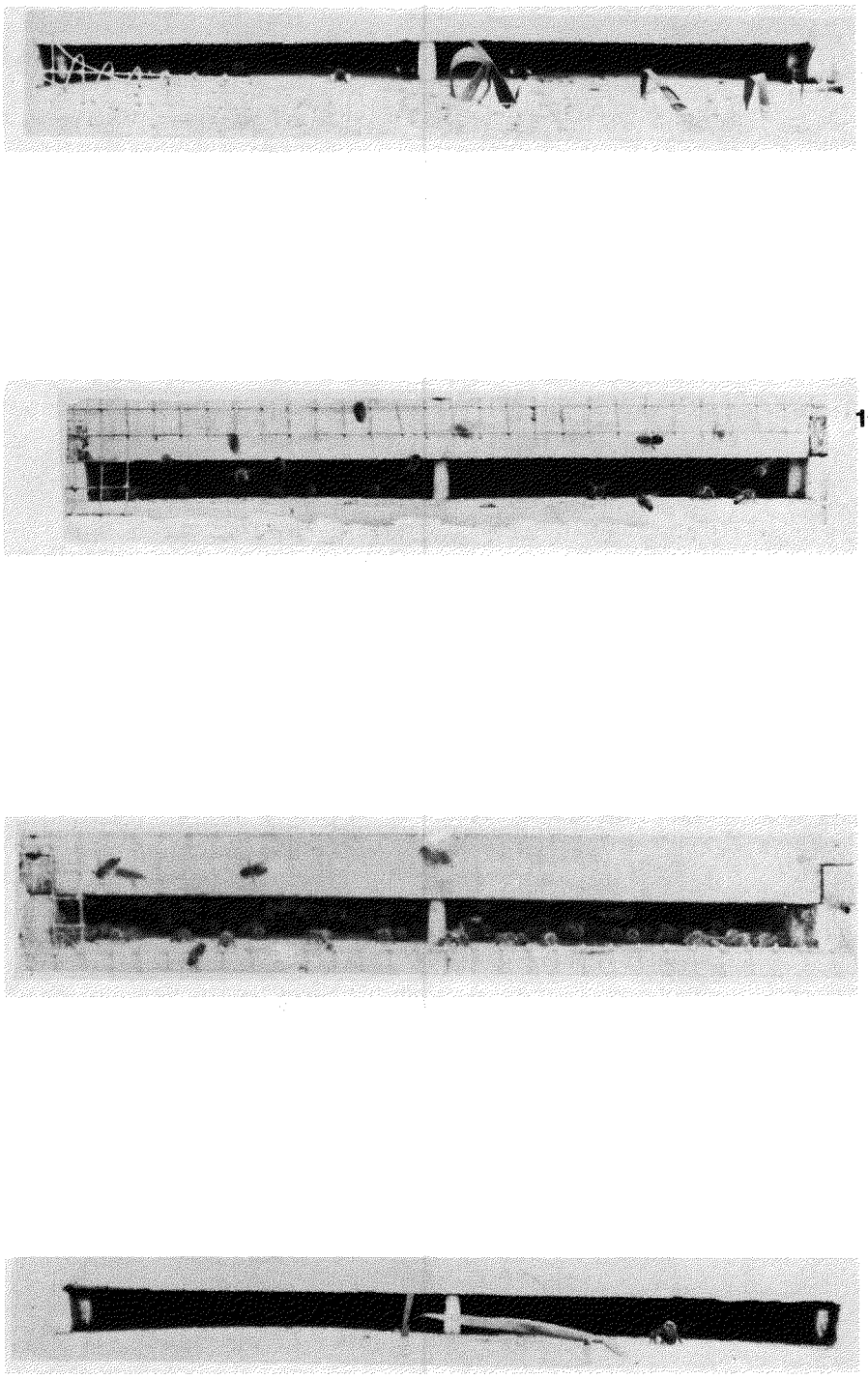


Figure 18. Shielded Metal-free Control Hives Showing No Propolization of Hive Entrances After 8 Weeks.

also abnormal propolis deposition on the tops of frames, generally in the lower chambers, but also in the upper honey supers.

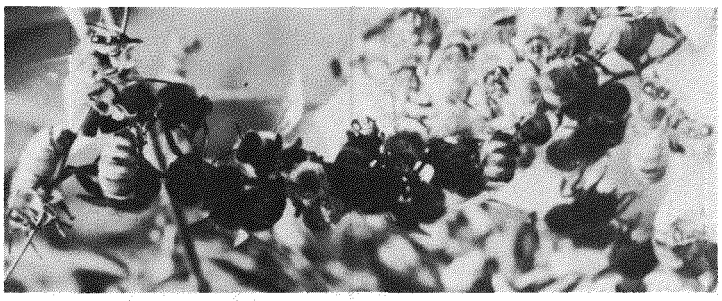
In general we observed greater irritability among bees flying under the 765 kV line. Arriving at the hives, we were often pursued for 50 yd by angry bees, without provocation. In contrast, the control bees were noticeably calmer. Also, there were typically 50 or more agitated bees at the lower front corners of metal-free unshielded test hives, especially where vegetation makes contact with the hive. These bees exhibited aggressive behavior, e.g., biting and stinging of plant material as well as hive-mates (Fig. 19). Propolization occurred at these foci of disturbance. To determine if discharge of current through bees to ground was a source of irritation, two bent rods ca. 20 cm long, one made of aluminum and one of glass, were placed near hive entrances. The terminal portion of each rod was wound with 100% gum rubber bands, leaving the extreme 5 mm of the tip exposed. To improve the grounding of the metal rod, a steel wire was attached to the rod and then to a deeply driven grounding stake. Rods were placed at hive entrances or front corners wherever larger aggregations of bees were present. The rods were pushed into the soil until their tips were about 1 cm from the hive surface. Each hive type was tested. Bee reaction to the rods was observed over a 15-min period, including number of stings implanted in the rubber band. Figure 19 illustrates these responses. It is evident from Table 7 that only the unshielded metal-free hives under the line elicited stinging behavior.

G. IN-HIVE ACOUSTICAL LEVEL

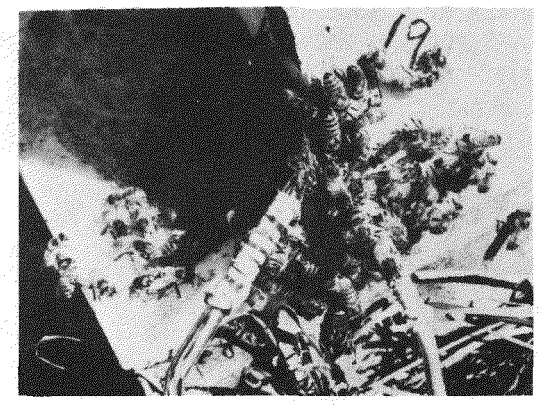
Table 8 presents a summary of the acoustical data. The readings for each point in the measurement (pre-disturbance, disturbance, etc.) were averaged for each frequency to yield these figures. Figures 20-23 compare the decibel levels at each point in the sampling period for various groups of hives.

Given the sensitivity of the instrumentation and the technique of analysis, the only case where there seems to be a significant difference is the 125-Hz situation, metal-containing hives. Here, the unshielded test group, following disturbance, exhibited a marked drop in dB level, whereas the others showed an increase. None of the metal-free hives, including unshielded tests, showed significant differences.

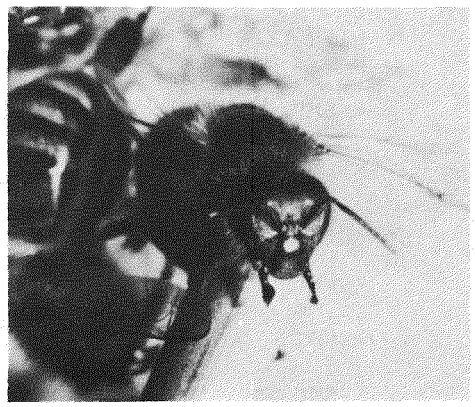
ADDENDUM: When the hives were inspected on March 2, 1978, it was discovered that 15 of 26 hives (58%) under the line had died during the winter, while only 2 of 16



a



b



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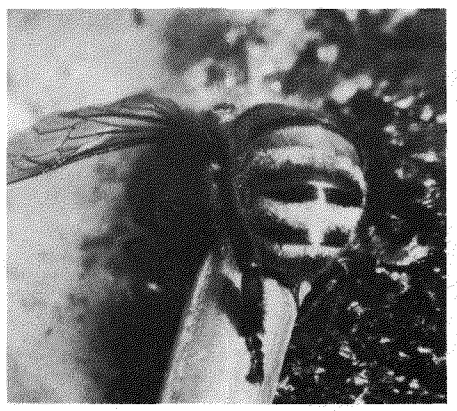
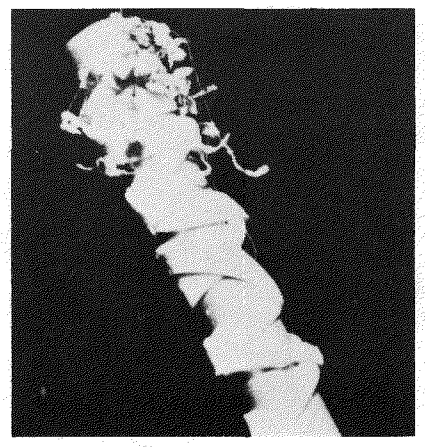


d



e

Figure 19. Angry Bees Cluster at Lower Front Corners of Metal-free Hives under Line: a. Garland of Stinging, Biting Bees on Blade of Grass Touching Hive; b. Corner of Hive Showing Placement of Glass (left) and Aluminum Rods; c. Biting Behavior Elicited on Aluminum Rod; d. Bee Stinging Aluminum Rod; and e. Stingers Implanted in Rubber Band on Aluminum Rod.

Table 7

Reaction of bees to contact with grounded rod.

HIVE TYPE	NUMBER OF HIVES	NUMBER OF STINGS IN 15 min	
		GLASS ROD	METAL ROD
SMT	3	0	0
UMT	1	0	0
SNT	1	0	0
UNT	5	0	76[a]
SMC	2	0	0
SNC	1	0	0
UNC	2	0	0

[a] 15 = mean per hive per 15 min.

Table 8

Mean dB Levels

FREQUENCY (Hz)	AMBIENT	UMT	SMT	SMC	UNT	SNT	UNC	SNC
31.5	46.5	50.6	52.7	52.7	48.7	47.8	49.0	46.4
63	53.0	63.1	61.4	60.2	61.5	60.8	62.0	62.3
125	55.2	56.7	63.5	63.4	58.9	61.2	60.8	61.5
250	44.0	62.7	64.0	61.6	63.8	63.4	61.2	61.1
500	43.5	62.7	61.0	58.9	59.4	58.7	58.4	56.0
1000	44.0	56.2	55.6	54.8	53.4	53.0	53.1	51.7
2000	43.0	54.3	51.5	51.7	52.1	52.1	51.2	48.5
4000	49.2	56.5	55.3	54.6	54.7	54.6	54.9	53.5
8000	55.4	62.5	62.5	60.6	60.9	61.0	60.7	59.8
16000	58.0	60.8	61.7	62.5	61.4	61.1	61.3	60.5

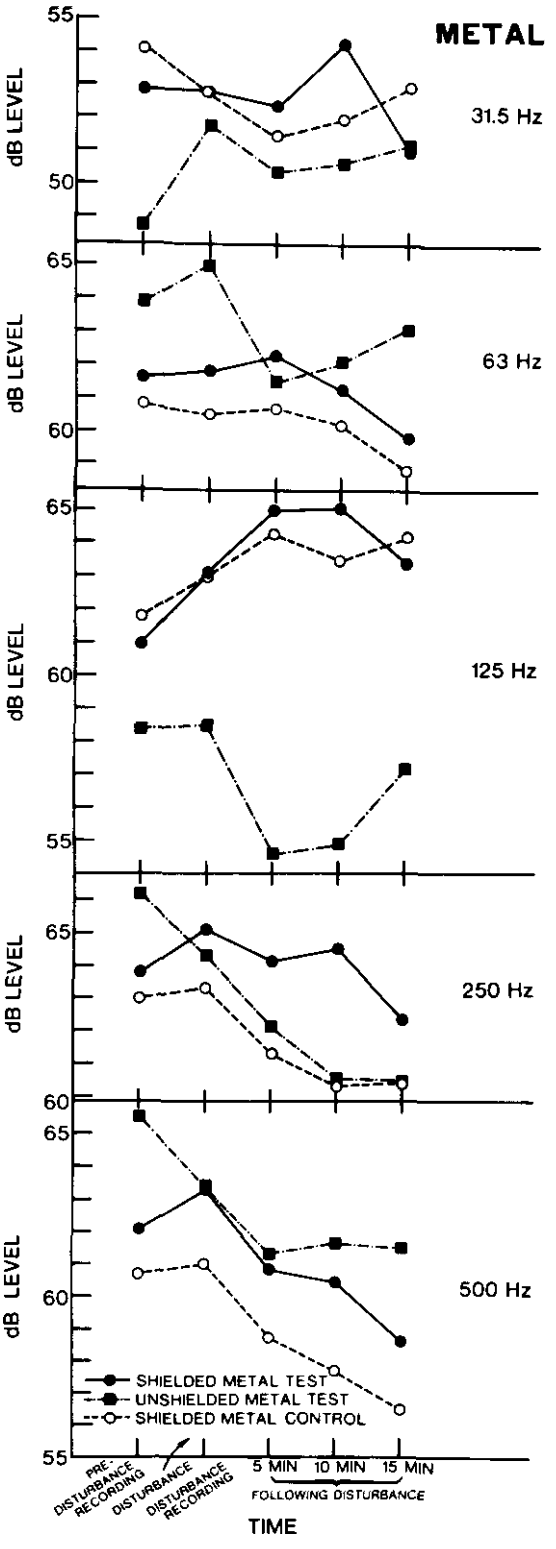


Figure 20. Octave Band Analysis of the Frequency/Energy Characteristics of In-Hive Bee Sounds before and after Disturbance. Each Point is an Average of the L_{50} Decibel Level Determined Visually from a Printout. 31.5 Hz-500 Hz, Metal-containing Hives.

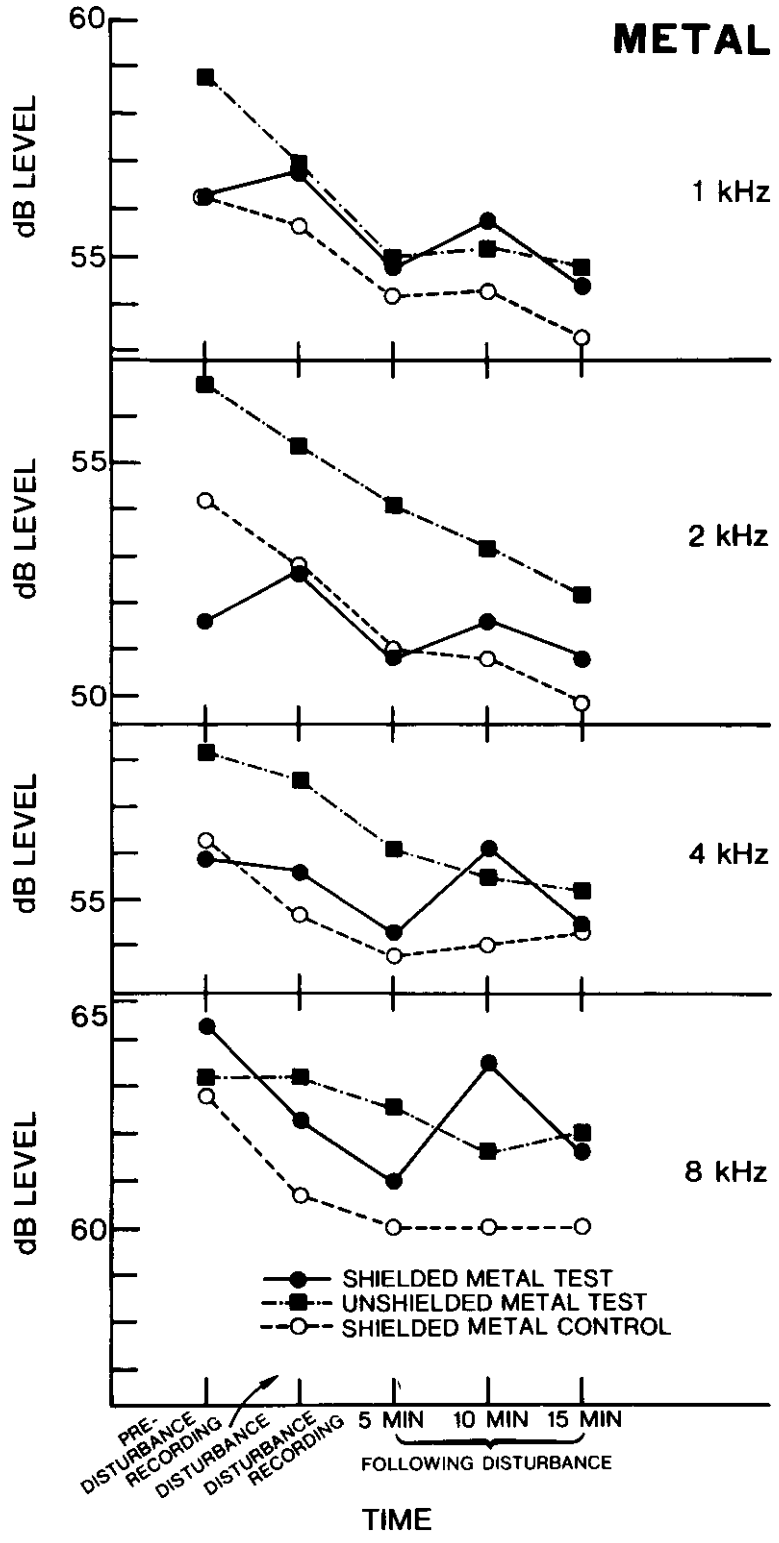


Figure 21. Same as Figure 20. 1 kHz-8 kHz, Metal-containing Hives.

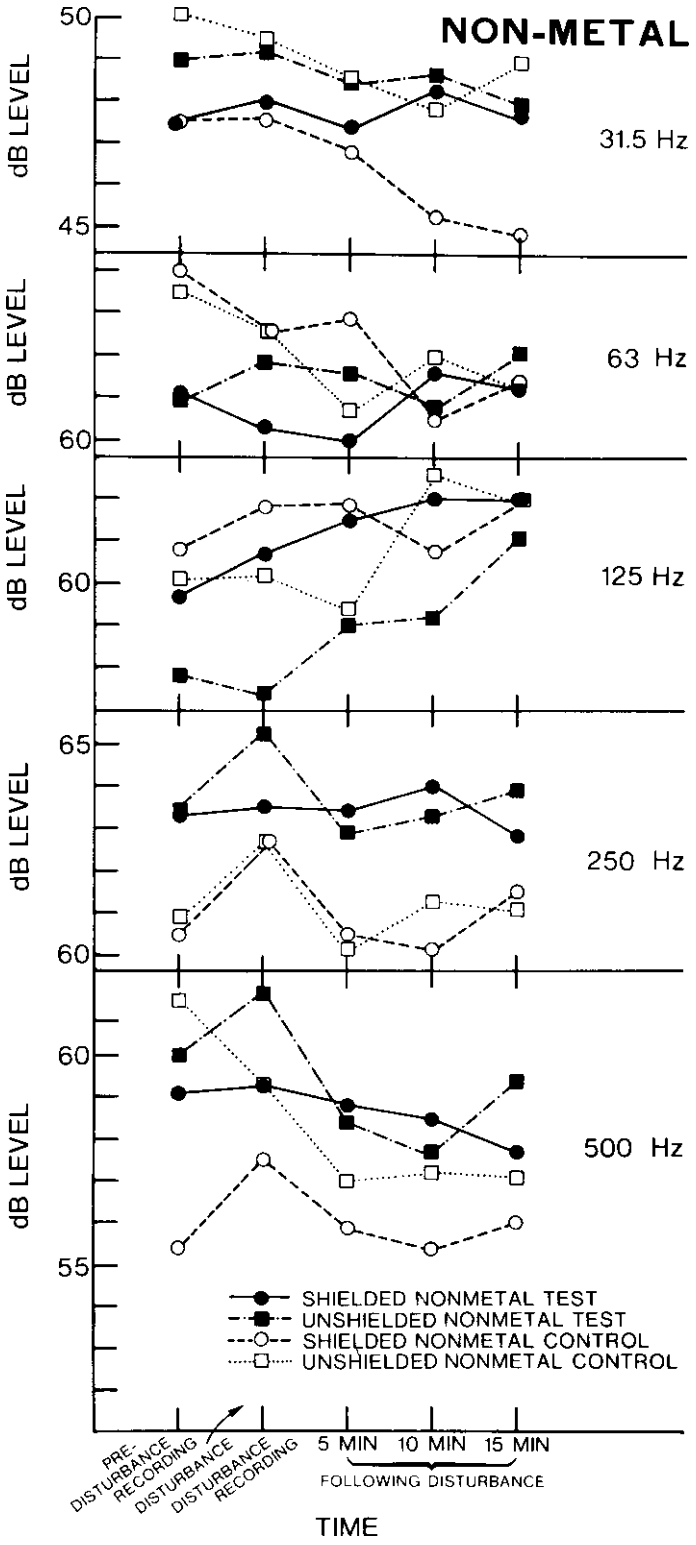


Figure 22. Same as Figure 20. 31.5 Hz-500 Hz, Metal-free Hives.

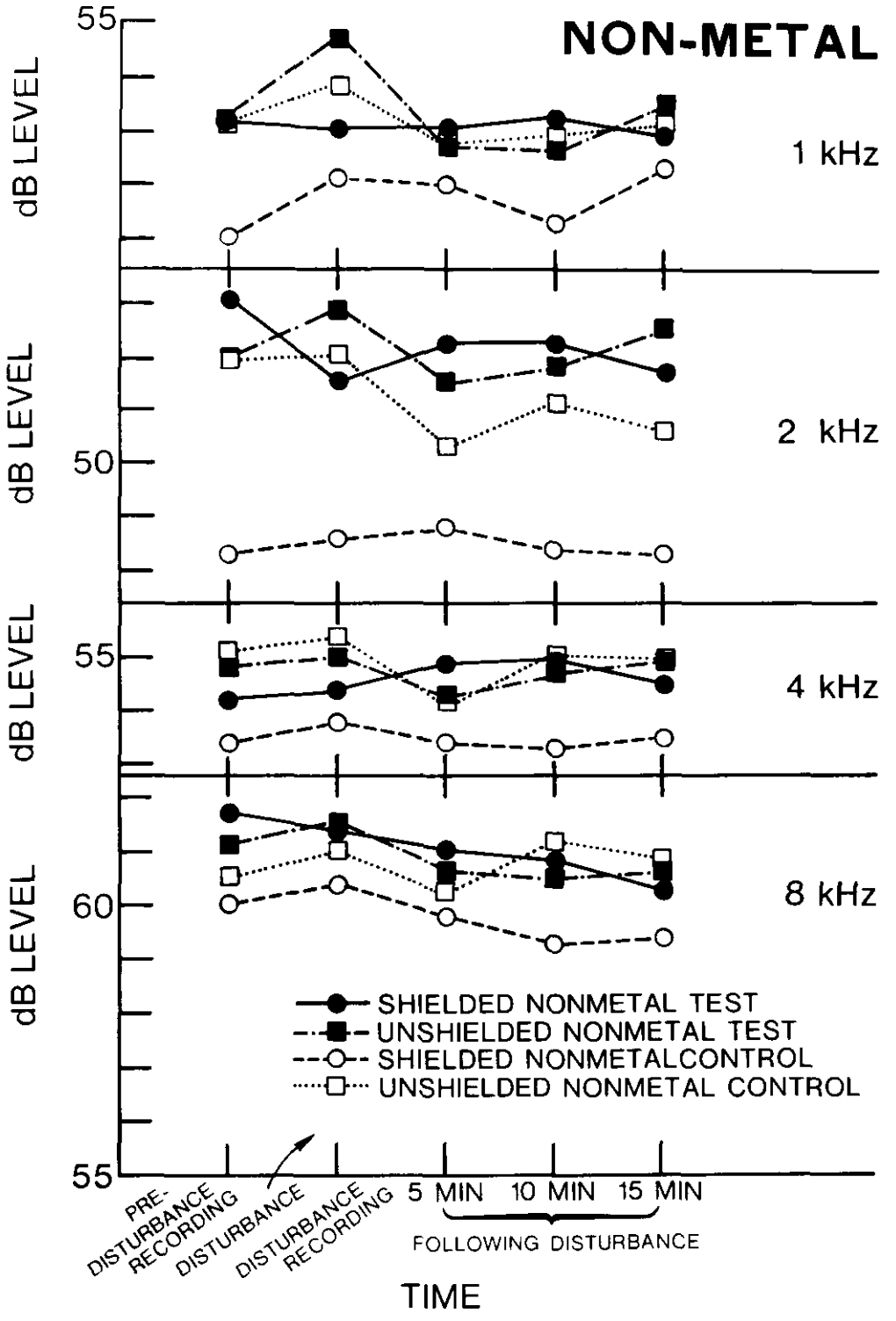


Figure 23. Same as Figure 20. 1 kHz-8 kHz, Metal-free Hives.

(13%) at the control site had failed to survive. At the area under the line, casualties included all 5 of the unshielded metal-containing hives, 2 of 5 shielded metal-containing hives, 7 of 9 unshielded metal-free hives, and 1 of 7 shielded metal-free hives. The control area casualties included 1 of 3 shielded metal-containing hives (another 2 were taken out of operation in fall because of excess wax moths), and 1 of 5 shielded metal-free hives. All 8 of the unshielded metal-free hives at the control area survived the winter. Among the surviving colonies, brood rearing activity had already begun at the time of inspection. In general, food stores among the colonies under the line seemed to be depleted to a greater extent than at the control area.

Table 9

Successive brood counts in various hive types, sampling periods 1 and 2.

HIVE NUMBER	EGGS 1	EGGS 2	LARVAE 1	LARVAE 2	PUPAE 1	PUPAE 2
40	3492	3161	4526	5274	5522	13196
38	2973	3611	6742	7947	14808	14176
35	3640	2688	7128	5754	13528	12710
33	0	0	0	0	9390	0
31	3372	3513	4973	4532	7173	9396
39	3818	4969	6666	4637	9969	4914
37	2572	2516	5148	5202	15065	8436
36	3095	2705	5403	4011	8879	5727
34	1788	2317	5605	4562	15570	3387
32	2770	1794	6508	3430	14871	1512
18	2576	3427	3985	4845	12979	13952
0	0	2927	0	2575	0	9142
13	3277	2587	7323	5802	14161	13149
7	1865	2959	2681	4432	2765	8967
4	1766	2228	3285	3258	10294	10143
14	1723	2602	3913	4431	7868	12533
25	1808	0	3220	0	10870	4401
8	1319	2608	3853	3936	8092	10988
11	3310	3040	5075	7323	10482	16691
5	2038	1947	1940	5135	7551	10755
29	1666	3081	2670	4555	7520	10647
12	6905	3343	7630	7048	12992	15987
23	3535	3354	5609	5501	12071	11568
19	4842	2029	5000	4783	10406	10771
16	2663	3016	3853	3180	10365	13500
3	3307	5253	6053	5253	14147	15794
41	3285	3727	5824	6731	16492	14392
42	2435	2407	5007	5804	8852	6110
43	2638	1530	3793	2990	3524	5526
44	1603	1630	4371	4086	10153	9318
45	2693	2858	5153	4569	12555	8319
28	1094	2048	3057	3105	1325	6124
20	1415	3245	1423	4582	8056	10380
21	4635	3862	6727	6682	12838	16121
10	2381	2247	2860	4465	10973	12992
9	1411	0	3559	0	9484	7344
6	1550	0	4391	5599	7190	16629
24	1863	2385	3743	3728	1991	6509
2	4737	2971	6049	7252	12759	17352
1	2110	0	3511	220	7004	8571
27	3035	4133	4036	5705	16136	17037
15	2176	2421	3729	4581	15098	15068
17	1727	2958	3887	5338	10371	15068
30	3396	2794	5899	4011	13397	14920

Table 9 (continued)

Sampling periods 3 and 4.

HIVE NUMBER	EGGS		LARVAE		PUPAE	
	3	4	3	4	3	4
40	2720	3621	5337	1810	12829	16066
38	2849	3858	5492	6367	12657	13188
35	0	0	0	0	0	0
33	1559	2347	3120	3246	5033	8201
31	2553	3401	5532	5397	14287	14633
39	4516	2785	5414	5018	10409	8256
37	3519	4194	3541	2457	8368	6409
36	2357	2845	2633	2358	5780	3894
34	1796	1098	2736	699	3555	1592
32	1565	1629	10	2378	1	2569
18	2798	3248	5547	6287	15568	13143
0	2526	4428	4539	7794	14264	16435
13	1726	3227	3625	5189	10263	14118
7	3506	3627	4953	7840	15897	16617
4	1984	2455	2948	3743	8643	6638
14	1671	3620	4381	6021	11779	12105
25	1743	484	4008	5025	6674	10744
8	2506	3409	4489	4755	11173	2702
11	2368	3679	5024	8461	9929	17700
5	1845	1796	10	5072	4	10303
29	3148	3647	3348	4758	9798	10553
12	3484	3631	5157	4791	17058	12008
23	3025	3813	3246	6291	11873	11493
19	2979	3231	4230	3875	11015	9402
16	4335	3392	5484	6756	13928	15772
3	2263	4321	2991	5336	10292	13166
41	3467	2514	6129	6978	13831	13807
42	2436	412	5428	5708	11077	5484
43	0	566	0	0	0	533
44	1442	891	2517	2896	8131	4094
45	0	1044	0	1720	0	1237
28	2724	2391	4981	5763	11500	13408
20	2219	3769	5250	6009	12458	14479
21	2154	3410	4382	5886	13516	13403
10	1802	2855	5165	7372	12131	13530
9	0	133	0	131	0	0
6	3992	3455	6467	6632	16105	16243
24	3179	3431	4033	4935	12207	12254
2	2488	4088	4216	5911	16284	14600
1	3616	4553	5634	6423	16005	12077
27	2747	3736	5227	7025	13929	15832
15	2357	3020	4151	5609	11934	10946
17	3478	3887	6797	7341	15935	15058
30	3806	3416	4602	6402	12496	13498

Table 10

Weights (kg) per hive before (6/13) and during experiment; metal-containing hives are gross weight, metal-free hives are net weight (see Section II, Materials and Methods).

HIVE NUMBER	6/13	6/22	7/2	7/13	7/20	7/27	8/3
40	53.9	57.6	62.1	73.2	88.1	94.3	94.1
38	64.8	71.1	78.4	95.2	112.5	114.3	116.8
35	69.2	72.9	80.0	94.3	107.1	109.3	113.9
33	65.8	65.2	64.8	65.2	65.6	65.2	65.7
31	59.3	64.4	67.5	77.7	91.9	98.4	102.3
$\bar{x}$	62.6	66.2	70.6	81.1	93.0	96.3	98.6
39	54.7	53.8	55.5	59.3	62.3	61.1	60.2
37	63.0	60.7	59.3	60.9	61.4	60.5	58.6
36	64.8	64.1	62.5	63.6	63.9	62.3	62.3
34	55.0	57.1	55.7	55.5	55.4	54.1	54.1
32	67.9	65.2	64.3	67.8	65.2	64.3	64.5
$\bar{x}$	61.1	60.2	59.5	61.4	61.6	60.5	59.9
41	69.1	78.9	83.9	98.4	111.6	117.1	115.7
42	66.4	65.5	67.1	72.5	79.7	82.7	82.3
43	64.6	65.5	68.7	70.5	73.6	74.3	74.0
44	65.1	62.7	69.6	78.4	85.6	89.8	90.3
45	59.6	63.4	66.8	74.3	77.5	78.6	83.4
$\bar{x}$	65.0	67.2	71.2	78.8	85.6	88.5	89.1
18	19.4	20.6	26.8	42.0	55.3	56.1	58.8
0	---	7.7	9.8	11.6	16.9	18.6	17.6
13	22.9	31.0	35.4	45.2	53.1	54.1	50.0
7	7.4	9.9	12.3	17.0	25.3	31.3	28.4
4	15.9	17.4	21.6	31.1	38.7	40.7	40.7
14	13.5	15.7	19.8	26.6	38.3	39.3	38.8
25	6.7	8.7	9.3	11.4	13.4	12.9	12.7
$\bar{x}$	14.3	17.2	20.8	28.9	37.4	39.1	38.2
8	14.7	16.9	20.0	23.9	30.4	31.4	37.7
11	20.8	25.4	31.8	42.0	50.2	51.1	50.2
5	6.1	10.7	12.0	14.3	21.6	24.8	23.9
29	7.7	12.1	14.1	17.7	23.3	25.7	30.0
12	12.8	17.0	21.6	32.7	34.3	41.3	42.9
23	10.0	14.4	17.3	24.3	29.9	30.7	29.1
19	7.8	11.9	15.9	21.1	25.9	26.6	28.9
16	11.2	13.2	18.9	25.7	32.8	35.2	37.5
3	17.6	19.8	24.8	33.8	38.7	43.4	42.5
$\bar{x}$	12.1	15.7	19.6	26.2	31.9	34.5	35.9

Table 10 (continued)

HIVE NUMBER	6/13	6/22	7/2	7/13	7/20	7/27	8/3
28	10.8	10.7	12.3	17.5	24.6	28.9	29.8
20	7.0	13.6	18.9	27.3	34.9	41.4	45.3
21	19.4	28.7	32.2	42.9	49.9	54.5	57.7
10	11.8	19.9	27.7	36.3	42.4	49.5	49.1
9	16.2	23.2	26.6	31.8	34.0	35.9	34.3
$\bar{x}$	13.0	19.2	23.5	31.2	37.2	42.0	43.2
6	13.4	18.4	24.5	34.1	45.8	46.2	46.5
24	10.4	16.7	19.9	23.8	30.7	31.9	31.7
2	12.9	18.8	24.2	32.7	41.6	40.7	42.0
1	13.7	20.3	23.6	29.2	35.0	36.8	36.9
27	14.8	21.8	25.6	28.7	37.1	37.7	38.4
15	17.3	23.4	27.4	35.8	44.3	46.1	46.7
17	17.9	21.4	23.4	29.4	37.8	38.5	39.4
30	18.4	27.9	35.7	42.9	49.8	50.4	51.3
$\bar{x}$	14.9	21.2	25.5	32.1	40.3	41.0	41.6

Table 11

Bee weights.

HIVE TYPE	HIVE NUMBER	MEAN WEIGHT PER BEE (gm)
	40	0.1307
	38	0.1243
SMT	35	0.1235
	33	0.1280
	31	0.1280
	39	0.1161
	37	0.1201
UMT	36	0.1056
	34	0.1187
	32	0.1180
	41	0.1281
	42	0.1260
SMC	43	0.1202
	44	0.1308
	45	0.1281
	18	0.1247
	0	0.1253
SNT	13	0.1322
	7	0.1240
	4	0.1327
	14	0.1257
	25	0.1244
	8	0.1270
	11	0.1259
	5	0.1317
	29	0.1321
UNT	12	0.1220
	23	0.1242
	19	0.1297
	16	0.1262
	3	0.1241
	28	0.1283
	20	0.1249
	21	0.1314
SNC	10	0.1279
	9	0.1242
	6	0.1314
	24	0.1289
	2	0.1318
UNC	1	0.1241
	27	0.1260
	15	0.1282
	17	0.1262
	30	0.1268

IV. DISCUSSION

A. BROOD

Among the metal-free hives, it is highly significant that no differences were found in pre-adult population structure for any experimental condition. This shows that all of the highly complex processes and interactions involved in egg production and brood rearing were not demonstrably affected by 84 days of exposure to the 765 kV line, in the absence of metal hive parts.

In unshielded metal-containing hives under the line, egg production and larval density were normal but pupal counts were significantly lower than in shielded counterparts under the line and in the control area. This difference is not due to our sampling periods being asynchronous with the brood cycle, since if this were the case, the larval and/or egg counts should be higher, given the more or less continuous brood production system we recorded. Evidently something may be happening to the larvae, preventing their survival to the pupal stage. Warnke and Paul (12) reported that bees exposed to 7-11 kV/m carried off brood from the hive. We did not observe this, but further investigation is warranted. In our expanded study in 1978, we plan to use all new instrumentally inseminated queens - Cale 876 three-way hybrids - to minimize genetic differences in fertility and performance.

Another drawback here is the small sample size, and the fact that the population of a given colony depends so heavily on a single individual. Queen losses due to accident or failure forced us to exclude some data for the purpose of statistical analysis (two of the shielded metal-containing tests, one unshielded metal-containing test, and two shielded metal-containing controls). Non-acceptance of replacement queens sometimes prolonged the effects of queen loss, as in the case of hive No. 33. A much larger sample size for each category of metal-containing hives, as well as an unshielded metal-containing control group, is planned for 1978.

B. HIVE WEIGHTS

There are highly significant differences in this parameter. None of the unshielded metal-containing test hives attained a final weight as large as that of the lowest shielded metal-containing test or control. This effect was not due to lower populations of the unshielded metal colonies since even hive No. 39, which had normal

population size throughout the sampling period, gained only 5.5 kg as compared to an average gain of 36 kg for the shielded metal tests and 24.2 kg for the shielded metal controls.

The question becomes: were the bees foraging less, consuming more, miscommunicating, or some combination thereof? The first possibility is plausible when one considers the extensive propolization of the entrances, tops of frames, and other portions of the unshielded metal-containing test hives. Warnke and Paul (12) found that bees in 7-11 kV/m sealed themselves completely with propolis within a few days, and died. We observed a slower rate of propolization which fell short of a fatal end point. Another reason for the zero weight gain could be depletion of honey stores due to increased metabolic rate from E-field exposure (5, 14, 15).

C. BEE WEIGHTS

Our average value of 127 mgm per worker agrees closely with Mitchell's (17) figures for workers taken at random from the hive. The lower weight of unshielded metal test bees may be associated with their depleted food reserves and higher metabolic rates. Next season it is planned to obtain dry weights of returning foragers, drones, and newly emerged workers in order to validate a conclusion.

D. IN-HIVE TEMPERATURE

Because our hives were variable in the distribution of brood, honey, and bees, we used only the highest, brood chamber, temperature readings in our analysis in order to insure that we were sampling comparable areas in each hive. Hüsing et al. (20) and Altmann et al. (21) found that the temperature in a beehive did rise under the influence of artificially induced E-fields, but that the brood chamber region was relatively constant in temperature. Therefore, our finding of no difference among the experimental groups is not definitive, since we restricted our sampling to the brood area. It would be desirable to use built-in thermistors, as did the above researchers, permanently contained within the hive. This would eliminate the need to open the hive for sampling purposes and would provide long-term data, but there are complications. The presence of extraneous metal parts within the hive would alter the internal E-field distribution and increase the risk of spurious effects, a problem not solved by past workers. Also, they used individual hives, and exposed them to different stimuli at different times whereas we used hives permanently exposed to the various experimental treatments. Our results provide considerable evidence for the perturbing influence of metal parts on a colony's general condition

when under the 765 kV line, and any additional metal would doubtlessly further complicate matters. Also, such internal equipment would hinder or prevent other experimental manipulations.

One possible solution would be to use each hive as its own control; initially the various hive regions could be sampled with a shield placed over the entire hive. Then, the shield could be removed, without disturbing the colony in any other way, and a second set of readings could be taken, some period of time later. Control hives would be sampled in the same manner, as a check on the possible shifts of in-hive temperature due to removal of the shield, or other factors. This method would allow us to greatly reduce the problem of internal variability from hive to hive and provide a more sensitive index of internal temperature fluctuations.

E. MOISTURE CONTENT OF HONEY

The small amounts of honey present in the unshielded metal-containing test hives have limited the amount of sampling, and as such the results are questionable, especially since they are only of borderline significance statistically. The great degree of consistency among all other groups and the scarcity of honey in the unshielded metal-containing test hives seem more significant than the small perceived difference.

F. GENERAL BEHAVIOR

Excess propolization of hive entrances, tops of frames, and hive corners in unshielded hives exposed to E-fields under the line may have been the workers' attempt to seal off irritating electrical 'hot spots.' Propolyzing is an inherited trait, much more common among Caucasian than Italian bees. Our hives were stocked with Italian queens. Among the unshielded metal-free hives under the line, the lowest hive body typically has the highest internal E-field. For example, hive No. 11 had fields of 3.6-4.5 kV/m in the bottom box, compared to 0.9-1.6 kV/m in the next higher super, and 0.8-1.4 kV/m in the third box. Although E-fields vary from hive to hive (< 3-fold), this general trend is basically constant. In the case of the metal-containing unshielded test hives, the greatest internal E-fields were generally found in the middle boxes rather than at the bottom, but was dependent on the nearness of the probe to the next super. The bottom entrance to the hive, as well as the small space between each super, is a region of high E-field strength (16), and coincides with the areas of abnormal propolis deposition. Investigations on the electrical properties of propolis are now in progress.

Aggression, e.g., stinging and biting, was observed among bees at the lower front corners of metal-free unshielded test hives. Since these hives contained bottom boards of low-conductivity polyethylene it is plausible that electrical charges built up on the hive body and were discharged through the bees to the grounded vegetation with which they made contact. Such discharges elicited unmistakeable aggressive behavior, as the data in Table 7 and Fig. 19 clearly show, and served to maintain small clusters of agitated bees throughout the day. Unshielded metal-containing hives never exhibited this phenomenon, presumably because their bottom boards were fabricated with wood and nails and were therefore more conductive. For next season, it is planned to map the induced currents in the different hives under the line.

G. IN-HIVE ACOUSTICAL LEVEL

Little detail is known concerning bee-produced sounds. Simpson and Greenwood (22) reported that sinusoidal 650-Hz vibrations, pulsed in a manner similar to that of the piping sound made by queen honey bees, increased the tendency of the bees to swarm. Esch (23) found that bees produce a buzz with a frequency of 180-250 Hz while performing the straight run of their communicative dance, and produce a buzz with 500-Hz frequency when they contact a hivemate. Minneman (24) recorded substrate vibrations in a colony, and reported that the sound level is generally correlated with the collecting activity and particularly with the recruiting of new foraging bees. During high foraging activity, the sound frequency ranged from 100-5000 Hz.

By using an acoustical filter we determined that the sounds produced at 125 Hz were indeed bee sounds. At this frequency, the post-disturbance sound level was markedly depressed only in the unshielded metal-containing test hives compared with their shielded counterparts. At all other frequencies, and for all hive types and experimental conditions, sound energy outputs following disturbance were remarkably similar. In the absence of further information concerning the nature of bee sound no conclusion can be drawn with respect to this difference in unshielded metal-containing test hives.

ADDENDUM: The death of all the unshielded metal-containing hives under the line during the course of the winter was anticipated, given their failure to accumulate adequate honey stores in fall (Figs. 8 and 9). However, the unshielded metal-free test hives had collected normal amounts of honey and pollen by fall, and yet

suffered extensive overwintering mortality (78%) compared to their shielded counterparts (14%) and both controls (9%). In many cases it seemed that their food stores had been depleted, resulting in starvation. Among the survivors as well, food stores generally seemed to be lower at the test area than at the controls. It will be important to check whether or not this trend manifests itself again during 1978.

PART 2

V. INTRODUCTION

Stress may be defined as the effect of a factor which extends any homeostatic or stabilizing process beyond its normal limit at any level of biological organization (25). Considerable research effort has focused on vertebrate responses to stress. Rothballer (26) demonstrated the release of neurosecretory substances in stressed rats and Barnett (27) reviewed stress resulting from social interactions in rat societies. Marino et al. (28) report significant changes, as yet unconfirmed, in rat serum proteins following exposure to 60-Hz electrical fields. Recent reviews and compendia attest to mounting interest in stress and other biological effects of extremely low frequency electric and magnetic fields (29-32).

Stress phenomena are not restricted to vertebrates, and their occurrence in insects is extensively documented. Sternburg (33) reviewed the literature concerning autointoxication and other effects resulting from physical, chemical, and electrical stressors, especially in cockroaches. Marek (34, 35, 36) demonstrated stress proteins in the hemolymph of the wax moth, Galleria mellonella, subjected to cold or injury. Jones and Tauber (37) and Jones (38) found changes in total and differential hemocyte counts in the mealworm, Tenebrio molitor, subjected to chemical stressors. Elevated hemocyte numbers have been correlated with several pathological states and physiological conditions in various Orthoptera as well (39). Pence et al. (40) described autointoxication and other effects associated with the release of an unknown factor into the hemolymph of adult honey bees subjected to physical and 'psychological' stressors. Altmann (14) has shown that adult bees exposed in a Faraday cage to an electrostatic field of 1.4 kV/m for 40 min had a 36% increase in oxygen consumption, increased food intake, and diminished longevity (15). When exposed to electric fields of 50 Hz at less than 10 kV/m, honey bees show variable metabolic rates; at 20-40 kV/m, metabolic rates increase in proportion to field strength; and at field strengths greater than 50 kV/m, the bees sting one another. In bees, peak aggressive response to the flow of electric current occurs around 50 Hz and with as low as 20 μA they bite and attempt to sting the electrodes before succumbing to apparent stress paralysis (41).

This preliminary study was undertaken to determine whether honey bees hived under a 765 kV transmission line exhibit symptoms of stress manifested by changes in hemolymph proteins and hemocyte counts.

VI. MATERIALS AND METHODS

A. PREPARATION OF BEEHIVES

Two non-metal hives were started in spring 1977 with three-pound packages and Dadant's "Starline" Italian queens. The hives were kept in the control area where ambient electric fields were 10 V/m and by September 24, 1977, when the experiment began, each hive contained about 2-2.5 medium-depth supers of brood and about 2 full supers of honey. Baseline hemocyte counts were made 3 times every other day during the week before the hives were relocated. On September 24, hive 22 (exposed) was placed under the 765 kV transmission line and hive 26 (control) was moved to another control area at the bee site at the Joliet Ammunition Plant. Both hives were in the same foraging area, separated by a distance of approximately 400 meters. The area is described under Materials and Methods in Part 1 and the electromagnetic environment is characterized by Frazier (16). A normal antibiotic regimen was followed in spring and fall and no disease was evident during the course of the experiment.

B. HEMOCYTE COUNTS

We limited our sample to bees taken from the upper honey supers. Different areas of the supers were sampled to minimize disturbance and possible release of alarm pheromone. Smoke was not used because its effect on hemocytes was undetermined. The use of disposable polyethylene gloves to avoid build-up of alarm substance did not work because bees were able to slip away and the gloves' clumsiness probably alarmed more bees than otherwise. A grasped bee was quickly punctured with a micropipet in the conjuctiva between the second and third dorsal abdominal tergites, a special effort being made to avoid the gut; the procedure took about 5 sec. Pipets were made from disposable 50 µl microsampling pipets (Corning No. 7099 S) drawn to a fine point and siliconized by a 5-sec immersion in a 1% Siliclad solution (Clay Adams Co.) to prevent hemocyte adhesion to the pipet walls; the borosilicate glass reduced, or eliminated, pH changes due to glass/hemolymph interaction.

Hemocytes were counted in an A.O. Bright Line hemocytometer at 125 X under phase microscope. Hemolymph of approximately 6-7 bees was necessary to charge the counting chamber and obtain a count, and up to 50 bees were used to obtain replicate

counts per hive per date. To insure uniform mixing of the hemolymph for each chamber, hemolymph was discharged onto sterile, plastic petri dishes, stirred, drawn back into the pipet, and the process repeated before introducing the sample into the counting chamber. Nine squares in an 'x' fashion were counted from the central hemocytometer divisions. Chambers with uneven cell distributions were not counted. Counts were made in the field immediately after hemolymph was drawn, because our previous work showed that hemolymph settled out in the pipets a few minutes after sampling, and hemolymph melanized in about 1/2 hr, even when stored on ice. Counts in foul weather were made inside a steel-framed, canvas tent near the hives, or if conditions were severe, in the tent over the hives. Also, to minimize cold stressing the bees, the super to be sampled was removed from the hive and covered with pallets, and the hive was normally covered.

A one-way analysis of variance was performed on the mean numbers of hemocytes in bees of each hive type before exposure to the 765 kV line because of the small number of replicates. A two-way mixed model analysis of variance was performed to determine the effect of exposure on the hemocyte counts; the effect of foraging activity was analysed by this method and by a nested two-way analysis of variance.

C. ELECTROPHORESIS

Hemolymph was sampled for electrophoresis before it was taken for cell counts. Hemolymph was obtained with pipets previously coated with a film of 1% phenylthiourea in acetone and air dried; this inhibited hemolymph melanization during prolonged storage. Pipets contained about 10 µl hemolymph each and were placed in corked test tubes on dry ice in the field for later storage at -30 C. A total of 110 µl of hemolymph per hive per date, representing 70-80 bees, was collected. Pre-treatment sampling was begun September 3, 1977.

Disc electrophoresis of hemolymph proteins employed a modification of the method of Lensky (42). The separation gel consisted of 17% acrylamide (Sigma), 0.25% bis (Eastman) with a tris-glycine buffer at pH 8.9. Hemolymph was not centrifuged because hemocytes are trapped in the stacking gel and give identical patterns to centrifuged samples (43).

The gels were run at 4 C and at 1 mA/gel for 1/2 hr, then at 2.5 mA/gel for 2 hr; current was supplied by a Buchler Model 3-1500 constant rate power supply. Gels were stained with 0.2% coomassie brilliant blue G-250 (Sigma) in 10:10:1

methanol:water:acetic acid for 15 min at 40 C and destained overnight in a large tank where the purity of the destaining solution was maintained by circulating it over an activated charcoal bed. Gels were scanned at 455 nm with a Gilford 240 gel spectrophotometer coupled to a Gilford 6040 recorder.

VII. RESULTS AND DISCUSSION

Insect hemolymph manifests stress reactions, due to physical, chemical and biotic agents, which are amenable to the precision techniques of electrophoresis and hemocytometry. It has been shown that DDT-induced stress in the beetle, Tenebrio molitor, significantly raises hemocyte counts in specimens that are not heat-fixed, but does not change the counts in heat-fixed specimens (38). Previously, Jones (44) showed that variance among duplicate hemograms of unfixed beetles was significantly less than among heat-fixed samples, suggesting the probability of greater tissue adhesion by hemocytes in the latter case. The use of unfixed honey bee hemolymph is clearly the method of choice in determining hemocyte counts in honey bees.

Data on hemocyte counts in Table 12 indicate differences in exposed and control honey bees. During a 6-week exposure under the 765 kV line, mean hemocyte counts were higher in exposed bees 11 out of 14 times ($p=0.01$). The difference first appeared about one week after exposure and only once thereafter did the control honey bees have higher mean numbers of hemocytes. On this particular day, non-foraging conditions prevailed during the time when exposed bees were being assayed. When control bees were assayed, the weather had cleared and active foraging had been going on for 2 hrs. A possible association between foraging activity and higher hemocyte counts, particularly in control bees, is discussed below.

Counts made on days when bees are foraging appear to be higher than counts taken on non-foraging days. This is statistically significant only in the control hive. The largest differences in counts between exposed and non-exposed bees occur on non-foraging days (Tables 12-14). One might tentatively conclude from the limited data that not only do exposed bees have higher hemocyte counts than non-exposed bees, but that in the absence of high electric fields, bees in an actively foraging hive have higher counts than bees from the same hive when no foraging is going on. Whether this is due to the degree of activity in the hive, the individual nutritional state and metabolism acting on hemolymph volume and number of circulating blood cells, and whether meteorological factors are involved we cannot say. It is noteworthy that exposed bees, even under non-foraging conditions, have high hemocyte counts and the 765 kV environment could be a possible cause. Unfortunately, the small sample size in this exploratory study limits the emphasis that can be

Table 12

Hemocyte numbers in honey bees before and after placement under 765 kV line. Means are based on number of samples indicated, 6-7 bees per sample.

Sampling date	Hive 22 (pre-exposure) $\bar{x}$	n	Condition	Hive 26 (control) $\bar{x}$	n	Condition
9/20	7069	4	nf[1]	7314	7	nf
9/22	8505	7	nf	10209	2	nf
9/24[3]	8255	6	f[2]	8177	8	f
	Overall mean = 8080			Overall mean = 8061		
	Hive 22 (exposed)			Hive 26 (control)		
9/27	6500	5	nf	8200	5	nf
9/29	11067	5	f	12144	6	f
10/1	9952	7	nf	8867	5	nf
10/4	13556	6	f	13133	5	f
10/6	12787	6	f	10907	6	f
10/11	12833	3	nf	9130	3	nf
10/18	15489	5	nf	8483	5	nf
10/20	14287	3	f	13130	3	f
10/22	13771	4	nf	12486	4	nf
10/25	12111	4	nf	9630	3	nf
10/27[4]	12467	5	nf	13153	4	f
11/1	12660	4	nf	8083	3	nf
11/3	13852	3	f	10954	3	f
11/8	14194	3	nf	9750	3	nf
	Overall mean = 12538			Overall mean = 10575		

1. Bees not foraging.
2. Bees foraging.
3. Hives moved to test locations after counts were obtained.
4. Sampling order of hives and hemolymph reversed.

Table 13

Hemocyte counts in relation to foraging and non-foraging weather conditions.

Foraging conditions					
Hive 22 (exposed)			Hive 26 (control)		
Sampling date	$\bar{x}$	n	Sampling date	$\bar{x}$	n
9/29	11067	5	9/29	12144	6
10/4	13556	6	10/4	13133	5
10/6	12787	6	10/6	10907	6
10/20	14287	3	10/20	13130	3
10/27	12467	5	10/27	13153	4
11/3	13852	3	11/3	10954	3
mean = 13003			mean = 12237		
Non-foraging conditions					
9/27	6500	5	9/27	8200	5
10/1	9952	3	10/1	8867	5
10/11	12833	3	10/11	9130	3
10/18	15489	5	10/18	8483	5
10/22	13771	4	10/22	12486	4
10/25	12111	4	10/25	9630	3
11/1	12660	4	11/1	8083	3
11/8	14194	3	11/8	9750	3
mean = 12189			mean = 9329		

Table 14

Analysis of variance of hemocyte counts.

Test condition	Significance
Pre-exposure hive 22 vs. hive 26.	n.s. ($P \sim 0.5$)
Exposed hive 22 vs. control hive 26.	sig. ($P \sim 0.01$)
Foraging conditions: exposed hive 22 vs. control hive 26.	n.s. ($P \sim 0.10$)
Non-foraging conditions: exposed hive 22 vs. control hive 26.	sig. ($P = 0.001$)
Exposed hive 22: foraging vs. non-foraging conditions.	n.s. ($0.50 > P > 0.25$)
Control hive 26: foraging vs. non-foraging conditions.	sig. ($P = 0.001$)

placed on the differences between data obtained.

There is the possibility of a sampling bias on hemocyte counts, since the control hive was counted before the exposed hive at approximately the same time on each sampling day. Reversing the order on and after October 27, 1977, did not alter the trends - exposed bees continued to have higher counts than control bees (Table 12).

Similarly, the sequence of first taking hemolymph samples for electrophoresis and then taking hemolymph for hemocyte counts was reversed starting October 27 in order to avoid artifacts from possible stress reactions resulting from taking 70-80 bees for the former. Again the data do not show any change in observed cell count patterns (Fig. 24).

The brief use of a tent over the hives during inclement weather did not interfere with the bees nor influence the cell counts because the bees were not foraging at this time.

The preliminary nature of this study did not justify diversion of considerable man-hours from the major field study. In future, a proper study will include more hives, instrumentally inseminated queens for greater genetic homogeneity, a longer sampling period beginning in early summer and running through fall, treatment reversal of some of the hives, and finally, a blind system of coding hemolymph samples to avoid counting bias.

Forty-four hemolymph samples await electrophoretic analysis within the next few months. Our trial gels have reproduced the degree of separation reported by Lensky (42). About 21-23 bands are discernable visually or by direct gel scanning (Fig. 25).

Handling of animals must be considered in studies of stress. Handling of American cockroaches induces a stress-related increase in blood trehalose (45), but this increase was not observed until 5 min after handling, considerably longer than the 5 sec required to bleed a bee. We are aware of no other reports of acute effects due to handling, but if handling effects do occur we would expect them to occur in both exposed and control bees, ruling out spurious transmission line effects.

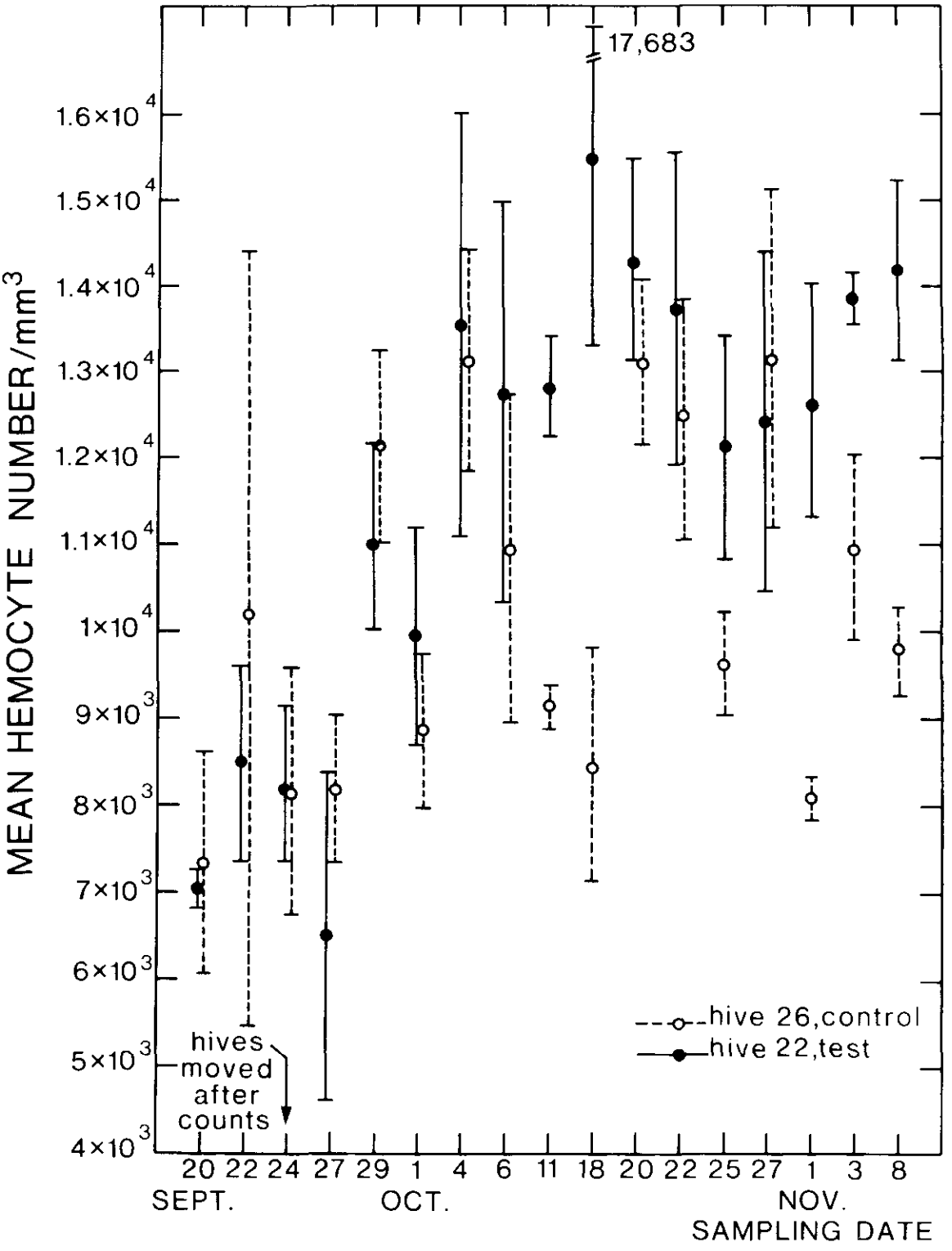


Figure 24. Mean Hemocyte Counts (±1 S.D.) in Worker Bees from a Metal-free Unshielded Hive before and during Exposure to 765 kV Line, Compared with Control.

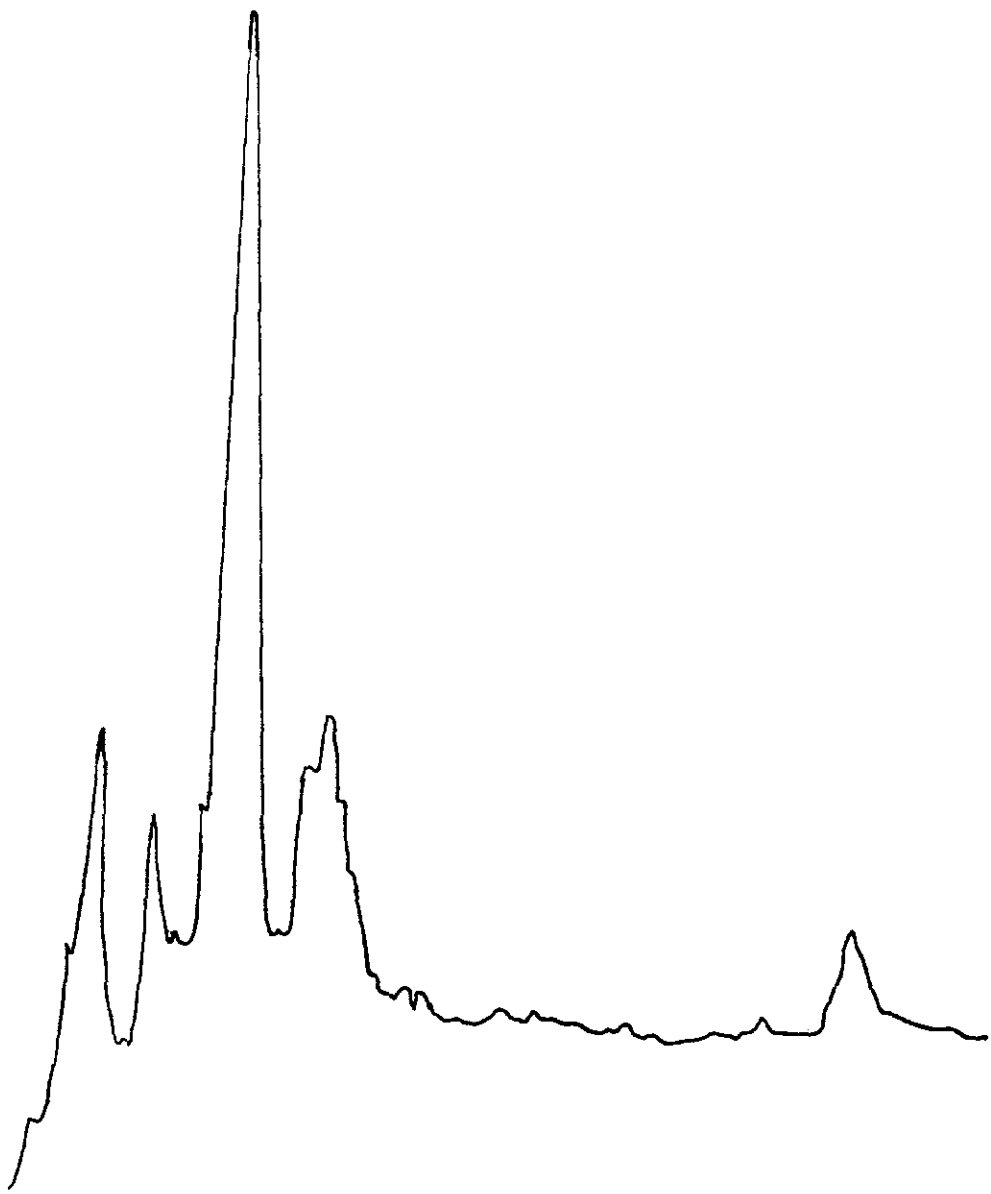


Figure 25. Typical Gel Scan of Worker Bee Hemolymph Separated Electrophoretically.

Storage of hemolymph samples at -30 C is universally practiced despite reports of artifacts in protein separations, some of which appear to be correlated with duration of storage (46). Therefore, only those samples of exposed and control hemolymph taken and frozen on the same day will be used for comparisons and these will be electrophoretically processed at the same time for analysis of possible transmission line effects.

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