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CONTRACT NUMBER DAMD17-95-C-5072

TITLE: New and Improved Methods for Monitoring Air Quality and the Terrestrial Environment

PRINCIPAL INVESTIGATOR: Jerry J. Bromenshenk, Ph.D.; Garon C. Smith; Bruce E. King; Robert A. Seccomb; Ghassan Alnasser; Colin B. Henderson; Christopher L. Wrobel; Matthew R. Loeser

CONTRACTING ORGANIZATION: University of Montana
Missoula, Montana 59812-1002

REPORT DATE: November 1996

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1. AGENCY USE ONLY *(leave blank).*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	November 1996	Annual (15 May 95 - 13 Oct 96)

4. TITLE AND SUBTITLE New and Improved Methods for Monitoring Air Quality and the Terrestrial Environment

5. FUNDING NUMBERS

DAMD17-95-C-5072

6. AUTHOR(S) Jerry J. Bromenshenk, Garon C. Smith, Bruce E. King, Robert A. Seccomb, Ghassan Alnasser, Colin B. Henderson, Matthew R. Loeser, and Christopher L. Wrobel

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of Montana—Missoula
Missoula, Montana 59812-1002

8. PERFORMING ORGANIZATION REPORT NUMBER
GESL-96-01-ANN/DEF

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Material Command
Fort Detrick, Frederick, MD 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT Honey bees (*Apis mellifera* L.) have been shown to be multi-media monitors of chemical exposures and biotic effects. However, most methods of measuring honey bee colony population dynamics have been time-intensive and subject to observer error. This four-year project has developed an automated system to assess continuously (in real-time) colony flight activity, temperature and relative humidity in the brood nest, total hive biomass, pollen collected, and a full spectrum of ambient weather information. Pollen, dead bees removed by housekeeping bees, and the chemical composition of the air inside the hive can be sampled daily. Field applications are ongoing at the Aberdeen Proving Ground—Edgewood area, where 21 electronic beehives have monitored three sites. These studies also address the applicability of artificial neural networks to quickly decipher patterns of bee behavior and to fingerprint hive chemistry. The primary intent of this project is to improve the ability to monitor stressors, both natural and anthropogenic. Preliminary results indicate that colonies respond to some environmental factors in less than two minutes. Trace amounts of bioavailable inorganic elements and chlorinated hydrocarbons were found in bee colonies from all Maryland sites. Industrial solvents exhibited the greatest between site differences, with the highest levels occurring in Old O Field hives and the lowest in Montana hives.

14. SUBJECT TERMS Biomonitoring, real-time monitoring, hazard assessment, acute toxicity, chronic toxicity, honey bee colony populations, environmental exposures, air quality, terrestrial environment, aliphatic hydrocarbons, heavy metals, military unique chemicals.

15. NUMBER OF PAGES
194

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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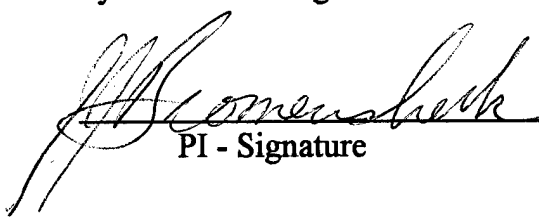

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EXECUTIVE SUMMARY

The primary objective of this five-year study is to develop real-time monitoring capability for using honey bee colonies as biomonitoring systems for military unique chemicals. Realization of continuous monitoring of honey bee colonies followed by controlled field and laboratory dose-response assays should provide the ability to discriminate and link stressors with observed responses. Because environmental contaminants rarely occur as single chemicals or as originally released forms, colonies were deployed at an actual military site to develop and refine methods. The Aberdeen Proving Ground—Edgewood (APG) area provided appropriate test locations for conducting *top down* (field to laboratory, colony to individual, effects to exposures) testing.

This report covers three distinct but related subjects relevant to real-time monitoring of colony condition and behavioral responses:

- Honey bees as environmental samplers.
- Development of equipment and methods for real-time measuring of the behavioral responses of bees colonies exposed to environmental contaminants.
- Evaluation of the use of honey bee colony populations to provide real-time monitoring of environmental conditions at APG.

We have made considerable progress toward accomplishing real-time measuring of colony performance. During the summers of 1995 and 1996, we deployed mini-hives containing nucleus (small) colonies of bees at APG. These hives were equipped with: 1) sensors that continuously measured several colony performance parameters, 2) sorption traps that sampled hive atmospheres over 8-10 hour periods, and 3) in-hive traps that continuously collected pollen and dead bees. These tests produced an extensive data set needed to: 1) determine the sensitivity, variability, and usefulness of several population-level assessment endpoints, 2) refine and validate PC BEEPOP, our honey bee ecotoxicological model, and 3) conduct a preliminary hazard assessment at APG. The equipment provided:

- Real-time measures of critical colony performance parameters, including levels of foraging activity, variability in flight activity among the colonies at a site, net loss of forager bees, changes in hive biomass, and the stability of colony homeostasis (i.e., thermoregulation).
- Real-time graphical displays at each field location of the monitored behavioral responses of bee colonies.
- Chemical fingerprint profiles of hive atmospheres, including the volatile (VOC) and semi-volatile (SVOC) organic chemicals given off by honey bees, hive stores, hive components, and bioavailable contaminants brought into the hive.
- Preliminary quantitative ranking of bioavailable inorganic contaminants and semi-quantitative ranking of VOC and SVOC contaminants at a Montana, a rural Maryland, and eight APG sites.

- Preliminary ranking of colony performance at two APG and a rural Maryland reference site.
- Identification of three colony performance parameters that offer promise for real-time monitoring of environmental contaminants—net loss of forager bees by one or more colonies, coefficients of variation for flight activity for colonies at a given location, and broodnest core temperature for each colony.

Real-Time Measuring of Colony Behavior

In 1989, the U.S. Environmental Protection afforded honey bees a Class 1 designation (i.e., an off-the-shelf, ready to use monitoring technique) for *in situ* Ecological Assessments of Hazardous Waste Sites. A 1991 National Research Council report on animals as sentinels of environmental health hazards concluded that honey bees are excellent monitors of air quality. However, prior to the investigations covered by this report, biomonitoring programs that utilized bee colonies had to rely upon periodic measurements of colony condition combined with intermittent chemical sampling to determine the effects of exposures to contaminants. Achievement of the capability to conduct reliable real-time monitoring of honey bee colonies should provide the ability to more directly and quickly associate colony responses, such as bee mortality or altered behavior, with exposure to specific stressors. However, accomplishment of real-time monitoring capability requires a new technological approach.

During the first year of this project, we developed the equipment and methods necessary for continuously measuring colony responses to military unique chemicals and also developed the techniques needed to detect volatile and semi-volatile organics in hive atmospheres. We designed, fabricated, and field tested specialized hives, electronic sensors, clock-driven samplers, electronic circuits, and computer interfaces needed to continuously characterize many of the parameters needed to evaluate the complex dynamics of honey bee colony populations.

In August, 1995, we transported six bee colonies with a full array of sensors and chemical sampling probes from Montana to Maryland for a pilot study. The hives were deployed for two weeks along the West Branch Canal Creek at Aberdeen Proving Ground, where a removal action and several geochemical studies were being conducted. This pilot effort provided a test of the efficacy, robustness, and usefulness of the overall method, as well as specific performance appraisals of equipment and software with respect to the goal of achieving real-time monitoring capability.

Following the APG pilot test, we focused our efforts on re-design and improvement of the hive electronics and interface software. For the 1996 field season we fabricated 21 electronic beehives, delivered them to APG in July, and deployed the hives for a biomonitoring study that ended in mid-November. We placed bee condos (plywood boxes containing mini-hives,

nucleus colonies of bees, dead bee and pollen traps, and a full array of electronic sensors and chemical sampling probes) in groups of seven at three sites. These sites included a reference site in rural Maryland near Churchville, and two sites at APG; namely Old O Field and West Branch Canal Creek. At the time of deployment of the colonies, the landfill at Old O Field was being actively capped and the removal activity site at the West Branch Canal Creek had been completed.

An additional 16 nucleus (small) test colonies were set out at six additional locations across the Edgewood post, covering work, recreation, and housing areas. These units provided additional survey points for chemical sampling to determine exposures to any environmental contaminants and to provide an indication of any acute colony response to these exposures, such as bee losses. To assess the influence of weather on colony behavior at the 'condo' sites, we maintained electronic weather stations at each site.

The 1995 pilot study and the 1996 biomonitoring study provided preliminary field applications of bees as real-time measures of environmental conditions. The primary objective of this project is to monitor honey bee colonies in real time, not the specific contaminants or mixtures of contaminants. The long-term goal of this approach is to reduce the need for frequent or periodic chemical sampling to determine whether the bees have been exposed to contaminants. Instead, bees or hive components would be sampled for contaminants when some critical colony parameter exceeds a warning threshold. This approach would minimize costly chemical analysis and eventually should be able to link specific stressors or groups of stressors to observed colony responses.

Based on a variety of experiments, weather conditions, and field applications, the hive electronics provided continuous, or real-time measures of several colony performance endpoints such as flight activity, colony core temperatures and relative humidity, and hive biomass. The flight counters collected data at a rate of more than 150 counts per second, which was averaged over 30-second intervals for each colony and continuously displayed on the computer screen as a time-series graph for each colony, including a cumulative plot of all of the colonies at a site. The output from each of the temperature, relative humidity, and biomass sensors was sampled at 30-second intervals and averaged over five-minute periods for each hive. These data were continuously displayed on another computer screen in a tabular form. Similarly, the meteorological data were updated at two-second intervals, averaged over one-minute intervals, and continuously displayed in graphic form on a third computer screen. The computerized colony monitoring systems at the three primary sites (i.e., Old O Field, West Branch Canal Creek, and Churchville) yielded over 10 Mb of colony performance data per day. Each site had a unique colony activity profile.

The flight activity counters were found to be the most promising of the devices employed for continuous monitoring of colony parameters. The counters demonstrated fast response times and provided a detailed profile of colony activities. More importantly, the counters provided a means of examining both acute and chronic adult bee mortality. When forager bees

are exposed to toxic chemicals in the field, such as insecticides, some of the bees die in the field. Of those that return to the hive, the sick and dying individuals will be removed by 'undertaker' bees. Hive mounted traps, often called Todd dead bee traps, collect bees removed from the hive. However, the bees in the trap represent only a portion of the acutely affected bees (i.e., those that managed to return to the hive versus those that died in the field).

Our internal, hive-mounted traps provided this classical measure of acute bee mortality. However, the entrance mounted flight counters proved capable of determining the actual reduction (net loss) in numbers of forager bees returning to the hive compared to those that left the hive each day. In addition, the counters clearly demonstrated alterations in patterns of bee flight activity in response to a variety of non-lethal stressors, including time of day and changes in weather. For a given site, the monitored colonies exhibited distinct diurnal patterns of flight activity which were markedly consistent from hive to hive. In addition, coefficients of variation (C.V.) for total daily flight activity between hives appears to be a promising metric for assessing overall colony condition. C.V.s increased on stormy days and at the end of the foraging season. Total flight activity was most variable at Old O Field and least variable at West Branch Canal Creek.

Flight activity and colony core temperature yielded the most useful information about colony condition and performance. From these two parameters, we were able to determine not only foraging activity levels but also whether the colony had brood (e.g., eggs, larvae, pupae) and a laying queen. Core temperatures and flight activity decreased markedly in queenless colonies and exhibited a differential decrease in those colonies lacking eggs, eggs and larvae, or all brood stages including pupae. Because a single queen lays all of the eggs that result in the brood that produces the entire compliment of bees that make up the colony's population, flight activity in combination with core temperatures provide a metric that shows considerable promise for determining the reproductive status of each colony.

Colony responses to a variety of stressors, including exposures to chemicals and oncoming storms often could be visually detected in the graphs and tables that were displayed on the computer screens at each field site. Ongoing research is directed at providing curve smoothing, statistical, machine-learning, and other techniques that will identify and 'flag' significant deviations from normal colony performance (adjusted for factors such as weather and food availability) and provide an immediate, electronically transmitted alert of a parameter that is out of compliance—exceeds warning or control limits. Accomplishment of that capability is a major task scheduled for the summer of 1997. Based on the 1995 and 1996 field studies at APG, it appears that colony performance data can provide an early warning of the presence of a wide array of external stressors including chemical exposures and storm events. Typical response times were under one hour, sometimes occurring in less than two minutes.

For both summers, on screen displays provided minute by minute and daily summaries of the sensor output. Data summaries were saved to hard disk and to a floppy disk at 24 hr

intervals. Data collection continued through November 14, 1996. Given the large size of the resultant database, final data analysis and interpretation is an ongoing activity. Preliminary results concerning colony responses are presented in this report. Also included is a discussion of the specific methods that we are developing to provide reliable real-time monitoring of environmental contaminants and more immediate detection of changes in colony conditions that may warrant colony inspection or chemical sampling to determine the cause of the variation.

Monitoring of Exposures to Bioavailable Contaminants

Our honey bee biomonitoring system tracks both acute and chronic effects as evidenced by changes in colony behavior, structure, and performance as well as by exposures determined through chemical residue analysis. A major focus of our Army sponsored project has been the addition of methods for detecting volatile and semi-volatile organics in bee colonies. The resultant procedure samples colonies for volatile and semi-volatile organic compound residues by pulling hive atmospheres through sorption traps and subsequently using thermal desorption/gas chromatography/mass spectrometry (TD/GC/MS). Air samples were drawn through sorption tubes connected to low-flow sample pumps. Hive clusters (plus external ambient air) were periodically sampled at all sites over both years. Pumping periods varied from 8 to 25 hrs. Sample tubes were thermally desorbed into a capillary GC/MS instrument.

Heavy metals and three other inorganic elements of interests (As, Be and Se) were assayed by microwave digestion of whole bees or pollen for analysis by inductively-coupled plasma mass spectrometry (ICP/MS). For determinations of inorganic contaminants, whole bees and pollen were oven dried and chemically digested in a microwave oven. Following digestion, sample concentrations were quantified on an ICP/MS.

APG Assessment Evaluation

The air inside beehives placed at West Branch Canal Creek in August, 1995, showed little evidence of bioavailable contaminants, nor was there any evidence of acute toxicity to bees. The colonies at West Branch Canal Creek did as well, if not better, than similar colonies left behind in Montana. The 1995 hive samples allowed us to establish a good baseline of volatile and semi-volatile organic compounds found in healthy bee colonies. Additionally, we were able to institute some artificial neural network training for recognition of normal hive atmosphere composition.

In 1996, we pumped more aggressively and found chlorinated solvents and other organic contaminants in hive atmospheres. The most significant chemical found was perchloroethylene (PCE), a degreasing agent used extensively at APG. The highest levels were found at the Old O Field landfill, where PCE has been reported at levels up to 1030 ppb in ground water tested prior to the capping activities. Other chlorinated compounds detected were tetrachloro-methane, trichloroethene (TCE), hexachloroethane (PCA), and 1,4-dichlorobenzene. These,

too, were reported in ground water samples prior to cleanup activities at Old O Field and the West Branch Canal Creek. Naphthalene, a polycyclic aromatic hydrocarbon (PAH) present in petroleum-based products, was found at all sites.

Many contaminants were found in hives at levels noticeably higher than in the ambient air. This suggests that these substances may be bioavailable to forager honey bees from media other than air, such as water, or that they were bioaccumulated through their assimilation from the atmosphere. For most contaminants, the highest levels were associated with the hazardous waste landfill at Old O Field. In general, colonies placed across the residential part of the Edgewood post displayed higher readings than those found at either the West Branch Canal Creek site or the off-post, rural reference site. This is not unexpected because several of the hives were in locations of past spills, decommissioned plant facilities, or active building renovations. It should be noted, however, that levels reported at these locations displayed widely varying individual values, ranging from non-detectable to readily detectable, depending on location, colony sampled, and sample collection date. Also, hives from the Churchville reference site contained low levels of chlorinated solvents, naphthalene, and some substances such as 1,4-dichlorobenzene that were first found at Churchville and later detected at APG. Because the Churchville site is distant and upwind from APG, these contaminants are not suspected to have been atmospherically transported from APG. By comparison, colonies in Montana also contained organic contaminants that arise from ubiquitous sources such as naphthalene resultant from gasoline and diesel fuel residues, but did not display most of the chlorinated solvents found at APG or Churchville.

Overall, heavy metals, As, Be, and Se concentrations in honey bees and pollen were low, approximating background levels for bees from other parts of the United States. Slight elevations of inorganics such as arsenic, cadmium, nickel and strontium were observed in some of the samples from Old O Field and other APG sites compared to the Churchville reference site. Previous studies have shown that arsenicals are known contaminants at Old O Field and some other post sites. However, the distribution is generally restricted to isolated hot spots. Our previous studies have shown bees to be excellent biomonitors for terrestrially dispersed arsenic. Our APG results did not reveal any widespread bioavailability of arsenic during the foraging period (mid-summer to late fall) of our 1996 measurements.

Colony performance also indictated little evidence of exposure to acutely toxic contaminants. Pooled across the summer for 1996, there was no statistically significant difference ($P < 0.415$) in bee mortality between the three instrumented sites—Old O Field, West Branch Canal Creek, and Churchville. On the other hand, statistically significant ($P > 0.001$) time by interactions for adult bee loss were observed. Throughout the season, bee loss at West Branch Canal Creek was as low or lower than at the other sites. Bee loss at West Branch Canal Creek gradually increased with time. Depending on the date, colonies at Churchville or Old O Field displayed higher bee loss than colonies at West Branch Canal Creek. In addition, some stress responses were observed in both the instrumented hives and the nucleus colonies placed at Old O Field. Queen disappearance occurred in 50% of these

colonies, resulting in decreasing foraging activity and lower colony core temperatures in the affected hives. These same hives exhibited the highest detected levels of organic solvents.

Hazard Assessments

Detectable levels of a variety of hazardous volatile chemicals were found at ***all*** sites, including the off-post reference site. With the exception of the waste dump that is being capped, these contaminants occurred at exposure levels that had minimal impacts on honey bee colonies during the period of the study. However, in both years, our field trials were begun during the summer, after the major nectar flows had ceased. To adequately address bioavailability of hazardous chemicals at these sites and to detect any adverse effects, monitoring activities should be extended through the spring and early summer when different floral resources are available, such as dandelions in lawns and nectar yielding trees.

In general, contaminant levels in the instrumented hives at the West Branch Canal Creek removal activity location were as low or lower than the levels found at the off-post reference site. Similarly, colony flight activity, thermoregulation in the brood nest, and queen status was as good or better than for the colonies at the reference site. Given the wide variation in the levels of organic solvents in bees from relatively closely spaced locations on the post, it appears that non-military sources probably account for the contaminants observed at the Churchville reference site.

The present study demonstrates that bees can be effective for evaluating the bioavailability of organic contaminants known to have been released from the hazardous waste sites where they foraged. Bees sampled an extensive environmental setting with placement of single clusters of hives. Equivalent monitoring with stationary instrumentation for air contaminants and sampling grids for soil, water and vegetation, would have been cost prohibitive. When our analytical chemistry results are combined with data from electronic flight activity counters; sensors for temperature, relative humidity, and biomass in hives; traps for collecting pollen and dead bees; and meteorological data, real-time observations of bee behavior can provide continuous measurement endpoints for ecological monitoring. These results can be used to compare and rank hazards to bee populations at different sites.

ACKNOWLEDGMENTS

We would like to thank Ms. Cindy Powels, Mr. John Wrobel, Ms. Allison O'Brien, and Mr. Frederick Brunton of the Environmental Preservation, Conservation and Restoration Division of the Directorate for Safety, Health, and the Environment for their support during the study. Mr. David Simmons and Mr. David Zink provided their expertise concerning proper management of honey bee colonies in east coast habitats as well as providing queens, bees, and off-post reference sites. Matthew Loeser, Rachelle Adams, and Danny Sullivan managed our Montana apiaries and coordinated more than a dozen undergraduate students who helped design, build, and test these methods and equipment. We also wish to thank Mr. Alan Rosencrance of the U.S. Army Center for Environmental Health Research (USACEHR)(provisional, formerly U.S. Army Biomedical Research and Development Laboratory), Fort Detrick, Maryland, for conducting the heavy metal and other inorganics analysis. Mr. Tommy Shedd of USACERH provided critical guidance as the contracting officer representative. We acknowledge the Research Methods Branch of USACEHR for supporting the study through U.S. Army Contract No. DAMD17-95-C-5072

SECTION 1

INTRODUCTION AND OVERVIEW

The primary goal of real-time biomonitoring with honey bees (*Apis mellifera* L.) is to predict the effects of exposures to chemicals (single elements, compounds, and complex mixtures) and other stressors (weather, food resource availability, disease, mites) on the health of individual bees, bee colonies, bee populations, communities, and ecosystems. Because bees are pollinators of many flowering plants, any stressor that adversely affects the numbers or flight activity of forager bees may indirectly affect plant communities. Because bees share environments with other animals and humans, including air quality, often collecting food and water from the same sources, and experiencing similar stresses, they can act as sentinel monitors to warn of potential ecological and health hazards in the terrestrial environment.

1.1 Honey Bees as Cumulative Effects Monitors

U.S. Army facilities must access complex mixtures of contaminants, including many military unique chemicals. Rapid, inexpensive methods are needed to discover and to manage chemical distributions and impacts. Terrestrial monitoring often relies on sampling of several media—air, water, soil, vegetation, and food. An alternative tactic is to use a mobile sampler that covers a large area, takes thousands of samples, and returns to a fixed location where sampling and other measurements can be conducted.

Honey bees fit these criteria and have been proven to be useful as *in situ* monitors of contaminant exposure (1-23) and associated effects (3-9, 24-38) reviewed in (2, 4, 8, 21, 22, 39). They address cumulative effects including altered land use, the availability of floral resources and changes in plant diversity, weather, anthropogenic chemicals, and biotic stressors including diseases, parasitism, and predation. The population dynamics of bee colonies provide measures of productivity and colony performance, while chemical analysis of bees and the products they produce fulfill the roles of monitoring and tracking of hazardous chemicals, including potential for transfer to humans via the food chain. Mammals and birds can serve similar functions, but their use is technically difficult, costly, and animal-welfare issues pose significant problems (23, 39).

Honey bees have been afforded a Class I (i.e., a ready to use) designation for Ecological Assessments of Hazardous Waste Sites by the U.S. Environmental Protection Agency (23). The National Research Council characterized honey bees as "excellent monitors of air quality (39). However, despite this recognition of the utility of bees for use as biomonitors, previously employed methods have had to rely upon periodic measurements of colony performance and condition combined with appropriate sampling to determine whether the bees had been exposed to harmful contaminants. The primary objective of the present study is to develop methods for monitoring honey bee colonies in real time with the goal of eventually linking the colony response to a specific environmental stressor or group of stressors, including any biotic, physical, or

chemical entity that may induce an adverse response. Additionally, the developed methods should be able to differentiate, or at least provide substantial weight-of-evidence, including any uncertainties, concerning the probable causative agent.

With respect to chemical stressors, bees monitor several media—air, water, soil, and vegetation and pick up contaminants in gaseous, liquid, and particulate forms (6). Branched, electrostatically charged body hairs combined with widespread foraging make them nature's flying dust-mops, effectively sampling anything in the air or on plant surfaces. Collection of pollen, resin, and nectar adds to capability of monitoring contaminants in or on plants. During hot weather, a colony of bees collects up to a kilogram of water per day to evaporatively cool their hive (40). Studies conducted in a forested area on the east coast of the United States by Seeley (41) documented that a single bee colony can collect and consume 20 kg of pollen and 60 kg of honey each year, flying 20 million kilometers overall on several million foraging trips. Most of the bees from a full-sized colonies foraged within 600-800 m of the hive; however the mean foraging radius could extend to more than 2000 m.

Bees have been used as biomonitors for more than 20 years at EPA Superfund sites, large federal facilities, and in rural and urban settings (2, 8, 23, 39). Exposure is measured via bee body burdens or by chemical residues in nectar, pollen, propolis, wax, and hive atmospheres. The results can be used to detect sources of pollutants, determine contaminant bioavailability, and produce dispersion maps of chemicals. We have used bees to map the distribution of a wide array of pollutants on scales ranging from small waste sites to landscapes.

Considered with effects assays, exposure verifies the co-occurrence and the interaction of the chemical stressor with a part of an ecosystem, including biotic and abiotic constituents or the ecosystem itself. Biologically, the potential for effects to populations, communities, and ecosystems is of greater concern than simply exposure. Short- and long-term bioassays, especially those addressing population effects, are often used to assess the hazards posed by environmental contaminants, mostly as single chemicals, and occasionally as complex mixtures of potentially harmful substances.

1.2 Diagnostic Evaluations

Until recently, biomonitoring with honey bees has focused on toxicity as the hazard assessment endpoint and has relied upon periodic chemical sampling and evaluations of colony condition (8). This approach provided evidence of exposures to contaminants and documentation of acute and chronic effects, but it more often yielded a postmortem assessment than a diagnosis of environmental conditions. In addition, time consuming and relatively costly procedures such as chemical sampling and analysis were usually conducted at fixed intervals rather than when an exposure event or environmental impact occurred. Frequent examinations of bee colonies to conduct measurements imposed additional stresses.

The goal of this project is to provide the Army with a means of using bees as real-time (continuous) monitors to more rapidly detect the presence or introduction of hazardous chemicals. Our task is to develop the equipment and methods necessary to realize real-time capability. The challenge is to differentiate change in colony population dynamics induced by anthropogenic stressors from those produced by variable such as weather. The goal is to use colony responses to trigger appropriate chemical sampling to recognize the cause of the observed effect.

During the summer of 1995, continuous monitoring was initiated using bee colonies deployed at Aberdeen Proving Ground (APG)—Edgewood. Because environmental contaminants rarely occur as single chemicals or as originally released forms, colonies were deployed at an actual military site to refine the methods. The approach utilized *top down* (field to laboratory, colony to individual, effects to exposures) testing. By combining continuous monitoring of colony behavior with controlled field and laboratory dose-response assays, the aim is to be able to discriminate and link specific stressors with observed responses.

1.3 Real-Time Measuring of Colony Resposnes at APG

To fully achieve monitoring of bee colonies in real time requires:

- Equipment and methods to continuously measure colony behavioral responses.
- Immediately accessible and continuously displayed output data.
- Identification of response variables that can be linked to exposures.
- A warning system that provides an immediate alert that a predetermined response threshold for a single colony or a group of colonies has been exceeded.

The first three of these steps was the focus of the 1995-1996 field studies. Ideally, to provide a real-time alert, a decision-making software program would provide an alarm of an out-of-compliance parameter. It should be feasible to transmit this signal to a distant computer and operator. Also, the lag time between the response and the alert should be relatively short. Depending on the monitored response variable, this lag could vary from a few minutes (e.g., exposure to a chemical that quickly alters flight activity), to a day (e.g., a net loss of foragers returning to the hive before nightfall), to several days (e.g., exposure to sublethal or chronic toxicity).

Because the initial field applications targeted development of continuous measurement methods and identification of appropriate measurement endpoints, the real time data displays provided a form of real time monitoring data. However, it was impractical to provide an observer to continuously watch the displays of the three computers at each of the three sampling locations. In addition, access to the Old O Field site was restricted to the daylight hours at the end of each work day, after the heavy equipment operators working on the landfill cap had departed.

For 1997, response thresholds will be determined based on the extensive database obtained in 1996. From this information, software programs will be developed that can transmit an electronic alert (i.e., a screen message or audible alarm) to an operator, either at an individual site or at some central location. For acute exposures that result in short-term responses such as a sudden reduction in the numbers of foragers returning to the hive, real time monitoring should be feasible by the summer 1997. For chronic exposures, the response may only be discernible by a assessing a trend that may take several days or weeks to be manifested. For example, an exposure to low levels of a toxin that slowly accumulates in the colony may not be immediately discernible. Also, the ability to identify specific exposure-response terms should improve with experience, experimentation, and advanced data analysis.

Initially, real-time measurements and real-time displays of colony responses to environmental stressors were feasible, but data analysis and interpretation, as well as transmission of the data from the field site to the central data repository, precluded true real-time monitoring. However, visual examination of the real-time data displays at each field site provided a means of identifying acute responses in the field as they occurred. This capability provided a form of real-time monitoring of colony activities, but would have required that a human observer continually monitor the displays to be truly real-time. The development of software feedback systems that would provide an electronically transmitted alert is a major goal of the investigations planned for the summer of 1997.

1.3.1 Electronic Hives

Real-time measuring of colony dynamics was accomplished by outfitting mini-hives placed inside protective wooden boxes, termed condos (Fig. 1.1), with:

- Electronic sensors to measure colony performance.
  - Entrance mounted bi-directional bee counters to monitor flight activity.
  - Integrated circuit temperature probes inserted into the brood nest to observe colony homeostasis.
  - Relative humidity in the brood nest as an additional check on colony homeostasis.
  - Pressure transducers or strain gauges under the hive to measure biomass.
  - Hot wire anemometers in the hive to determine air flow direction and rate as influenced by bee fanning.
- Bottom-mounted traps.
  - Clock-driven traps to sample pollen on a diurnal basis.
  - Hopper-shaped traps to collect dead and dying bees removed from the hive by housekeeping bees.

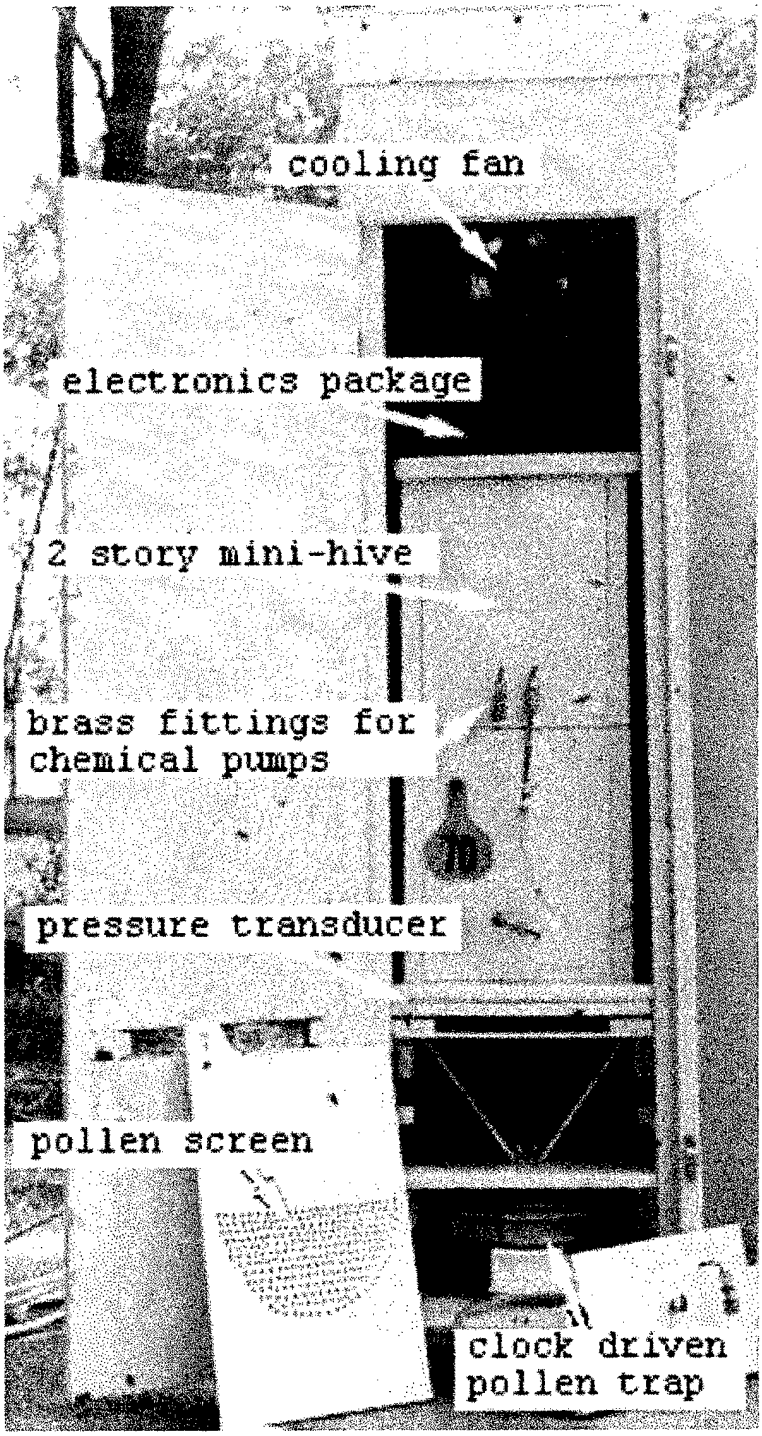

Figure 1.1. Honey bee condo containing an electronic hive fitted with sensors to monitor colony behaviors and probes for chemical sampling. The flight activity counter mounts to the outside of the open door ad aligns with the horizontal slot.

- Chemical sampling sorption traps
    - Copper tubes inserted into hive bodies, brood nests, dead bee, and pollen traps

Instrumented hives were set out in clusters of six to seven hives per site. At each site, an electronic weather station continuously logged ambient conditions, including:

- Air temperature.
- Relative humidity.
- Wind speed and direction.
- Precipitation.
- Barometric pressure.
- Light intensity.

1.3.2 APG—Edgewood Sites

Under funding from USACEHR (formerly USABRDL), Fort Detrick, Maryland, an initial pilot study was conducted in August 1995 using six condos placed adjacent to the west branch of Canal Creek. The results of this pilot study established the feasibility of conducting real-time monitoring using honey bees on a larger scale and indicated great promise for detecting volatile and semi-volatile organic compounds. In 1996, the Directorate of Safety, Health and Environment (DSHE) Environmental Conservation and Restoration Division (ECRD) commissioned an expanded study to assess various strategic locations within the Canal Creek, Lauderick Creek, and Old O Field Study Areas.

In late July, clusters of seven instrumented colonies were deployed at the:

- West Branch Canal Creek near the block of trailers belonging to the U.S. Geological Survey (USGS) and USACEHR.
- Old O Field hazardous waste landfill.
- Off-post reference site near Aldino road east of Churchville.

Additional groups of two or more nucleus colonies to be used for chemical sampling and assessment of acute toxic events were placed (Fig. 1.2) at the:

- Eastern-most area of Beach Point.
- West of the Youth Services Center off of Wise Road.
- Open field west of the Maryland National Guard Armory, south of the DSHE offices.
- Abandoned railroad tracks just west and north of the G Street area, south of the post boundary.
- Lauderick Creek area, just behind the fence adjacent to the golf course.
- East Branch Canal Creek, southeast of the horse stables.

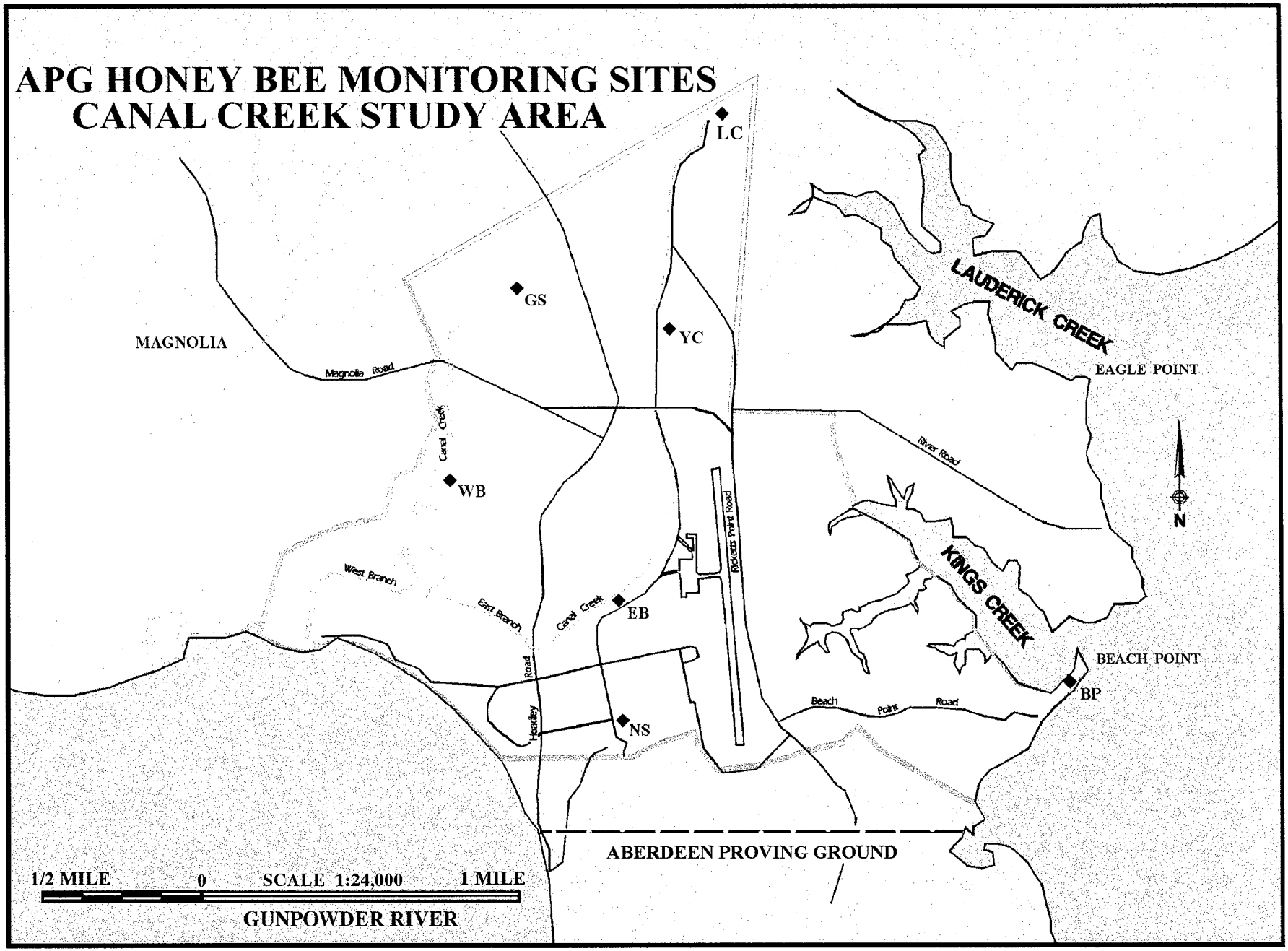

Figure 1.2. Honey Bee Sampling Sites at APG—Edgewood, 1996.

1.4 Objectives

The primary objectives of our ongoing four-year study are to:

- Provide real-time monitoring capability for military unique chemicals, especially volatile and semi-volatile organics.
  - Provide real-time monitoring for effects and calibrate bees for an array of chemicals and environmental conditions.
  - Provide real-time monitoring for exposures to evaluate the dose-response functions as indicated by monitoring of effects (an ongoing activity).
- Validate bioassessment methods employed for real-time monitoring.
  - Determine the variability of effects assays chosen for monitoring so that field trials can be designed to detect a defined change in response to chemical contaminants.
  - Refine and validate ecotoxicological models for use in designing ecological assessments using bees and for predicting and interpreting population response.
  - Establish standards for Quality Assurance.
- Determine which methods are the most cost-effective and useful predictor of population responses

The primary objectives of the field applications at Aberdeen Proving Grounds were to:

- Conduct a pilot study to test the feasibility of real-time monitoring of volatile and semi-volatile chemicals at military sites (August, 1995).
- Apply real-time measuring of honey bee colony behaviors as a means of surveying the overall quality of the terrestrial environment at multiple locations for the presence or introduction of hazardous chemicals (May - November, 1996) and any associated short- or long-term toxicity.
- Provide a biomonitoring assessment evaluation for selected areas at the Aberdeen Proving Ground—Edgewood area.

1.5 Aberdeen Site Description

The Edgewood area of Aberdeen Proving Ground is located northeast of Baltimore and southwest of Aberdeen, Maryland. It consists of a peninsula extending into Chesapeake Bay. The northernmost portion is characterized by estuaries, hardwood forests, open meadows and lawns. Clusters of houses, barracks, offices, and laboratories occur throughout the area. Recreational facilities include a horse-riding stable, trails, and golf, archery, and trap-shooting courses. Wade airfield occupies the central area of the post. Decommissioned chemical plants and laboratories are scattered across the area, particularly near the West Branch Canal Creek. The southernmost part of the peninsula is closed to general access and is divided into several fields. This project focused on hazardous waste landfill at Old O Field. An off-site reference location was established upwind from Aberdeen on a small acreage just off Aldino Road east of Churchville. The hardwood forests, open meadows and lawns approximated the habitat found over much of the Edgewood post area.

1.5.1 Historical Use

The Edgewood area has been used for a number of activities which have contributed to the contamination of soils and water. Many of the chemical processes in this area used industrial solvents, by-products and breakdown products from rocket fuels, pyrotechnics, and nerve gas. These occur in localized hot spots such as small pits and landfills and have become more widely dispersed in groundwater and some soils and sediments. Multiple heavy metals, other inorganics such as As, Be, and Se, and a wide array of chlorinated aliphatic hydrocarbons have been found across the site. However, most of the monitoring and remediation conducted to date has focused on contaminants in groundwater. Much less is known about air quality and the terrestrial environment. Similarly, little information exists concerning the potential for migration off-site and exposures to people living both on- and off-site.

1.6 Honey Bee Biomonitoring at Edgewood

As described under Section 1.2, in 1996, we deployed pairs of hives at several locations and clusters of seven hives at three locations to assess exposures to volatile and semi-volatile organics, heavy metals, and other inorganics. These periodic observations were correlated with real-time data concerning honey bee colony population structure and performance. Superimposed on this assessment application were some additional experiments and equipment tests intended to further improve and refine our development of real-time monitoring, particularly with respect to providing the information necessary for achieving the capability of using honey bee colony responses as an early warning system of contaminant presence or increase. At the time of this study, much of the data collection was real-time, but data evaluation lagged considerably due to the enormous amount of data (i.e., hundreds of Mb/day) being generated.

The following sections of this report are focused around specific biomonitoring approaches followed by an overall conclusions section. Each section is intended to cover both the research

and development stage and the actual application in Maryland. Because the field season has just ended, we have just now collated all of the data. This report covers preliminary data analysis and selected issues. For example, the colony behavioral sections illustrate observed colony responses and an initial assessment of the utility of selected measurement and assessment endpoints. Comprehensive data analysis and interpretation by statistical and Artificial Neural Network procedures is an ongoing activity. Similarly, we present the results for inorganics and for seven volatile and semi-volatile compounds found in the beehives and known to be of concern as possible contaminants at APG. Over 200 additional chemicals have been identified. Many of these chemicals are associated with normal bee physiology or the materials from which the hive is constructed. Cataloguing of each of these chemicals and the sources (i.e., from the bees, hive components, or contaminants) are ongoing. All of the samples taken in 1995 and 1996 have been analyzed and the results stored in digital form.

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SECTION TWO

DESIGN AND FIELD APPLICATION OF ELECTRONIC FLIGHT ACTIVITY MONITORS FOR DETERMINING BEE COLONY DYNAMICS

2.1 Introduction

Electronic bee-counters were designed and tested to monitor honey bee forager flights. These devices were optimized for simplicity in construction and ease of use compared to other published designs (1-4). The electronic components were inexpensive, durable, and required little maintenance. The counters measured flight activity, a critical behavior that reflects colony strength and condition. It also responds quickly to weather and to other factors such as exposures to environmental contaminants.

The bee-counter system consisted of: 1) a counting unit built into a porch that attached directly to the front of the protective boxes (condos) enclosing each of the nucleus beehives, 2) a modular digital interface that transferred the data to a computer, and 3) an IBM-compatible, 486 or Pentium grade Personal Computer (PC) that collected and analyzed flight activity data. Up to ten condos could be connected to the central digital interface via 10 m cables. This provided for colony replication at a site as well as flexibility in layout and spacing among sample hives.

This design differed greatly from the counter described by Liu *et al.* (1), who used a microprocessor-based computer with a keyboard and display for each hive. Struye *et al.* (2) also used a hive-mounted, single central processing unit (CPU) which had a serial port connection that could collect data from more than one hive. In both of these designs, the data processing and storage unit was mounted to the front of the hive, where it was exposed to heat, cold and moisture as well as being vulnerable to theft, vandalism, or accidental damage. Because this complex circuitry was part of the counter, building more than one system was neither inexpensive nor simple to assemble. For maintenance and repair the entire counter assembly had to be removed from the hive.

Like Lui *et al.* (1) and Struye *et al.* (2), we used infrared photo-emitters and detectors to track bee activity. The bee-counters were built into a polyethylene bee-landing and take-off porch affixed to the entrance of each condo. The porch caused the bees to enter and exit the hive in an upright position. Each porch had fourteen side-by-side, 5mm x 7mm x 47mm long, entrance tubes for individual bee passage. Each tube had an infrared emitter inset into the ceiling and two photo-detectors mounted front-to-back underneath a clear, Lexan® floor. Using paired detectors allowed differentiation of incoming from outgoing bees, depending on which detector was first blocked by the bee. The spacing between detectors was adjusted to resolve the passage of a single bee from that of two, head-to-tail bees (1-2). Because these counters

were used with small (nucleus) bee colonies, fourteen passageways proved to be sufficient to avoid queuing at the hive entrance.

With this system, a 486 or Pentium grade notebook computer can simultaneously monitor up to ten colonies. A bi-directional, parallel port, digital interface system connected to a square-wave generator ensured fast and consistent sampling rates. Monitoring each passageway permitted investigation of preferential channel use (e.g., end or center passageways) and detection of any tube blockage by dead bees (2) or pollen buildup. The custom software summarized and stored the data from each counting tube at 30 second intervals. This system allowed data comparisons among the entrance tube counts for each hive, the overall flight activity among hives at each site, and ultimately, flight activity levels among several sites. Data from individual channels was also used to align the detector units for each passageway.

The software was written to record and display flight activity in real-time during daytime hours and to save the day's flight data to a user-accessible floppy disk at night when no bees were flying. In addition, off-line analysis software was developed that could detect abrupt changes in flight activity due to external (e.g., weather, food resource availability, environmental contaminants) and internal (e.g., queen condition, hive maintenance, smoke in the hive, chemical treatment to control mites) events. This software was recently expanded to detect long-term changes in overall flight activity on a site-wide basis. The strengths of this system include simplicity of the electronics attached to the hive, use of a single computer for data acquisition from up to ten hives, and real-time analysis and display, at relatively low cost.

2.2 Evolution of Counter Design

Prototype counters used during the summer of 1995 employed a W-shaped detector holder that reflected light from the emitters to the detectors. This system proved useful for assessing patterns of overall flight activity but did not provide accurate counts of individual bees. The system's counting resolution was lower than expected for several reasons: 1) the W-shaped holder allowed stray light reflected from other tubes to create spurious counts, 2) detector alignment was difficult, and 3) resistors used in the voltage drop part of the detector circuit also reduced detector sensitivity to bee passage.

Replacing the W-shaped detector mount with one that placed the detectors head to head in flat grooves improved overall resolution, but the counters still miscounted some of the bees due to insensitivity of the detector circuit. Lowering circuit resistance increased detector sensitivity and improved counter accuracy, which was tested with paper bee models. With the lower resistance (1KΩ), even a thin sheet of paper was sufficient to block a passageway and trigger a count. With the higher resistance (4.7MΩ), only thick cardboard blocked the signal to the detectors. Because detector alignment also affected counter resolution, the emitter and detector holders were redesigned to permit a broader range of adjustment.

These improved counters were tested on condos at The University of Montana—Missoula (UM) apiary at the start of seasonal flight activity in the spring of 1996. During the early

summer, the emitter and detector holders went through another stage of improvement. Detector alignment continued to be difficult due to small variations in the machining of the polyethylene holders. To improve tolerances, a custom built milling jig was fabricated. A steel guiderail on the jig provided consistent spacing of the detector and emitter grooves. The jig produced detector and emitter holders that could be interchanged among counters.

The new counters included weather-resistant, plastic skirts to protect the wiring and electronic circuits from rain and snow. Earlier tests revealed that on cold nights, bees were attracted to the warmth produced by the emitters and sometimes got into the counter circuitry. The skirts also prevented damage to the electronics by small animals such as mice and skunks. Flexible plastic seals above and below the entrance tubes kept bees from by-passing or getting inside the counters.

2.3 Data Acquisition Hardware and Software Description

The original interface system collected data for three hives at approximately 1 kilobyte per second. This was the maximum data transfer rate for the serial (RS-232), A-bus® interface system (Alpha Products, Fairfield, Connecticut) that was employed. This interface could not be expanded to collect data from ten hives. Several other interface systems were tested, but only the Digibook/72 parallel port interface system (IOTech, Inc., Cleveland, Ohio) achieved transfer rates greater than 150 kilobytes per second. This interface takes advantage of the high rates of data transfer achieved with bi-directional parallel ports. The Digibook interface allowed more hives to be connected to the computer and dramatically increased sampling rates, improving the ability to differentiate between bees closely following each other through the counters.

A single IOTech DigiBook/72 parallel port interface can be used with three, five, seven, or ten hives. To optimize data collection accuracy, a square-wave generator was built that provided consistently spaced reference pulses. Software was written that controlled sampling rates by referencing the generated pulses. This enhanced the ability to detect bees traversing the tunnels at high rates of speed. The computer-controlled sampling rate was optimized at 200 observations per second for good resolution and to reduce the time required for data analysis.

Two custom software programs were written to interface the flight activity counters with a computer. One provided a set of diagnostic tools for testing counter performance. The other conducted data collection, processing, and graphical display at the field site. Colonies were monitored and analyzed for flight activity from 6:00 AM to 9:00 PM, after which the program made multiple backups of the data on the central computer's hard disk. Daily data summaries were automatically stored in compressed form on a floppy disk. The software also tested the operation of the detector/emitter pairs and generated a daily systems audit report for each hive.

2.4 Data Analysis Software Description

Data collected from the interface system was processed using code written in BASIC. Originally, three algorithms were evaluated for counting bee flights. Using three different algorithms provided a method for comparing analytical consistency and accuracy. All three algorithms searched each detector pair to find when both detectors were blocked. One of these algorithms tested only the immediately preceding data point for detector blockage; whereas another examined only the succeeding data point. The most rigorous algorithm tested the preceding data point to decide which detector was first blocked and then searched the succeeding data points to find when only a single detector was again blocked. If this algorithm failed to detect complete passage through a detector pair, no count was recorded. For all three algorithms, the sequence of detector blockage indicated the direction of bee movement. Because bees sometimes balked and backed out of a counter passageway, only the most rigorous analysis method provided reliable counts. This algorithm was used to create the daily summaries of flight activity at each of the field sites.

2.5 Real-time Graphical Data Display

Because a major project goal is to use changes in bee behavior to provide a warning of the presence of environmental contaminants, real-time measuring of flight activity was needed to provide a feedback system for enacting the chemical sampling program. By the summer of 1996, a software program was added that provided a real-time, monitor-based, graphical display of counter data for each colony. The on-screen plots of flight activity facilitated assessment of the operational status of hardware, revealed departures from normal flight activity, and provided a means of examining the correlation of flight activity with colony population size and weather events.

The real-time monitoring display was accomplished by splitting the computer screen into two parts. The left half of the computer monitor displayed condensed data from each of the previous five days, and the right half displayed all of the current day's data (Fig. 2.1). In the evening, the program condensed the day's data by averaging flight activity over 2.5 minute intervals. The condensed data set was then added to the display of previous days, and the oldest day's data was removed. Plots of total flight activity for all seven colonies at each site and of the mean flight activity for all of the colonies at each site were simultaneously displayed.

2.6 Off-line Data Analysis Methods

A three-tier visualization system used color-keyed charts to facilitate comparisons of different aspects of flight activity data. Tier 1 charts focused on seasonal (i.e., long-term) contrasts among sites; while Tier 2 charts examined seasonal dynamics among the colonies within each site. Tier 1 (Fig. 2.2) provided time series assessments of the total number of flights for each of the three field sites. Tier 2 (Figs. 2.3-2.5) displayed the daily total flight activity for each of the colonies at a single site. Tier 3 charts (Fig. 2.6) evaluated shorter-term

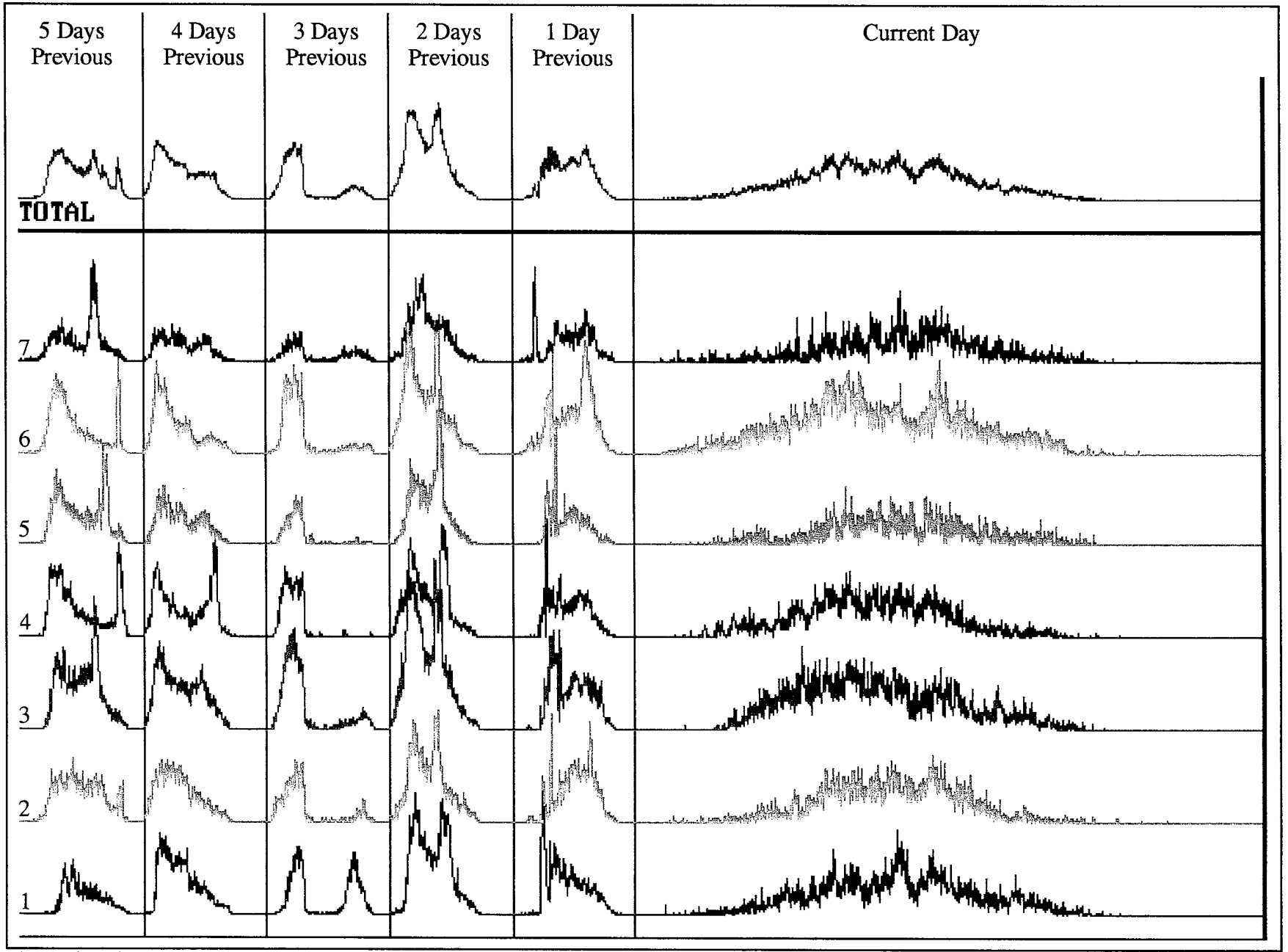

Figure 2.1 Screen-Capture of the Real-Time Graphical Display for Colonies at West Branch Canal Creek Displaying Bee-Counter Data from August 7, 1996 to August 12, 1996. In this figure the current day is August 12, 1996, and the remaining days fill from right to left on the left half of the display. The numbers along the left side indicate the number of the colony, with the top trace, labelled 'TOTAL' representing the mean activity for all seven colonies.

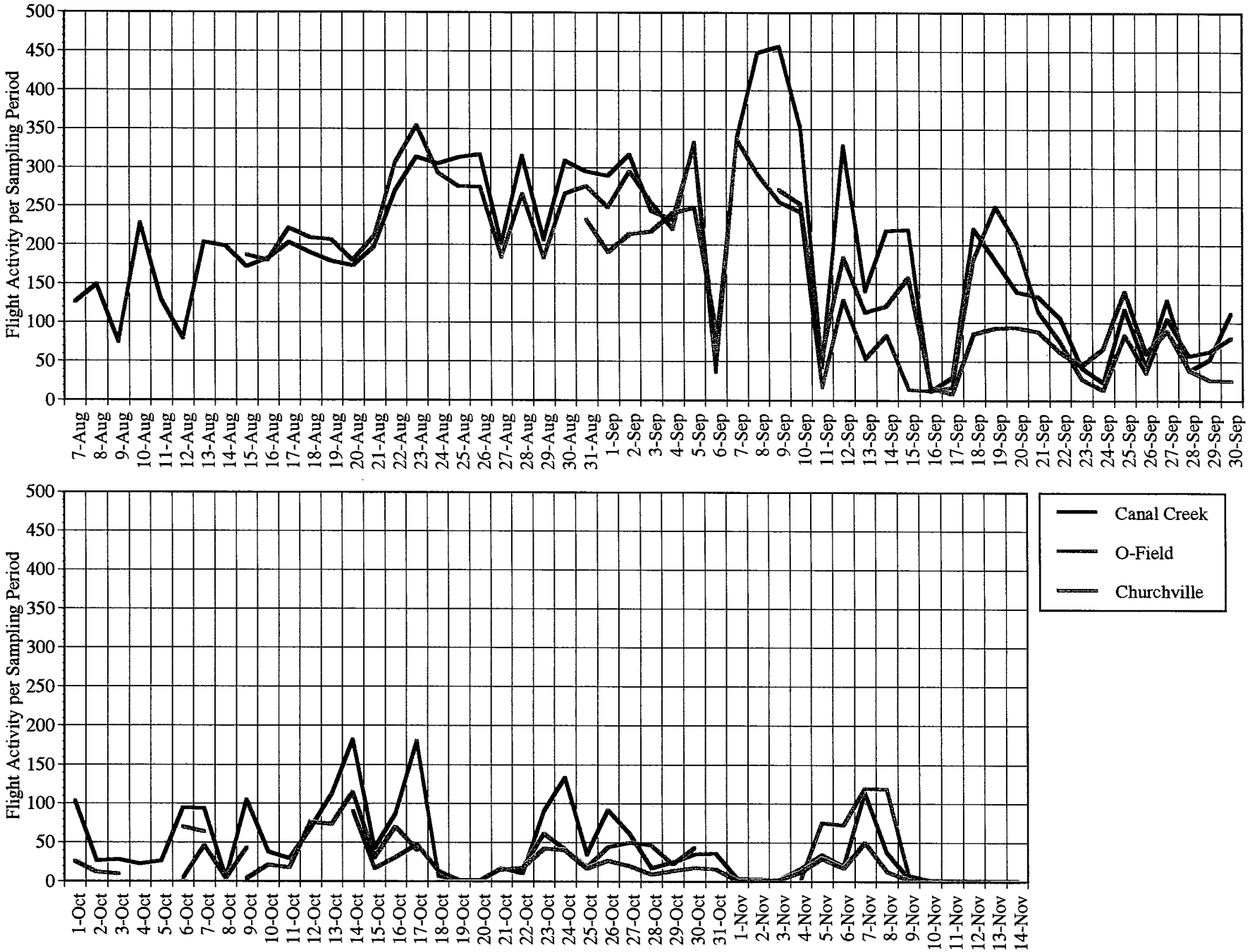

Figure 2.2 APG site by site comparison of total daily flight activity (entering + exiting) corrected for number of 30 second sampling periods collected per day. The x-axis contains dates and the y-axis is corrected for total flight activity.

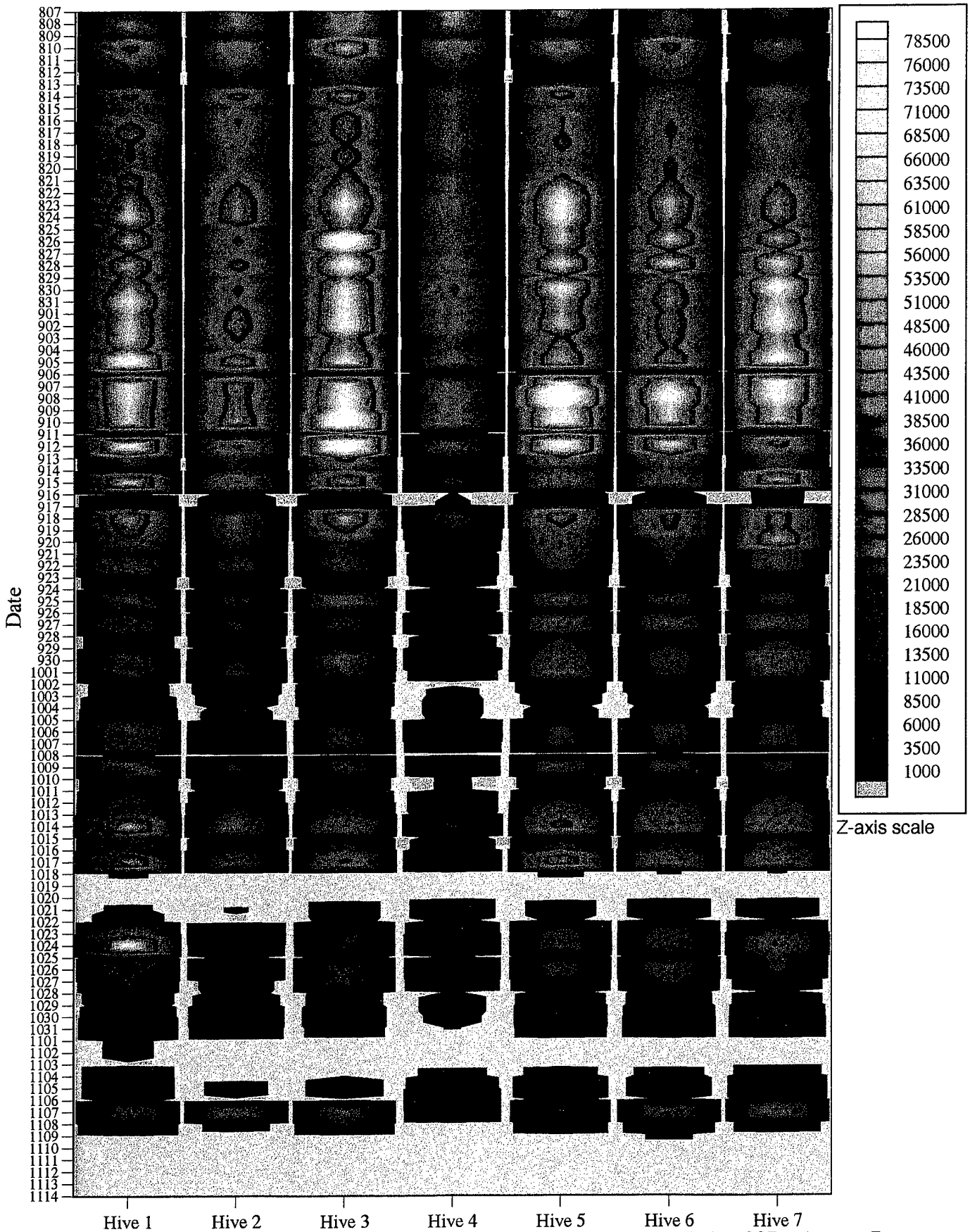

Figure 2.3 Total daily flight activity for Canal Creek hives. Dates are encoded as 807 = August 7, 1996. Hotter colors in the z-axis denote higher flight activity levels. Columns represent individual hives as labelled on the bottom axis.

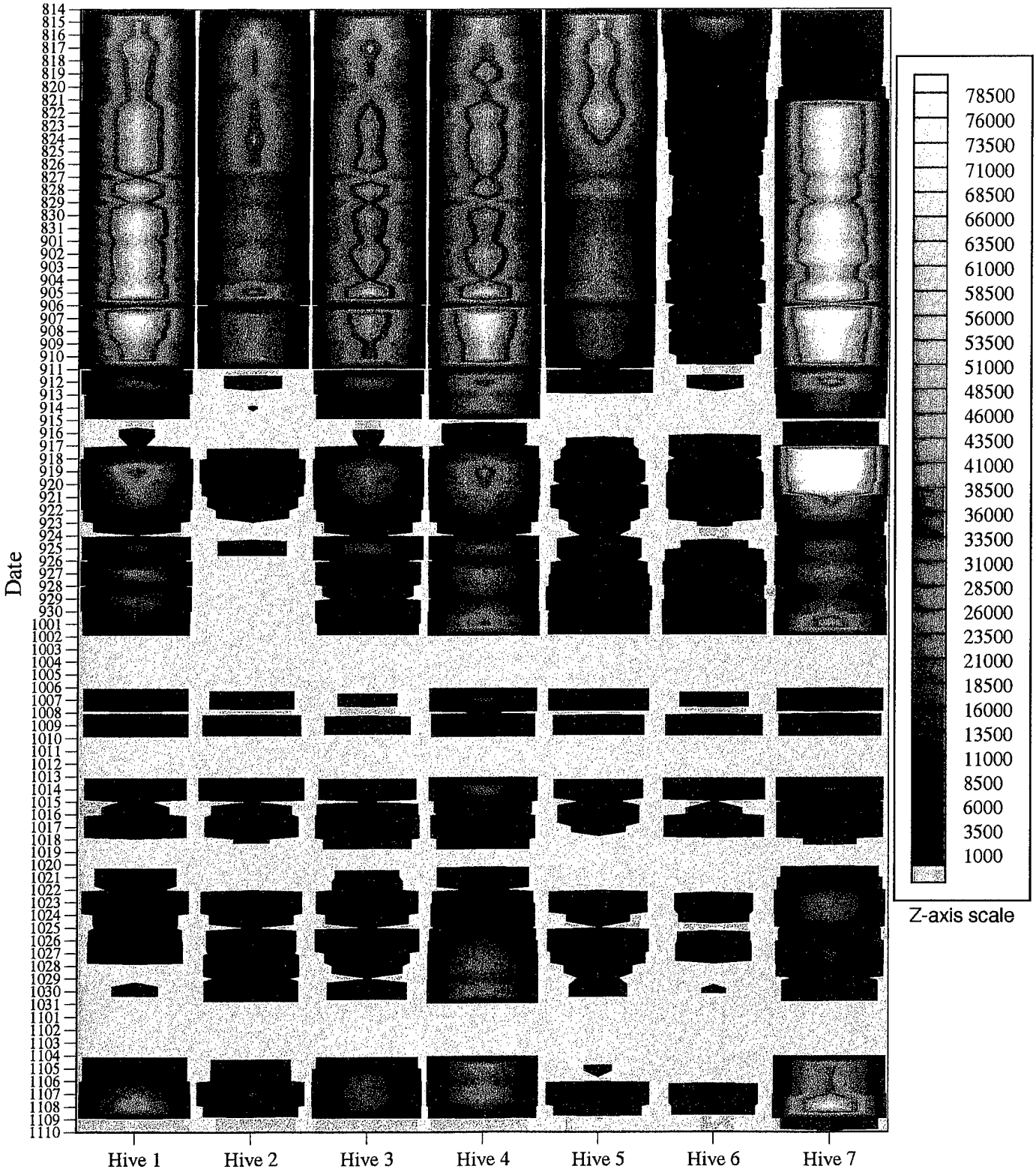

Figure 2.4 Total daily flight activity for O Field hives. Dates are encoded as 807 = August 7, 1996. Hotter colors in the z-axis denote higher flight activity levels. Columns represent individual hives as labelled on the bottom axis.

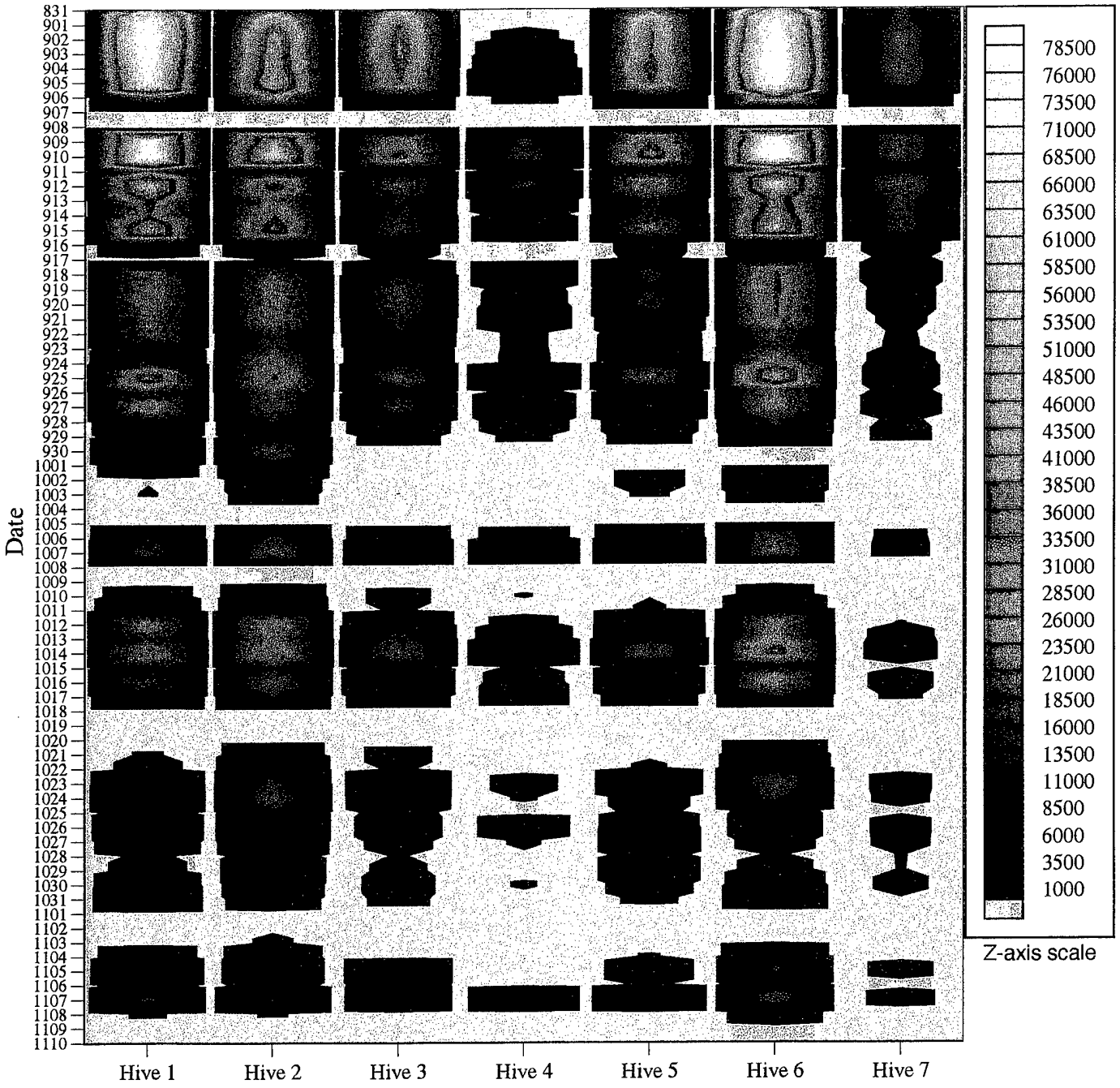

Figure 2.5 Total daily flight activity for Churchville hives. Dates are encoded as 807 = August 7, 1996. Hotter colors in the z-axis denote higher flight activity levels. Columns represent individual hives as labelled on the bottom axis.

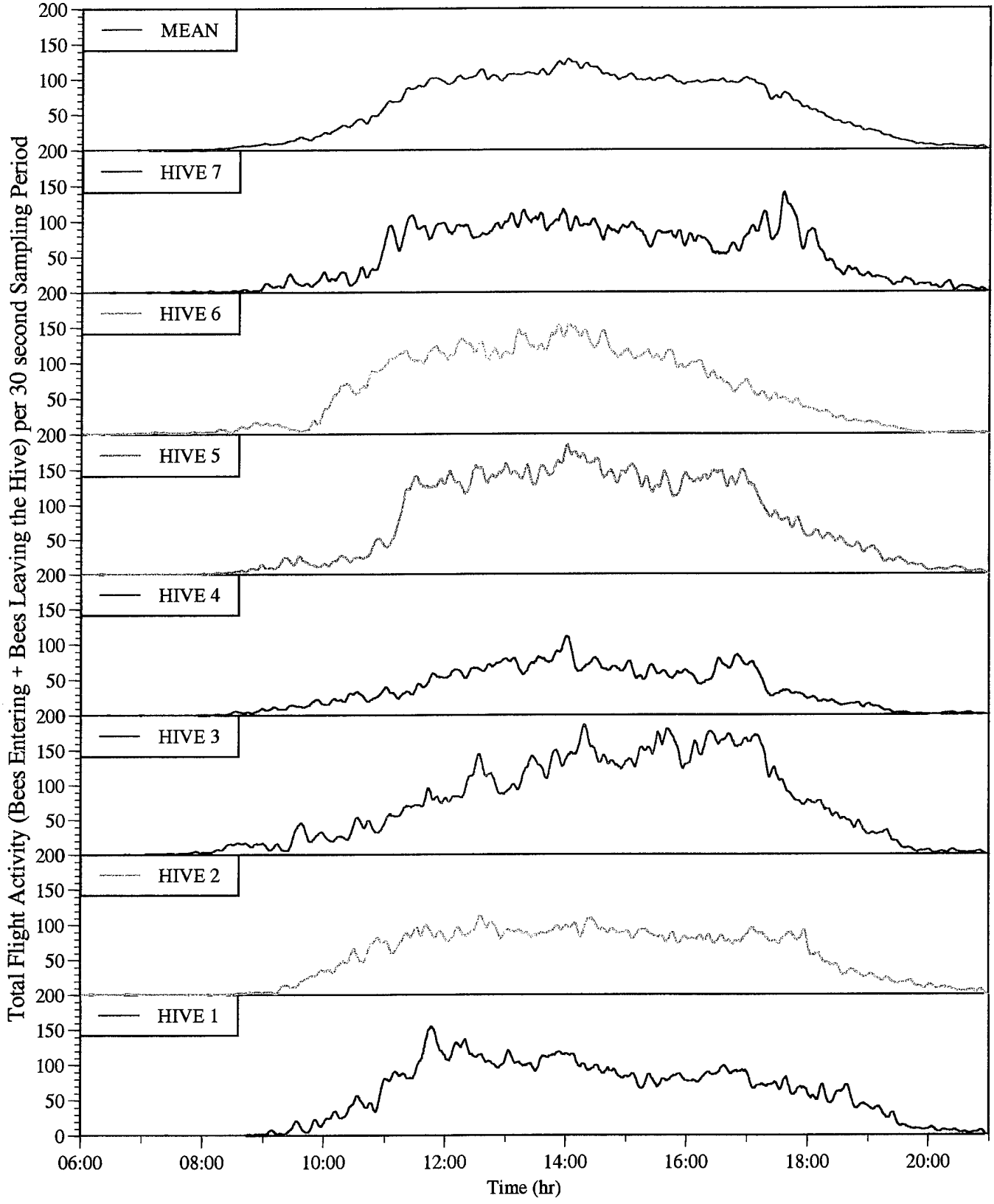

Figure 2.6 Total Flight Activity for West Branch Canal Creek Colonies on Sunny Day in August 1996. The combination of flight activity data from hives 1 to 7 produces the broad continuum exhibited in the mean activity trace.

data by depicting for each day the 30 second summaries of all flight activity variables (bees in, out, and total) for each colony within a site. In addition, the numbers of bees passing through each counter tube for each hive could be displayed. This provided a means of checking counter performance (e.g., whether all of the counting tubes were clear and the detectors working) as well as more closely inspecting the movements of bees in and out of each colony.

Tier 1 flight data was computed as the total number of flights per 30 second count period multiplied by the number of count periods that were recorded for a given day. The among colony coefficient of variation (C.V.) for total activity (bees entering the hive + bees leaving the hive) was used to further assess Tier 1 data for long term trends (Fig. 2.7) in flight behavior as well as differences among the field sites. The C.V. provided a benchmark on which to compare the relative strength and vigor of the colonies at a site.

If all the colonies at a location are alive, actively foraging, and queenright (i.e., a queen is present and laying eggs), the C.V. should be relatively low and constant from day to day (e.g., below 40% as indicated by Fig. 2.7a). If the total foraging activity of one or more colonies changes relative to the other colonies at a site, the C.V. should increase. The C.V. for total foraging should go to zero at night when bees don't fly, or if all of the colonies die.

The strength of the Tier 2 visualization of flight activity for each of the hives at a given site is that it provides a means of determining the source of variation in Tier 1 data. A quick glance at Tier 2 data will reveal any colony or colonies that differs from the others in foraging levels. With this information in hand, closer inspection of the weak colonies by an apiculturist and chemical sampling can be enacted to help identify the cause of the problem.

Tier 3 charts provided a means of displaying rapid changes in the incoming, outgoing, or total flight activity of any colony. Tabulating activity counts every thirty seconds yielded extremely dense data plots. The high frequency of data sampling tended to conceal short-term changes in bee flight. In other words, the sampling frequency noise concealed the event signal. To separate the colony response signal from the sampling noise, data smoothing techniques such as boxcar averaging described by Savitsky and Golay (5) for spectroscopic data were employed.

High frequency data smoothing was accomplished by first averaging consecutive data points within a selected range (usually 6 data points) using a two-point boxcar. The same smoothing technique was then applied to the resultant average. In order to enhance sensitivity to changes in flight activity, absolute derivatives of smoothed raw data were used to produce peaks that indicated the time and magnitude of an activity change. Data used for derivatives was smoothed using a two-part consecutive boxcar average with each boxcar containing six samples.

A QuickBASIC program was written to prepare data files for analysis using Deltagraph© plotting software, Excel© spreadsheets and Mathcad© workbooks. This program was capable of simultaneously displaying raw data for all colonies at one site for each day, and it included data smoothing, derivative analysis, and relative return ratio (i.e., numbers of incoming versus outgoing bees) analyses routines. An important feature of the software is the ability to retrieve a

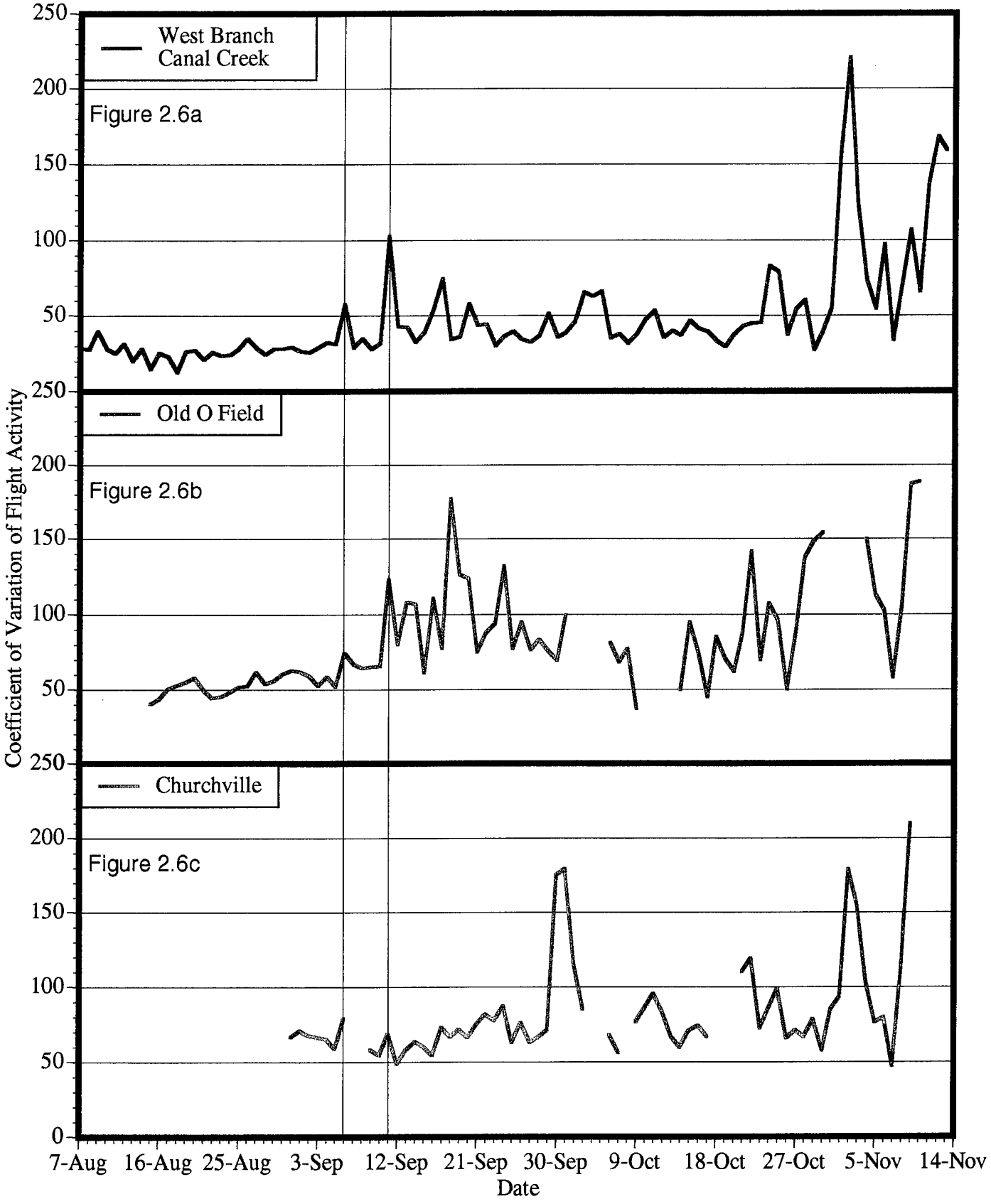

Figure 2.7 a, b, and c. Coefficient of Variation of Flight Activity Among Colonies at APG Sites During Late Summer 1996. The two solid lines common to all plots represent days of heavy rainfall, and thus, low flight activity producing high variability among colonies.

previously stored data file and to display the data in the same graphical format as seen in the field when the counters are operating (see Fig. 2.1). Another useful feature of this program is the ability to patch together broken data files that result from restarting the computer in the middle of the day. Recently, this program was ported to PowerBASIC for a substantial increase in speed.

2.7 Field Trials Conducted

Four sets of field trials were conducted. The first trial was conducted with six hives in Maryland in August of 1995 as a preliminary test and demonstration of the technology. The second trial at the UM apiary took place in the fall of 1995 using counters with redesigned emitter and detector holders. Over the winter, the detector circuits were modified and tested. The third field trial began in the Spring of 1996 at the UM apiary with three hives. These trials included the new parallel port interface, vastly improved emitter and detector alignment, enhanced detector sensitivity, and refined analysis software. The fourth trial was conducted from August to November of 1996 using 21 hives at three different sites in Maryland. Seven colonies at each site were continuously monitored for flight and other indicators of colony performance (see Section 3 of this report). The Maryland field trials included real-time analysis and display software, the finalized design of the counter emitter/detector system, and improved weather protection for the counters.

2.8 Results of the Final Methods Trials

The Three-Tiered Data Visualization system provided an organized approach in identifying changes in flight behavior as affected by acute and chronic exposure to internal (inside of hive) and external (outside of hive) stimuli. Internal stimuli consisted of factors such as hive manipulation (including smoke injected into the hive to calm bees, adding brood frames or bees to strengthen or equalize bee populations, and inserting Mite-A-Thol Menthol™ and Fluvalinate™ to control bee mites), egg-laying by the queen, brood rearing, and concentrations of environmental contaminants inside the hive. External stimuli primarily consisted of weather events, resource availability (e.g., nectar, pollen, resin, water), and bioavailability or release of environmental contaminants, including acute episodes such as crop spraying . Acute responses were characterized by a sudden change in flight activity, while chronic effects appeared as more gradual or longer-term changes (e.g., days, weeks) in flight activity.

The 1996 flight data revealed that flight activity can undergo drastic changes in less than one minute. Data collected with the original serial interface (Summer/Fall 1995) was collected at 5 minute intervals. During the Spring of 1996, the sampling interval was reduced from 2 minutes to 1 minute with the parallel port interface, and eventually to 30 seconds when real-time analysis was brought on-line. This increase in sampling capacity allowed characterization of events that previously would have been disregarded. Earlier work done with bee-counters by Struye *et al.* integrated data on a 15 minute interval. Data from the present study indicated that such a long integration interval can mask the effects of short-term internal or external events making it more difficult to assign potential causes.

Boxcar averaging and filtering techniques eliminated the high frequency sampling noise, but retained the important fine features used to detect changes in flight activity. The derivative technique accurately identified short-term responses to acute events, whether biotic or abiotic. Events that induced a simultaneous response in most or all of the colonies at a given site were particularly evident when derivative analysis was applied (Fig. 2.8). This method was less useful for detecting longer-term or gradual changes in flight behavior.

For distinguishing the effects of chronic events, the coefficient of variability among colonies for total flight activity proved to be more effective, especially for site comparisons (discussed in Section 9.2.2). Other methods being evaluated for detecting colony behavioral responses to chronic stressors include applications of advanced computer learning methods such as the use of an artificial neural network (ANN) to detect patterns in colony flight activity as affected by weather conditions and modified by other factors such as food availability and colony growth which are measured by other electronic hive sensors (described in Section 3).

In addition to the BASIC data analysis software, Mathcad© and Excel© proved to be the most useful software for viewing data files and for conducting exploratory data analysis. They allowed direct comparisons of smoothing techniques and also helped determine the minimum length of time necessary for a sampling interval.

2.9 Results of Field Trials of Equipment and Methods

During the August 1995 field trial at APG, the prototype bi-directional bee counters proved adequate for identifying general flight activity patterns. Bee activity was highest between 11:00 and 17:00 hours, except on the 20th when activity remained high until 20:00. All days showed some bee activity until 20:00 hrs.

The prototype counters exhibited some spurious nighttime counts. However, the general activity patterns appeared to be reasonable. During a second set of field trials in Montana, additional tests of the positioning of the infrared detectors were conducted. It was found that detector spacing affects counter resolution. For example, if one bee closely follows another through a pair of detectors, the counter has to be able to resolve whether one or two bees passed through within a fraction of a second. Decreasing the gap between counters and increasing sampling frequency (i.e., the number of times each detector was sampled per second) improved sensitivity. Bees sometimes investigated, hesitated or backed out of counter tunnels, resulting in spurious counts, especially with closely set detectors. Slightly wider spacing of the counters combined with a higher sampling frequency reduced the number of spurious counts without sacrificing resolution.

Further improvements made to the counter hardware and software (described in 2.4-2.7) eliminated spurious counts, increased sensitivity to bees passing through the tubes, provided better alignment of detectors with emitters, and resulted in faster and more accurate sampling rates. Whereas the original serial interface sampled the hive at approximately 12 Hz, the parallel port interface and more rigorous analysis software permitted reliable, user-adjustable, sampling

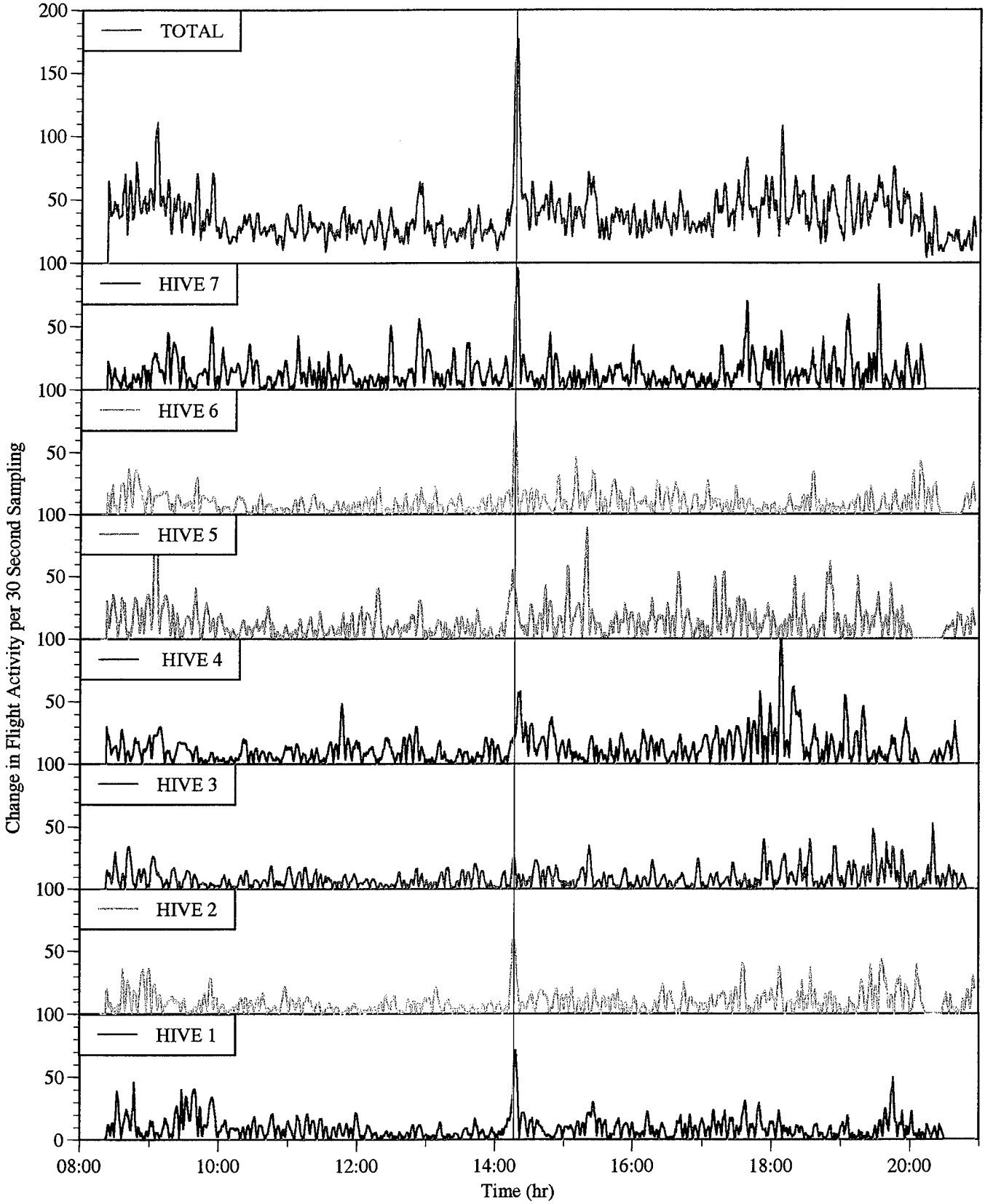

Figure 2.8 Derivative of Departing Bees on August 8, 1996 at West Branch Canal Creek During 'Smoke Event'. Hives 1, 5 and 6 were exposed to smoke puffs at 14:30. The solid line indicates a synchronous response for all colonies.

rates of up to 200 Hz. If sampling is done too slowly, bees pass through undetected which probably happened in the initial field study. On the other hand, overly fast sampling produced an enormous amount of redundant data that pressed the memory and data storage capacity of the notebook computer. Field trials conducted in Montana during Spring 1996 established that 150 Hz is the minimum sampling rate from which reliable bee passage data can be obtained.

Following additional testing and finalization of hardware and software design in Spring 1996, two hives were monitored through early July in Montana and 21 hives were deployed in late July in Maryland for monitoring through mid-November. Flight activity during late spring and early summer in Montana was similar to that of mid-summer through autumn in Maryland. Total daily flight activity per hive of about 70,000 trips per day in Montana. In Maryland, flight activity tended to be somewhat lower, with the most populous colonies only occasionally achieving the maximum observed in Montana. The high Montana counts appeared to be due to the intense foraging that occurred during the period of maximum population growth (i.e., May-June) combined with abundant resources of both nectar and pollen. Also, the number of hours that bees could forage was longer in Montana than in Maryland due to the increased day length (i.e., period of daylight).

In Maryland, the major nectar flows occurred before the colonies were deployed at the test sites. By August, the Maryland bees primarily foraged for pollen (as evidenced by pollen trapping), brought back water for evaporative cooling in the afternoon, and collected small amounts of nectar. As evidenced by pulses of incoming bees that exceeded the numbers of outgoing bees on several occasions, Hive 7 at Old O Field recruited bees from nearby colonies and as such was characterized by very high levels of flight activity. Several queenless colonies at Old O Field declined in population size, while the colony in Hive 7 increased in size. This robust colony probably benefitted from the tendency of returning bees to drift toward the outermost colony in a long row of hives.

For both years and in each state, the rigorous data analysis algorithm consistently gave the most accurate results. The less stringent algorithms were useful for aligning detectors and improving counter sensitivity, but these analysis programs were susceptible to errors induced by electronic noise or by bees backing out of entrance passageways. Only the more rigorous method could reliably distinguish between bees that fully entered or exited the mini-hives and bees that stopped and reversed direction in the counting tubes. Consequently, this method was utilized for the field applications in Maryland during 1996.

As the counter technology improved throughout the spring and early summer of 1996, the count accuracy and resolution also improved. Before being shipped to Maryland in the summer of 1996, the final improvements to the counter technology were completed and the sampling performance remained consistent throughout the 1996 Maryland field study.

2.9.1 Results of the 1996 Tests of Equipment and Methods at APG

In mid-September of 1996 a fault in the data analysis code that caused data from counting tubes 1 and 2 (out of 14 total) on even-numbered hives to be deleted prior to analysis was discovered and fixed. This problem was resolved by renaming the arrays passed to subprograms used to analyze flight data and by only deleting arrays after all data had been analyzed. Another bug was discovered at the same time that caused Hives 5 and 7 to repeat data for some counting channels. Despite these software problems, in each case sufficient numbers of count tubes recorded accurate count numbers to provide representative estimates of total flight activity, although not every bee was counted.

The real-time monitoring display made it easy to compare counter output graphs to actual bee flight at the front of each hive. By standing next to the computer and watching both the monitor and the bees at the front of the hives, the visually observed and computer recorded levels of flight activity could be easily assessed with respect to known colony population size and current weather conditions (e.g., sunny, windy, rainy, or advancing storm fronts). In all cases, the relative levels of flight activity seen at the front of the seven hives at each site were consistent with the recorded levels seen on the computer screen. Colony size was periodically addressed by opening the hive and inspecting the colony to determine overall colony condition and strength. Total colony biomass which provided an indirect measure of population size was continuously measured by strain gauges (discussed in Section 3). These examinations confirmed the relative colony strength and status (such as presence or absence of a queen) as indicated by the flight counters.

The software faults only became evident in Maryland, when the number of hives connected to the central interface was increased from three to seven, apparently inducing memory allocation problems that reflected inherent limitations of QuickBasic. Lowering the sampling rate to ease the demands on memory was considered, but not pursued in order to maintain data consistency throughout the year. Eliminating these memory problems and producing the next generation of software for networked bee counters has been a major component of the ongoing development of this technology.

Porting the QuickBASIC code to Visual BASIC was recently attempted. However, Visual BASIC allocated so many user interface resources that it was not capable of maintaining a consistent sampling rate. PowerBASIC offers good array management, access to upper memory, and faster compiled code. The entire OuickBASIC data analysis and manipulation program was ported to PowerBASIC in less than 4 hours. Additional code has been written in PowerBASIC to expand the capabilities of the data analysis and manipulation program. Currently, work is being done in collaboration with IOTech technicians to create a software driver for the Digibook/72 that is compatible with PowerBASIC. When this is done, the memory allocation problem should be resolved and both IOTech and the bee counters will benefit. IOTech will be able to offer PowerBASIC compatibility with their product, and the bee counters will have a faster and more stable software platform on which to run.

2.9.2 Results of the 1996 Tier 1 and 2 Evaluations of Flight Activity at APG

The flight activity from the Summer and Fall of 1996 was summarized using the Tier-based approach. Fig. 2.2 presents a Tier 1 summary comparing corrected (i.e., adjusted for the number of sampling periods per day) total daily flight activity throughout the season for each of the three primary test sites. West Branch Canal Creek was the first site to be put on-line, followed by Old O Field, and the Churchville reference site. Overall, each of the sites displayed similar day to day seasonal trends. At all sites, activity leveled-off from the third week of August through mid-September and then gradually dropped off as the foraging season ended.

As can be seen in Fig. 2.2, most of the major weather events affected all three sites. For example, greatly reduced flight activity at all three sites on September 6 and 11 coincided with heavy rain showers throughout the region. More localized weather events produced site specific differences in total flight activity. West Branch Canal Creek and Old O Field were in close proximity, both being located at APG—Edgewood on a peninsula extending into Chesapeake Bay. By comparison, the Churchville reference site was inland from the Bay and far from the APG sites. Weather conditions at the APG sites should more closely resemble each other than those at the more distant Churchville site. Preliminary analysis of the weather data suggests that this was a correct assumption.

Tier 1 graphical summaries indicate that through early September total flight activity at West Branch Canal Creek and Old O Field was similar. As the season progressed, the colonies at West Branch Canal Creek usually displayed the highest flight activity, while those at Old O Field tended to have the lowest flight activity and often more closely resembled the colonies at Churchville than those at Old O Field. This suggested a response that was not simply a consequence of differing weather conditions.

The differences in flight activities at these three sites were further investigated by plotting the coefficient of variation (C.V.) of flight activity among the seven colonies at each site (Fig. 2.7a-c). As seen in Fig. 2.7, the colonies at West Branch Canal Creek established a baseline level below 40% for variation in flight activity, which stayed below 50% as the season progressed. There were several brief periods where the C.V. exceeded this baseline, but most periods corresponded with inclement weather. At Churchville, the variation in flight activity hovered around 65% for most of the season. Colonies at Old O Field established a C.V. baseline at approximately 55% until September 6th, but were unable to make a consistent return to that level thereafter. On September 6, 1996, hurricane Fran swept through the east coast. Whether the change observed at Old O Field was a direct response to the storm or in response to some other factor is still under investigation.

The coefficient of variations for flight activity data were indicative of the site-wide colony condition. If all colonies at one site were thriving and showing similar activity patterns, the C.V. values remained at a relatively low, constant level (<40%, as was the case for West Branch Canal Creek). The same also could be true if all of the colonies at a site were in poor condition and behaving similarly. Therefore, the C.V. data must be used in conjunction with total flight

activity data and other measures of hive condition such as biomass (Section 3) in order to determine the source of the low variability. If the C.V. begins to deviate from a baseline level characteristic of the colonies at a site (<40% for West Branch Canal Creek, 55% for Old O Field and 65% for Churchville), then one or more of the colonies must be exhibiting a different flight behavior. This deviation could be due to a colony with a much increased number of flights compared to the other colonies as well as to a colony with a depressed activity level.

Tier 1 plots of total flight activity and the associated C.V. revealed site specific trends, but these plots did not reveal the strength or weaknesses of individual colonies. Tier 2 analysis (Fig. 2.3-2.5) provided the means of distinguishing weak colonies from strong colonies. Tier 1 analysis revealed when one or more colonies at a site were displaying atypical flight characteristics. Tier 2 analysis identified the specific colonies that were the source of this variation. Having identified these colonies, each colony could then be inspected to determine if it was queenright, whether it had brood and adequate food stores, or whether it showed any symptoms of disease or mite infestation. If the change in foraging could not be assigned to a difference in colony structure, a chemical sampling program could be initiated to look for the presence or an increase in exposure to chemical contaminants.

Tier 2 analysis provided additional insights regarding colony condition and equipment performance. From August 14 through August 22, one half of the counter channels on Hive 7 at Old O Field were out due to a defective power circuit. The fault can be easily seen in Fig. 2.4 by the abrupt change in colors (indicating flight numbers) on August 23 on the contour plot for Hive 7, following repair of the power supply.

The repaired counter clearly demonstrated that throughout the season the colony in Hive 7 made more foraging trips than any of the other colonies at Old O Field. Observations of dense populations of bees inside the hive with large masses (beards) of bees hanging outside the hive at night confirmed that this hive contained the strongest colony at O Field. Fig. 2.4 not only identified the strongest colony at Old O Field, but it also showed that colonies 2, 5, and especially 6 had the lowest flight activity. Upon inspection, these three colonies were found to be queenless, and two were broodless.

Tier 1 analysis showed that flight activity was most consistent among the colonies at West Branch Canal Creek. As at Old O Field, Tier 2 plots identified which of the hives differed from the others. Hive 4 displayed the least flight activity. When opened it was found to contain the smallest population of bees, although it had a queen and brood. Similarly, the plots show that at the Churchville reference site (Fig. 2.5), flight activity throughout the season was lowest in Hives 4 and 7. Hive 4 became queenless and was replaced mid-test with a colony that had a small bee population. Hive 7 contained only drone brood (indicating queen failure and possible replacement by a laying worker).

2.9.3 Results of the 1996 Tier 3 Evaluations of Flight Activity at APG

Whereas Tier 1 and 2 plots proved to be most useful for detecting site specific and long-term changes in colony activities, Tier 3 analysis had the advantage of plotting flight activity data for a single day for each colony. This provided information about the effects of sudden events such as storms, food resource availability, opening the hive, or sudden release of environmental contaminants. The strength of Tier 3 was to identify rapid changes in a colony's flight behavior and to compare the number of bees entering the hive to the number of bees exiting the hive during a given sampling period, resulting in a measure of net loss of forager bees.

Fig. 2.6 displays the total flight activity typical of a sunny day in August at West Branch Canal Creek. As mentioned earlier, boxcar averaging and other filtering techniques provided data smoothing to reduce the high frequency sampling noise. For each plot, the bottom line represents Hive 1, ascending to Hive 7, with the uppermost red line the mean of the total flight numbers for all seven hives. As can be seen from the plot, the overall flight activity for the day for each hive appears as a more or less continuous, broad continuum.

By comparison, Fig. 2.9 displays a large peak in the number of bees returning to the hive before a thunderstorm. Few bees left the hive until after the rain. Bees avoid heavy rain, which can knock them out of the air while flying. Many beekeepers believe that bees avoid rainstorms in response to changes in barometric pressure, but our bee-counter graphs in combination with digital weather data show that bees probably use multiple meteorological clues, not simply barometric pressure drops (see Section 3) to determine when to return to the hive to avoid a storm. In this case, foraging activity was clearly affected by an external factor that first influenced the behavior of foragers in the field, causing them to return to the hive, and then influenced the bees in the colony by inhibiting flight.

Fig. 2.10 shows the smoothed flight activity traces from a day on which six small puffs of cool smoke (from a bee smoker using burlap as fuel) were directed over a three-minute period into the entrances of three of the seven hives at West Branch Canal Creek. The smoke event can best be seen as a drop in activity for the bees leaving the hive. Fig. 2.8 shows derivative plots of the flight activity data for bees entering each hive at the site. The peaks corresponding to the smoke event are discernable in the total activity and the bee-exit data, but they are absent from the incoming bee data. Because the data smoothing and derivative procedures enhance the signal, one can not only see the response evidenced by the three smoked colonies (Hives 1, 5, and 6) but also by three other nearby colonies. We suspect that these colonies were responding to smoke drift due to gusty winds. Only one colony (Hive 3) did not demonstrate the response.

As evident in these plots, following injection of smoke into the hive, bees continued to return at the same rate, but the rate of outgoing bees decreased within a few minutes. Activity levels remained low for 20 minutes or more, possibly for several hours as indicated by the response of Hive 1. Beekeepers use smoke to calm bees while performing maintenance on hives. Apparently, the bees are fooled into believing the hive is threatened by fire. Instead of rushing out to fight an intruder, some of the bees gather up as much nectar as possible and prepare to

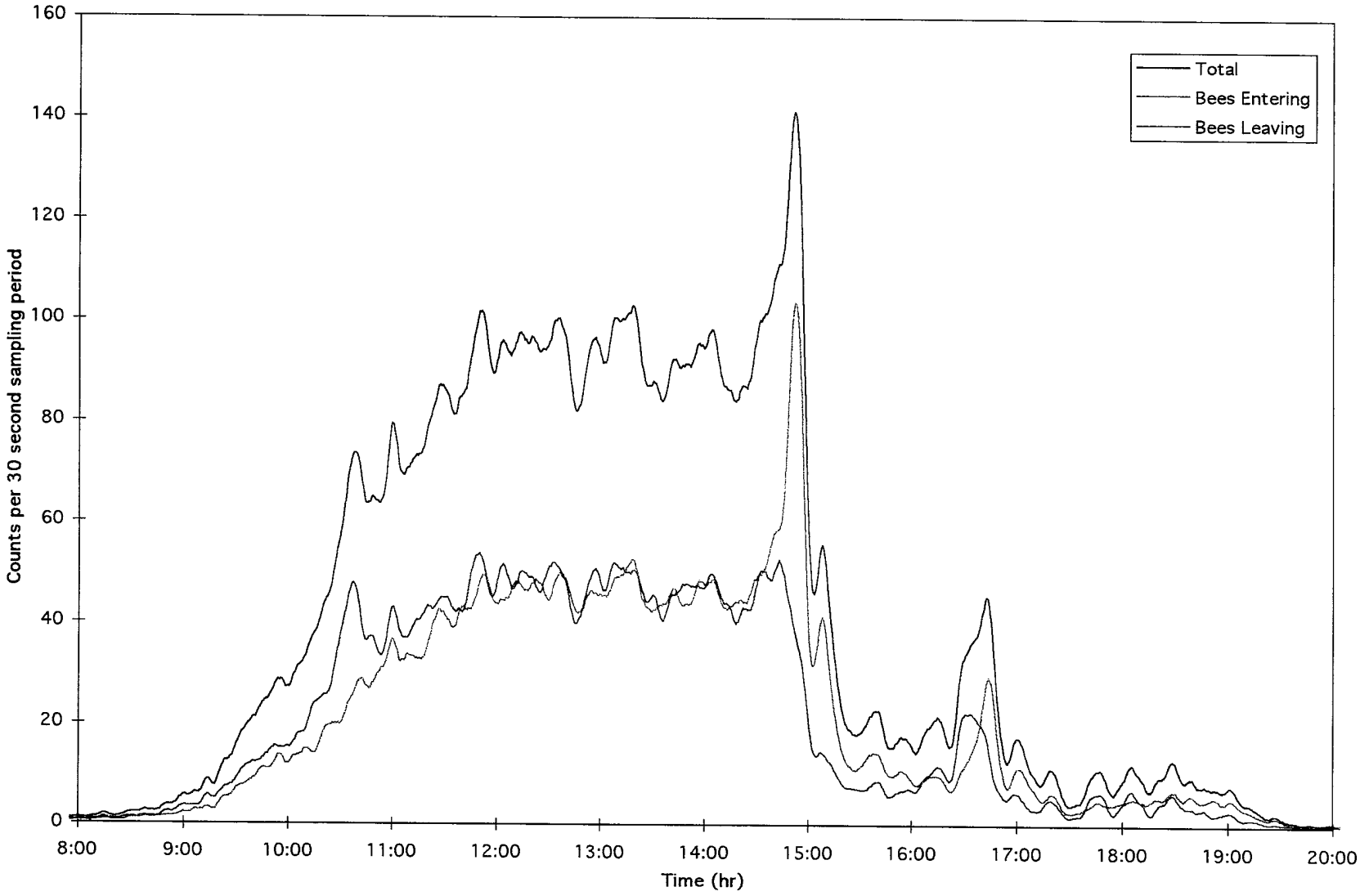

Figure 2.9 Expanded Flight Activity Data for West Branch Canal Creek Colonies on August 27, 1996 During Rainstorm. Sharp drop in activity at 15:00 represents beginning of rain.

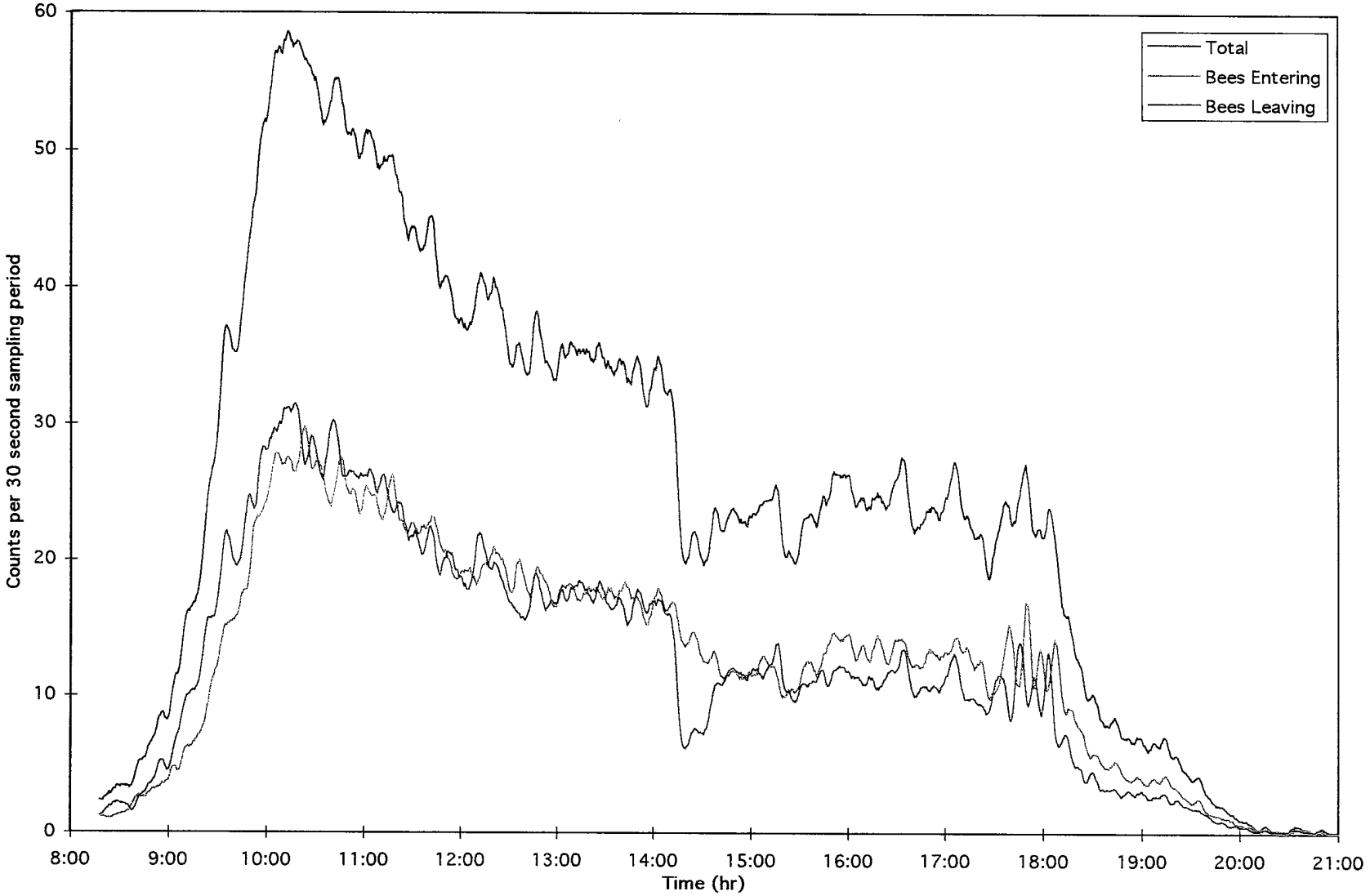

Figure 2.10 Expanded Flight Activity Data for West Branch Canal Creek Colonies on August 8, 1996 During 'Smoke Event'. Sharp drop in bees leaving the hive at 14:30 is due to smoke.

leave the hive. Others may fan to remove smoke from the hive. Since the smoke was injected directly into the hive, it should have been sensed primarily by the bees within the hive rather than bees outside the hive.

In the fall and early spring, a colony's foraging habits vary depending on the presence of brood in the colony and the availability of floral resources. At these times, daily flight activity was often inconsistent, characterized by peaks and valleys, rather than the broad continuum typically seen on days of high foraging activity. In order to be able to distinguish responses to external events of particular interest for environmental monitoring, such as exposure to contaminants, from normal variations in flight activity, the subsequent data analysis will have to consider relevant ancillary data for weather, food resource availability, and other factors that alter colony dynamics. To address these needs, the honey bee condos have been equipped with electronic systems for continuously measuring colony core temperature, time and rate of pollen gathering, and hive weight (see Section 3).

2.10 General Discussion

By continuously measuring colony responses (e.g., total flight activity, net loss of forager bees, colony biomass, thermoregulation in the broodnest) to a variety of internal and external stressors, an extensive database is being compiled. The goal is to associate colony responses to intrinsic and extrinsic factors as they occur or very soon thereafter. By combining the knowledge gained from this database with continuous monitoring of weather and food resource availability (e.g., clock driven pollen traps) monitoring, it should be possible to determine when chemical sampling is warranted to examine possible contaminant exposure. Based on the information obtained over the past two years, it is apparent that flight activity can respond quickly to a variety of stressors.

With sufficient data, various external and internal events can be characterized by unique behavioral responses. For example, an external event such as an approaching storm often induced a large positive change in the rate of bees entering the hive with a negative change in the rate of bees exiting the hive. One would expect bees to return to the safety of the hive and, once inside, stay until the weather improved. An internal event such as smoke in the hive was characterized by a large negative change in the rate of bees exiting the hive with little or no change in the rate of bees entering the hive. Again, one would expect bees in the field to be unaffected, because they were not exposed to the smoke. Similarly, swarming produced a rapid increase in the number of bees exiting the hive with some a increase in rate of bee returns after the main swarm had departed.

The bee counters have some inherent limitations with respect to identifying the presence of bioavailable toxins. First, bees normally only fly during daylight hours. This raises a concern about detecting toxins that are emitted at night, during storms, or when the bees are clustered inside the hive in the winter, especially in northern climates. Although chemical releases may occur at night from industrial sources, dumps, and various media, some potential sources of

harmful chemicals normally occur only during the daytime. For example, crop-dusters don't fly, nor do most clean up crews work at night.

In addition, bees can monitor air borne chemicals even when confined to the hive box. Bees switch from their daytime routine of sampling multiple media and wide areas to a point sampling system for air quality at night. They accomplish this by drawing air through the hive while fanning with their wings (discussed in Section 5). Thus, while the bees are enclosed in the hive they exhibit a sampling behavior that is similar to that of the carbon sorption tube traps used in more conventional sampling of air quality. During the day, they change to an area sampling mode that is less point specific than traditional methods. Taking into consideration the seasonal nature of foraging, the spatial averaging and sampling potential of bees is limited to the active foraging season, but the point specific sampling potential continues year-round. The advantage that the bees offer over traditional chemical sampling methods is their ability to indicate when to begin sampling for the presence of bioavailable, hazardous substances. This helps reduce the amount of chemical analyses being performed, thus reducing costs.

Our counters provided an accurate means of indicating both acute toxicity and colony condition. Traditional methods of determining acute toxicity (dead bee traps) have been limited to counting bees that died after returning to the hive and do not adequately evaluate the number of bees dying in the field. The bee counters provided a measure of bees that failed to return from the field (i.e., net loss for each hive for each day). Occasionally, bees are unable to return to the hive due to abrupt changes in weather at the end of a day, and these bees usually returned to the hive the next morning. Colony condition has traditionally been monitored by periodic inspections and estimates of population size that rely upon the observer's experience with colonies. The bee counters provide an unbiased metric (i.e., C.V. among hives at a site) that can not only evaluate the vigor of the colony (due to trends in total flight activity), but also indicate changes in colony function. Problems in the colony, ranging from exposures to toxins to the loss of the queen or lack of brood, will be reflected by changes in foraging levels.

Both raw and smoothed flight data, as illustrated by the Figures, clearly shows the influence of weather and of at least one complex chemical mixture smoke. Struye *et al.*(2) demonstrated that flight activity changed when bees were exposed to toxic chemicals (pesticides) and that these changes are different than those induced by weather. By monitoring multiple hives simultaneously at three sites, this study demonstrated a correlation in flight activity among the hives at these locations that can be attributed to meteorological conditions. Although some colony specific differences occur at each site, the overall patterns are remarkably similar.

Ultimately, the ability to accurately interpret the movement of bees into and out of a hive in conjunction with similar data for weather conditions and food resource availability depends on the use of artificial neural networks, advanced statistics or other advanced software tools (see Section 3). The goal of the portion of the study described in this section is to provide the tools needed to assess the normal foraging patterns of bees as influenced by natural factors such as weather and abiotic stressors such as exposure to pollutants.

This work is beginning to produce an extensive data base that will be invaluable in determining the sensitivity of bee flight to environmental stressors. Currently, two colony performance parameters have proven to be useful: net loss of forager bees and the coefficient of variation for flight activity at a given site. Net loss can quickly identify even small decreases in the number of bees returning to the hive. Because exposure to a toxic chemical is a likely cause of this response, a sudden or increasing rate of loss may warrant additional investigation and sampling to determine the underlying cause of the change. First steps would include examining weather conditions and the reproductive status of the colony (e.g., as indicated by the presence or absence of brood). If these actions fail to identify the likely source of the loss, then chemical sampling can be initiated. In combination with the use of the more traditional dead bee traps, samples can be taken for body burden analysis. Also, exposure to a toxic substance should increase the numbers of dead and dying bees removed by housekeeping bees from the colony and dropped into the traps.

The coefficient of variation addresses differences among colonies and appears to be a more useful metric for assessing overall colony condition. This statistic also provides a link between the Tier 1 (comparison of locations) and Tier 2 (comparison of hives at a location) graphical summaries. The latter helps identify the specific hives that are the source of the observed variability. Thus, higher C.V.s at Old O Field were in part due to the disappearance of the queen from three of the seven colonies. The sudden increase in C.V. values at this site following hurricane Fran, and a failure to return to the levels seen before the upheaval suggest that something changed at this site that had a long-term impact on the colonies. Whether this was due to a change in forage availability, exposure to toxins in surface waters, or some other factor is unknown. However, this is the type of response that could be used as part of a feedback system to provide a real-time alert of an alteration in the environment. Consequently, the change may signify a reason for initiating chemical sampling.

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SECTION 3

DESIGN AND FIELD APPLICATION OF ADDITIONAL METHODS FOR DETERMINING BEE COLONY DYNAMICS

3.1 Introduction to Effects Monitoring

The flight activity monitors that integrate flight activity data with factors such as weather and chemical exposure conditions to indicate overall colony health are an important biomonitoring tool. However, flight activity is only one of several measurement and assessment endpoints that can be used to assess colony structure and performance. Toxicity is the classical assessment endpoint and is typically monitored using traps to collect dead bees removed from the hive by housekeeping bees. We use this approach to monitor for acute or gradual increases in bee mortality. However, except for extremely acute toxic events such as exposure to insecticide spray drift, this parameter detects but does not predict bee death nor warn of episodic exposures to less toxic substances or of increasing levels of potentially hazardous contaminants.

Toxicity examines effects at the level of the individual bee. The thousands of bees living together as a social entity show consolidated group responses in food procurement and storage, defense, kin recognition, daily rhythms of metabolism and breathing, and temperature regulation (1). Colony levels of stability and control exceed those of individuals or the sum of behaviors of individuals comprising the group.

Colony biomass reflects the size of the bee population as well as production of wax and honey. Strong colonies produce more honey than weak colonies because the stronger colony has more adult bees available for honey production. As a colony increases in size, the number of bees needed for housekeeping chores does not increase proportionately. Thus, a colony with 60,000 bees can make twice as much honey as two colonies of 30,000 bees (2). Because honey and wax are marketable products, reduced productivity has a definable economic impact. For these reasons, we monitor total colony biomass. However, as with toxicity, this parameter serves mainly to document that a change has already occurred or to document a trend such as declining population size or food stores. More importantly, total biomass provides an estimate of colony size that can be used as a co-variable for interpreting differences between colonies in foraging activity and for providing a useful weighting factor. For example, a small colony and a large colony may both be healthy, but the total daily flight activity will be considerably different. By taking into account colony size, the flight activity data can be adjusted for colony size.

Also, because the availability of food stores can affect colony biomass, a decline in biomass would provide an alert to inspect the colony for total food stores. If needed, a colony

can be provided with supplemental food to avoid starvation. Thus, continuous monitoring of colony biomass eliminates the need to routinely open the hive for inspection and provides a warning of a condition, that if left uncorrected, could be confused with a contaminant exposure. In addition, we found in a previous study that colony responses to a food shortage differ significantly from those of exposure to a toxin (3).

One of the most studied and stable colony-level controls is that of core temperature regulation. The individual honey bee is poikilothermic. Its body tends to take up the temperature of its surroundings and it has no homeostatic system of temperature control other than heat in its muscles generated by activity. By comparison, through social homeostatic adjustments the colony maintains high (approximately 34-35 °C), relatively constant, core temperatures, especially if brood is present. Colonies have been shown capable of maintaining normal hive temperatures under environmental conditions where outside air temperatures hit extremes as high as 70 °C (4) and as low as -80 °C (5). Hive temperature control also has a stabilizing effect on humidity, which under normal conditions stays at about 40-50 per cent. Overheated colonies using water to evaporatively cool the hive and colonies removing excess moisture from nectar may have high humidity levels exceeding 70 per cent (6). Abnormally low humidity levels are thought to cause the death of eggs and larvae by desiccation.

Colony thermoregulation and humidity control depend on the concerted effort of the members of the social group. A variety of complex social behaviors including fanning, shivering to generate heat, and clustering (forming a ball and generating heat) are necessary to achieve the constancy of thermoregulation evidenced by healthy bee colonies. Maintenance of such a high degree of cooperation depends on the integrity of the social structure. Thus, core temperature and humidity offer readily monitored endpoints for which the signal to noise ratio is high. In other words, small deviations from the norm can be distinguished.

Thermoregulation is important to successful production of brood. In addition, larvae must be fed a diet rich in proteins, vitamins, lipids, and nectar, all of which are derived from pollen. A shortage of pollen can result in failure of the colony to develop optimum populations. Pollen traps attached to the beehive remove and collect pollen pellets from the pollen baskets of incoming forager bees. Clock-driven traps provide measures of both the amounts and times when pollen is gathered. The trapped pollen can be used both to document exposures to contaminants from plant sources, to determine what plants have been foraged, and to assess the adequacy of protein resources for normal brood development.

3.2 Experimental Methods and Materials

An important objective is to correlate colony activity with food resource, chemical, and meteorological data to determine which factors are associated with observed departures from expected activity patterns.

3.2.1 Meteorological Conditions

At each site, we set up a digital meteorological station (Weather Max™, Maximum®, New Bedford, Massachusetts) that recorded time and date, wind speed direction and velocity, temperature, wind chill, humidity, rainfall, and dewpoint (Table 3.1). The weather station had it own data logger and readout. However, to obtain continuous data rather than data averaged for the day, we connected each unit to a IBM-compatible PC. Initially, we used The Fourth Dimension history logging weather software (Version 3.4) supplied with the weather station. This software saved the data for hourly periods. During the 1996 field season, we replaced this software with Weather View for Windows, Version 2.3 (ControlWare, Amity, Oregon). Weather View can save data at one minute intervals.

To measure sunlight, we used a variable resistance cadmium sulphide photoelectric light sensor (CD-113, Alpha Products, Fairfield, Connecticut). As ambient light intensity increases, the photocell resistance decreases and provides a varying voltage to the A-Bus A/D converter which then returns the value of relative light intensity to the computer.

3.2.2 Pollen Collection to Assess the Protein Food Resource

To collect pollen, we used a removable 19 x 3 cm, 5mm plastic mesh (E.H. Thorne (Beehives) Ltd., Wragby, England) mounted horizontally below the bottom of each nucleus hive. The scraper mesh dislodged the pollen pellets from the legs of forager bees. The pollen fell into a glass-sided hopper that funneled it through a seven mesh plastic-coated screen onto a pie plate mounted on top of a 24 hr clock-drive mechanism (Intermatic # 2E021 Interval Timer, Granger®, Spokane, Washington).

3.2.3 Dead Bee Collection

The glass-sided hopper also served to collect dead or dying bees removed from the hive by the housekeeping bees. Normally, dead bees are dragged out of the hive by these bees, but the entrance tunnels of the flight activity counters were too narrow to permit easy passage of a bee pulling another bee. Some loss of adult bees occurs in healthy colonies due to normal attrition as bees age and wear themselves out from foraging.

3.2.4 Electronic Hive Sensors

Colony core temperature, relative humidity, and weight were measured using various sensing devices attached to an A-Bus™ analog to digital interface system (Alpha Products, Fairfield, Connecticut). This system is connected to an IBM-compatible Personal Computer (PC) running in-house developed software to scale and record the data. The software© was written in C language. This software will continuously poll each sensor attached to the system, convert

TABLE 3.1
Range, Resolution, and Accuracy of WeatherMax Functions

WeatherMax Specifications		
Function	**Measurement Range**	**Resolution**
Wind Speed	0-255 MPH	1 MPH
Wind Direction	16 Compass Points	22.5°
Temperature—Outdoor	-40 - 122°F	-1 or 1°F
Temperature—Indoor	50-122°F	-1 or 1°F
Barometric Pressure	27.5 - 31.5 in Hg	.01 in Hg
Relative Humidity—Inside	10-90%	1% RH
Relative Humidity—Outside	20-90%	1% RH
Rainfall	0-99.99" of Rain	.01"
Windchill	-119 to 122°F	1°F
Dew Point—Outside	-7 to 115°F	1°F
Pressure Rate of Change	-4 to +4 in Hg/HR	.01 in Hg
Time	24 hr/00:00 - 23.59	1 minute
Accuracy		
Wind Speed—Total System	± 2.1 mph	
Wind Direction—Indicator	0 error Display System	
Temperature—Indoor	± 1.5	
Temperature—Outdoor	± 1.5	
Barometric Pressure	± .08" Hg	
Relative Humidity—Inside	± 8% RH	
Relative Humidity—Outside	± 8% 20-80% RH	
Rainfall Indicator	± 0 error Display System	
Time	± 1 minute/month	
Temperature Sensor	± 1° F	
Wind Direction Sensor	± 11.25°	
Rain Collector	± 0.1"/inch	

millivolt input to the appropriate output units, and display the result. At 5 minute intervals the data for each sensor is averaged and saved into a text file on the hard disk drive, which can be imported into a spreadsheet program for analysis. At midnight the day's data is also copied to the floppy drive, and a new text file is created for the next day. In this manner the data can be examined without taking the data acquisition system off-line.

The sensors used for measuring core temperature are thermal transistors (Part # TS-111, Alpha Products, Fairfield, Connecticut). These sensors are accurate within $\pm 1°$ F (approximately 0.6 ° at room temperature). Calibration of these sensors entailed placing the sensor in a constant temperature water bath and recording the output millivolt value for temperatures from 0° to 50° C using 9 to 11 calibration points. Water temperatures were referenced to two ASTM traceable thermometers (ASTM 636 and ASTM 650) (Total IMM SGA CO, Blefd, New Jersey). Simple linear regression provides the slope and intercept values used to convert the input voltage to temperature. Each electronic bee hive contained one temperature probe placed in the center of the hive between the upper and lower story.

Relative humidity sensors were placed next to the temperature probes. We used EMD-2000 Micro RH sensors (Phys-Chem Scientific Corp. New York, New York) with a claimed accuracy of ±5% RH. These sensors required a conditioning circuit built from circuit diagrams provided by Phys-Chem. The construction and tuning of these circuits is time-intensive and problematic. The system used during the 1995 trail run tended to be overly sensitive and peaked out at 70 to 80% humidity. We added a tunable offset and gain to the existing circuit amplifier before the 1996 field season. RH probes were calibrated according to the procedure outlined in (6).

For 1995 pilot study, we used a hydraulic pressure transducer system to measure changes in colony biomass. Like the RH sensors, this system proved to be overly sensitive to environmental factors including temperature and the increase in barometric pressure due to Maryland being at a much lower elevation than Montana, where the system was originally developed. We replaced this system for the 1996 field season with strain gauges. Three of the electronic hives at each site were equipped with a proto-type weighing system consisting of 8 foil strain gauges (SG350-LY13 from Omega Engineering, Inc., Stamford, Connecticut) attached to 4 aluminum beams, which supported the two-story hive. As these were first generation prototypes, it was difficult to determine the size, shape and thickness of the beams needed to encompass the range of weight changes likely to occur over a field season in Maryland. We chose a conservative approach using beams that were "stiff", limiting the resolution of measured weight changes to 1 pound. We were concerned that more flexible beams might bottom out as the hive gained weight. Both the pressure transducer and strain gauge systems were calibrated using a standardized metal weight.

Prototypes of three hot-wire anemometers were installed in three electronic hives at the Canal Creek site during the summer of 1966. These devices were designed to measure air flow rates within the hive. Each anemometers consisted of two thermistors (Digi-Key, South Thief River Falls, Minnesota) connected to a Wheatstone-bridge resistor system. Air flow was determined as flow rate over time relative to each sensor.

3.3 Colony Dynamics, Results and Discussion

The flight activity counters described in the previous section are a critical component of our colony assessment system. However, honey colonies present an array of measurable hazardous assessment endpoints, some or all of which may be appropriate biomonitors for the particular problem to be solved. The 1995 and 1996 field trials provided the data sets necessary for deciding which of these endpoints could most effectively be employed.

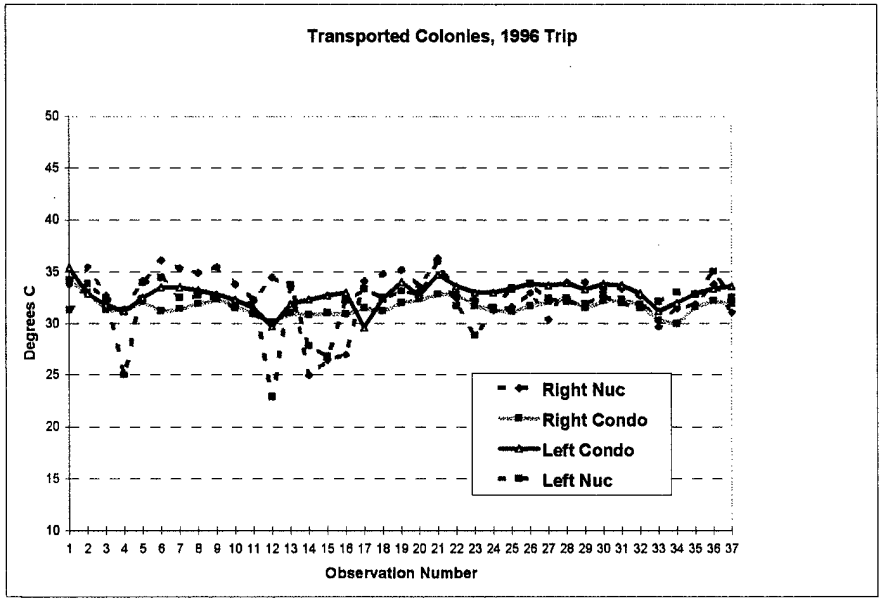
3.3.1 Transportation Stress

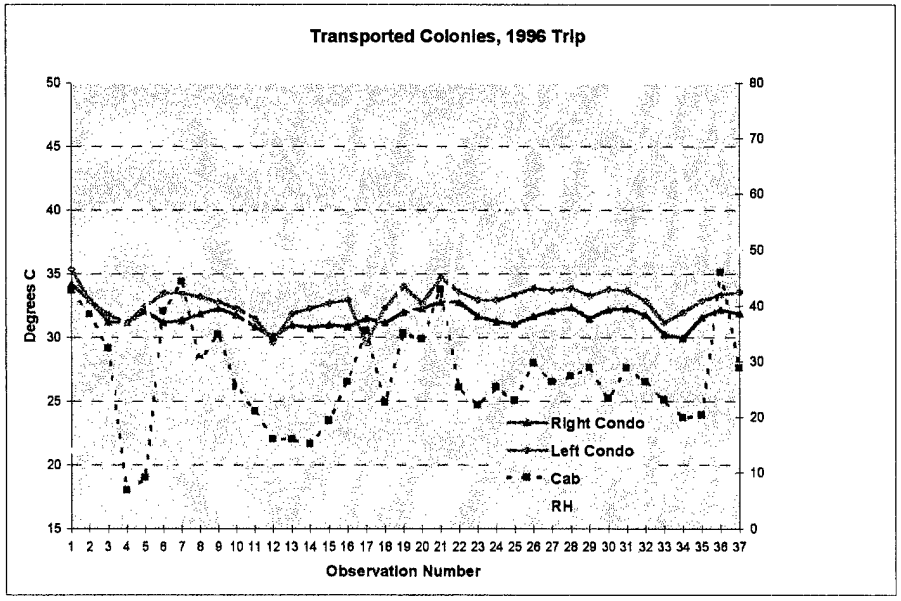
Heat stress is a major problem when transporting bees over long distances. Healthy colonies demonstrate a remarkable ability to thermoregulate. Not only can they generate and conserve heat, but they evaporatively cool their hives on hot days. Confined bees do not have access to water and as such cannot cool hives by evapotranspiration.

One of our challenges was to be able to deliver bees and fragile equipment over long-distances for biomonitoring where needed by the Army. Carrying bees from Montana to Maryland in August of 1995 provided a good test of our transportation techniques. The summer of 1995 was marked by record high temperatures across the mid-west. Based on the results of this first trip, we developed a strategy for safe delivery of many more colonies of bees in 1996.

During both of the trips, we monitored colony core temperatures using digital thermometers inserted into the brood nest and readouts mounted inside the cab of the truck. Fig. 3.1 displays colony core temperatures in nucleus hives transported from Montana to Maryland in August of 1995 and July of 1996. The observation periods occurred at approximately one hour intervals during the period in which the bees were being moved by truck (i.e., early morning through late evening). Juxtaposed against these values are those for the temperature and relative humidity of the ambient air (i.e., cab temperatures). In 1995 we also measured temperatures inside the condo boxes (shells). Shell temperatures (Fig. 3.1) approximated ambient air temperatures, while core temperatures in the nucleus colonies were much less variable. However, whenever the truck slowed or stopped, core temperatures increased rapidly in less than ten minutes. Wetting sponges and manually misting bees through the open screens on top of the nucleus hives brought core temperatures down by 2-3 degrees C.

For 1996, we mounted a gas-powered electric generator and a drip irrigation system on the trailer used to carry the hives. Generator-powered small fans in the top of each condo drew off excess heat. An electric water pump was used to simultaneously drip water onto sponges above the colonies whenever core temperatures exceeded 36°C. The trailer load consisted of 21 nucleus hives inside condos arranged in two front to back rows and 17 extra nucleus hives parallel to the condos along the outside of the trailer. As evidenced by Fig. 3.1, the





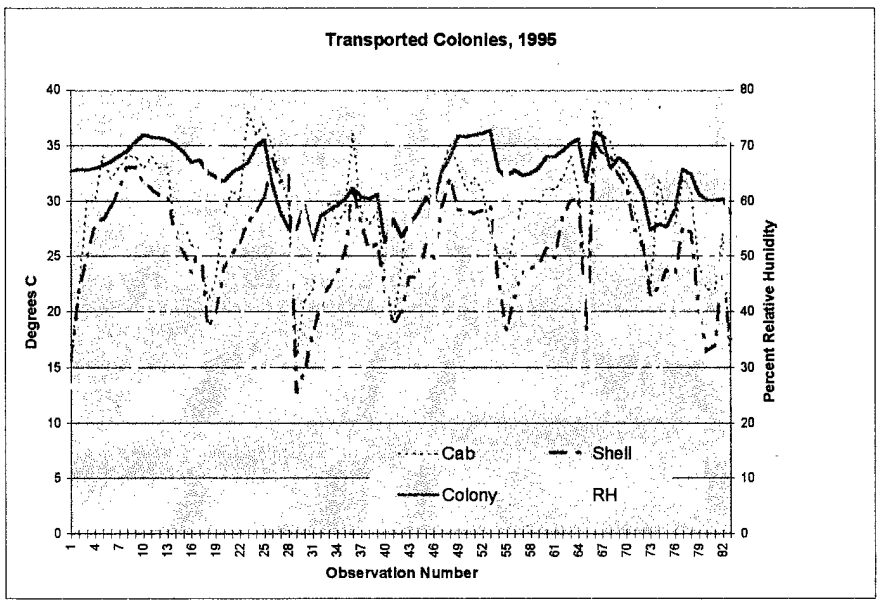


Figure 3.1 Colony temperatures during transport to Maryland from Montana. Truck cab temperatures for both years approximated ambient air temperature. For 1995, shell temperature was a measure of the temperature inside a box enclosing a single nucleus test colony (on the right side of the truck facing the sun), and core temperature refers to conditions inside the nucleus colony in the monitored condo shell.

In 1996, only colony core temperatures were measured. Two of the monitored colonies were enclosed in condo shells (left and right condo), and two of the nucleus colonies (left and right nuc) did not have condo shells. Left and right indicates position on the trailer used to carry the equipment. Relative Humidity, as indicated on the Y axis on the right side of two of the plots, was the level of moisture in the ambient air, not the colony.

generator-powered fans and watering system kept core temperatures from exceeding the normal range (i.e., greater than 36 °C). Also, colonies transported inside the condo shells maintained more stable core temperatures (Fig. 3.1, uppermost charts) than nucleus colonies without shells. The colonies in the condos remained warmer at night and cooler on hot days than the other colonies on the trailer. As can be seen from Fig. 3.1, supplying water and ventilation as needed allowed the confined bees to thermoregulate despite large changes in ambient air temperature and relative humidity.

Heavy rainstorms encountered in Ohio in 1996 contributed to the loss of bees from five of the nucleus colonies, probably due to water spray from the wheels of the vehicle that got under hive covers. The inside of these hives and the bees were drenched. Hives inside the condos were protected from the rain and spray. Initially, we were concerned that the condo shells would retain excessive heat. Our experiences in hauling bees over long distances indicated that the condos buffered external conditions, retaining heat at night, shielding the nucleus colonies from solar radiation, and protecting them from rain.

For both years, with the exception of the drowned bees, transportation mortality was minimal, ranging from a few bees to less than a cup of bees per hive. This is well within the level of normal attrition from the death of old bees. Considering the distances traveled (over 4,000 km), the length of confinement, and the high ambient air temperatures and relative humidity (as high as 38 °C, 80% RH), adult bee mortality was remarkably low.

3.3.2 Adult Bee Toxicity

Bees dying during the trip and after being placed on site were caught by dead bee traps located inside the condos. Following deployment, dead bees were collected at all three of the condo sites on three different dates during the 1996 field season. Monthly totals for August, September, and October were compared using Repeated Measures Analysis of Variance. Data for each hive, classified by site and date of collection were analyzed using the GLM repeated measures package in SPSS® for Windows (version 7.0). The data approximated a normal distribution and the variances were sufficiently equal to abrogate the need for transformation prior to analysis. Results of the analysis indicated that there were no overall differences among sites (Table 3.2). However, changes in mortality over time differed significantly among sites as indicated by the date-by-site interaction. Fig. 3.2 shows that there was a progressive increase in bee mortality at Churchville between August and October. During the same period, Old O Field and West Branch Canal Creek hives showed highly concordant patterns of a slight rise in mortality from August to September, followed by a decline from September to October. The cause for the significant interaction, shown by post hoc tests (Table 3.3) was the continued increase in mortality from September to October at Churchville when mortality was decreasing on the other sites.

TABLE 3.2

Repeated Measures Analysis of Variance for Adult Bee Mortality at West Branch Canal Creek, Old O Field, and Churchville.

Tests of Within-Subjects Effects

Sphericity Assumed based on non-significance of Mauchly's Test of Sphericity ($P \geq 0.242$)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Observed Power[a]
Date	1.282	2	0.641	0.990	0.000	
Date*Site	1.336	4	0.334	0.973	0.001	
Error (Date)	2.978	36	5.56-02			

Tests of Between-Subjects Effects

Intercept	10.480	1	10.480	96.275	0.000	1.000
NSITE	0.276	2	0.138	1.270	0.305	0.240
Error	1.959	18	0.109			

a. Computed using alpha=0.05

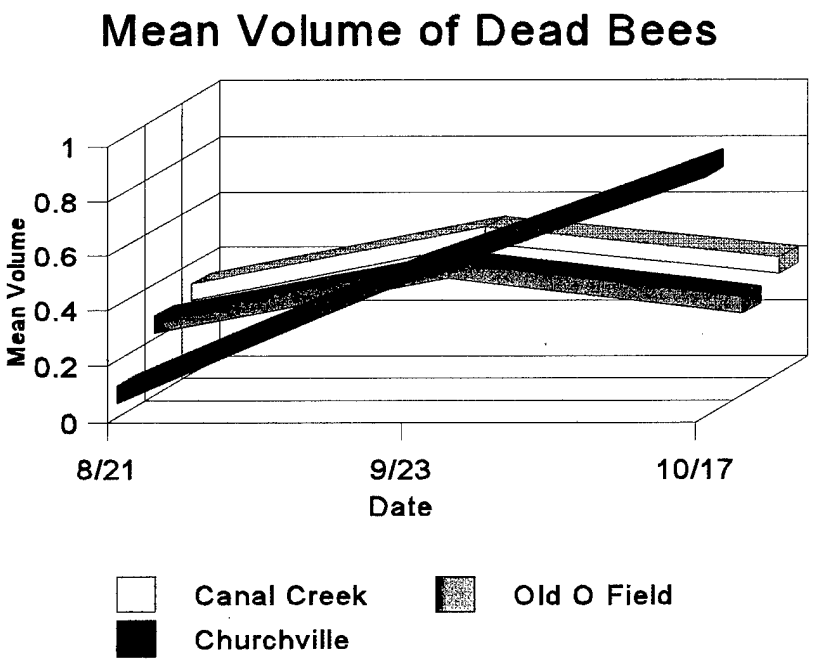


Figure 3.2 Seasonal Variation in the Mean Amount of Dead Bees Collected by Hive-Mounted Traps.

Table 3.3

Post Hoc Tests of Adult Bee Mortality at West Branch Canal Creek, Old O Field, and Churchville

Scheffe's Multiple Means Comparisons							
						95% Confidence	
Dependent Variable	(I) Site	(J) Site	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Lower Bound
Dead 8/21	Canal Creek	Old O Field	1.357E-02	0.099	0.991	-0.2498	.2769
		Churchville	0.1843	0.099	0.204	-8.E-02	0.4476
	Old O Field	Canal Creek	-1.3E-02	0.099	0.991	-0.2769	0.2498
		Churchville	0.1707	0.099	0.251	-9.E-02	0.4340
	Churchville	Canal Creek	-0.1843	0.099	0.204	-0.4476	7.9E-02
		Old O Field	-0.1707	0.099	0.251	-0.4340	9.3E-02
Dead 9/23	Canal Creek	Old O Field	4.143E-02	0.152	0.963	-0.332	-0.4460
		Churchville	-7.14E-03	0.152	0.999	-0.4118	0.3975
	Old O Field	Canal Creek	-4.14E-02	0.152	0.963	-0.4460	0.3632
		Churchville	-4.86E-02	0.152	0.950	-0.4532	0.3560
	Churchville	Canal Creek	7.14E-03	0.152	0.999	-0.3975	0.4118
		Old O Field	4.857E-02	0.152	0.950	-0.3560	0.4532
Dead 10/17	Canal Creek	Old O Field	8.429E-02	0.172	0.888	-0.3754	0.5439
		Churchville	-0.5114*	0.172	0.028	-.9711	-5.E-02
	Old O Field	Canal Creek	-8.43E-02	0.172	0.888	-0.5439	0.3754
		Churchville	-0.5957	0.172	0.010	-1.0554	-0.1361
	Churchville	Canal Creek	0.5114*	0.172	0.028	5.2E-02	0.9711
		Old O Field	0.5957	0.172	0.010	0.1361	1.0554

*** The mean difference is significant at the 0.05 level**

Strong colonies should exhibit higher natural losses of bees than weak colonies. The aforementioned results were not adjusted for colony size. Eventually, hive biomass, flight activity, and other indicators of colony strength will be used as co-variables. At the time of this report, we were still processing this supporting data.

We did not observe acute or severe adult bee mortality at any of the sites. Bee losses at West Branch Canal Creek appeared to be within the range expected from natural attrition. Late season bee loss at Old O Field may have been somewhat offset by re-queening three of the seven condo colonies in September. The progressively increasing loss at Churchville was in part due to the drone-laying colony. Drone-laying colonies do not replace worker bees. Thus, the worker population becomes progressively older and dies. Hive 3 (a queen right colony) demonstrated the highest bee loss. This may have been due to inadvertent trapping of bees inside the condo. Rather than exit the hive via the counters, the bees in this colony often avoided the counter by slipping through small gaps into the condo shell. Once inside the shell, they seldom found the way out and died. Although this problem was occasionally observed in other colonies and at other sites, it was pronounced in this unit. For 1997, the door mounted counter will be replaced by a slide-out unit that will be permanently sealed to the hive base. In addition, the condos will be fitted with conical bee escapes.

Based on the contents of pollen traps, observations of available food sources, and uptake of syrup from feeders, we believe that the late season attrition at Churchville was in part due to a declining food resource. Although the meadow and forest habitat at Churchville approximated that of APG, this site had no estuary floral resource—an important late season food resource at APG. However, some of the Churchville colonies displayed occasional high levels of individual contaminants, including some not seen at APG (discussed in Section 4).

The effect of food resource availability on the real-time monitoring capability of a honey bee colony system can be offset by supplemental feeding. Also, as mentioned, based on an earlier study (3), it appears that colony responses to food shortages differ from those of contaminant exposure. Because food shortages could be an indirect effect of a stressor acting on a supporting component (e.g., the floral resources of an ecosystem), the goal is to eventually be able to reliably differentiate colony responses to food shortages from those of contaminant exposure, rather than to suppress the effects of changes in the vegetation resource by supplemental feeding. The hypothesis being tested is that advanced statistical and computer intelligence programs such as artificial neural networks will eventually be able to distinguish the difference in response to a biological versus a chemical stressor.

Although useful for identifying acute or long-term chronic bee mortality, the classical assessment endpoint (i.e., bees caught in a hive-mounted trap) was less sensitive and took longer to be expressed than some of the other colony parameters that we monitored. Hive mounted traps only document mortality within the hive. Workers dying in the field, which is often the case with acute exposures to toxic chemicals, can not return to the hive. On the other hand, all outgoing and incoming bees are recorded by the flight activity counters. These

counters could identify small reductions (e.g, 3-6%) in numbers of bees returning to each colony on any day at any site. In addition, flight activity provides a rate metric that can be related to functional relationships such as pollination efficacy.

Unless severe, colonies survive bee losses. Bee death changes the life stage structure of the population and may affect colony performance. On the other hand, any breakdown in the integrated social behavior required to maintain thermoregulation threatens the integrity and success of the population. Therefore, the ability to thermoregulate is a direct measure of colony functioning and condition.

Other indicators of colony performance also indicated that the colonies varied within and between these sites. Flight activity (see also Section 4), queen disappearances (mentioned in Section 2, 4, and 5, and discussed below), colony core temperatures (described below), and the levels of contaminants, especially industrial contaminants and some inorganics (see Section 4), differed among these sites.

3.3.3 Queen Disappearance

No queen disappearance was observed at West Branch of Canal Creek during the pilot study of 1995 and the field trials of 1996. During 1996, the queen from hive 7 at Churchville absconded with part of the bee population within 24 hrs after transportation from Montana. We returned her to hive 7, but by late summer the colony contained only drone brood. Later in the summer, the queen from hive number 4 also absconded with most of the colony's population. In neither case did the colonies attempt to produce new queens as evidenced by a lack of queen cells. At Old O Field, by early September, the queens were gone from three of the seven condos and from three of the five nucleus colonies that had been placed on site in June for chemical monitoring. None of these colonies appeared to have swarmed or absconded as indicated by bee population size and by the presence of empty emergency queen cells rather than queen supersedure cells in the hives. In addition, our flight counters did not detect issuance of a swarm from any of the condo hives. Also, late season swarms are unusual. Finally, a marked queen from one of the nucleus colonies was found walking around on the ground in front of the hive, which is an unusual behavior. Whether the queen abandoned the hive or was expelled is unknown.

The 50% queen disappearance, both from the condo and other nucleus hives suggests a stress response at Old O Field. Transportation stress seemed to be responsible for the loss of at least one of the two queens at Churchville, given that the queen absconded within a day of being brought from Montana. Transportation stress would not account for the loss of queens from the nucleus colonies at Old O Field that were set up in June for chemical monitoring. These colonies were moved less than 25 km in June and had been doing well through mid-August. Again, transportation stress did not seem to be a likely explanation for the queen disappearances from the Old O Field condos. These hives were the first to be deployed, followed by the hives at Churchville which were stockpiled for a couple of weeks at an

alternate site near Jarretsville, Maryland, and finally, the hives at Canal Creek. Because the Churchville colonies were moved from Montana to Jarretsville and then again to Churchville, they received more handling than the other colonies.

By itself, the 50% disappearance of queens at Old O Field, the 28.6% loss at Churchville, and the 0% loss at Canal Creek (for both years) indicate site specific differences but little information about the cause of the loss. Occasional queen disappearance is not uncommon, but 50% loss of all of the queens is notable. Our chemical analysis results provided a possible explanation. The highest seasonal and site levels of several industrial chemicals were observed in the queenless colonies at Old O Field (see Section 4 and 5). We intend to further examine this issue by conducting dose-response tests next summer to determine whether these organic chemicals preferentially affect the queen. The queen is exposed for a longer period than any of the workers in the colony. Whereas worker bees live only a few weeks or months, a queen may live for two to eight years. For our experiments, we only use queens grafted and mated during the preceding spring, so all of the lost queens were relatively young.

Because an objective of this project is to apply artificial neural networks and advanced statistics to quickly identify colony differences or responses to environmental factors, we needed to collect real-time data for healthy, queenright (i.e., a queen is present and laying eggs) colonies and for colonies with known problems. On the other hand, we had decided before initiation of the experiment to keep the number of replicate hives at a site to a minimum of five units. Therefore, we elected to re-queen or re-place some of the queenless colonies and left others so that we could continue to collect colony response data. We replaced all of the queenless colonies used for additional chemical monitoring at Old O Field with colonies in good condition. We replaced the queens in condo hives 5 and 6, but left hive 2 queenless. We replaced the absconded colony from hive 4 at Churchville with a small, queen-right colony, but did not replace the drone-laying colony (hive 7).

3.3.4 Pollen Collection

The contents of the pollen traps were used for chemical analysis to monitor a primary route of entry into the colonies of environmental contaminants (see metal analysis, Section 4). The pollen samples also provided an indication of the time of day of collection and amounts of pollen (protein) available to the colonies. This information is being evaluated as one of many inputs used for advanced statistical, artificial neural network analysis, and eventual modeling of colony behaviors.

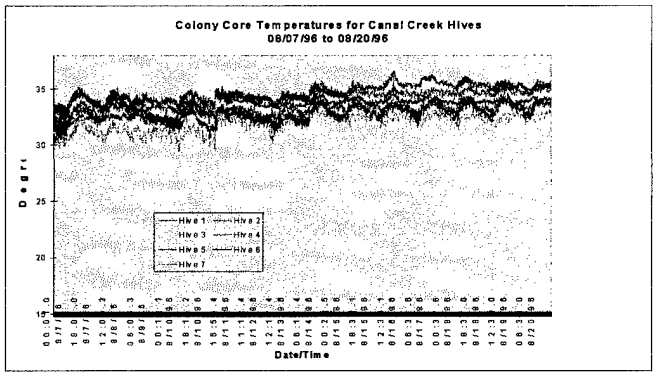
3.3.5 Flight, Temperature, and Relative Humidity

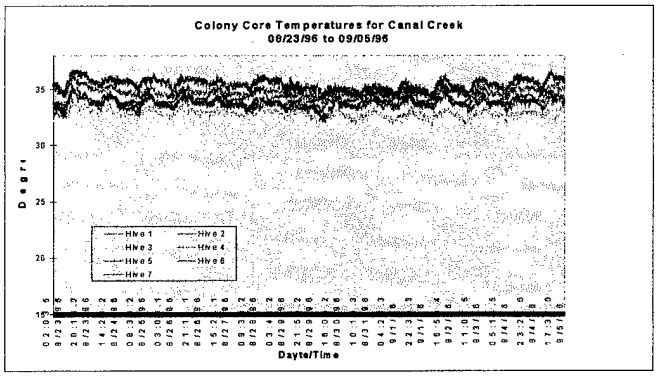
As discussed in Section 2 and shown in Fig. 2.2-2.5, hive flight activity patterns clearly showed depressed flight for the queenless colonies at Old O Field, as well as for the small replacement colony for hive 4 and the drone-laying colony of hive 7 at Churchville. Hives 4 and 7 at West Branch Canal Creek exhibited lower flight numbers than their companion colonies, but both proved to be queen-right.

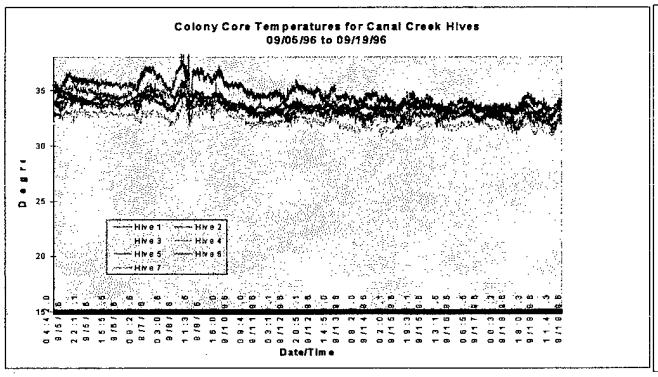
Hive core temperatures provided another indication of colony condition. When brood was present, core temperatures in the brood nest were maintained within ± 1 °C of nearly constant temperatures of 33-34 °C (Fig. 3.3). This agreed with published studies indicating that during the annual period of brood rearing, the nursery region of each colony's nest is stabilized between 33 ° and 36 °C, averaging about 34.5 °C (8). When colonies are without brood, from late autumn to mid-winter, the temperature within broodless clusters of bees drops somewhat, but remains above freezing, never falling below 18 °C (8). Temperatures near the outside of the cluster stay above 10 °C. Our data verified these reports (Fig. 3.3 and 3.4). Example plots are provided for Canal Creek colonies for most of the 1996 field season. The plots from Churchville are shown to demonstrate that all of the sites exhibited similar seasonal trends in thermoregulation. Core temperatures were kept within narrow limits as long as brood was present. With the approach of fall and reduction of brood rearing, core temperatures became more variable.

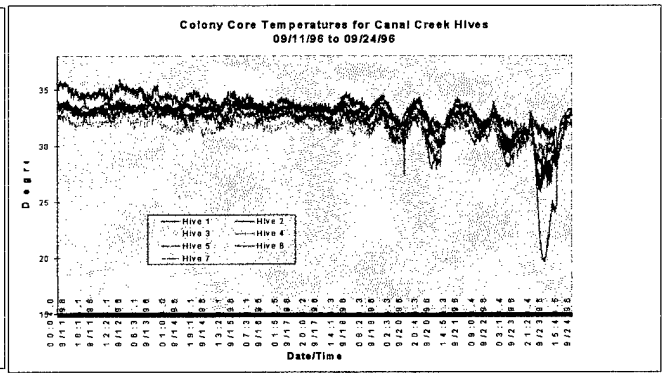
The real-time monitor displays of colony core temperature clearly showed the absence of a queen or a lack of uncapped brood. Generally, colonies containing capped brood, but no uncapped brood, averaged about 2-3 °C lower than colonies with all stages of brood. Colonies without brood usually followed ambient temperatures, thermoregulating only when ambient temperatures dropped low enough to threaten the health of adult bees (i.e., below 10-18 °C). This temperature constancy is best illustrated by Fig. 3.4. Because the pupae are contained in capped cells, do not feed, and do not require any additional care by the adult bees, this life stage is known to be less susceptible to variations in temperature and other external factors. Conversely, the eggs and larvae are more susceptible to temperature fluctuations that may affect either the developing brood or the nurse bees that care and feed the larvae.

The top six charts of Fig. 3.4 show core temperatures within individual hives (left column) and the mean core temperature $\pm$ 1 SD for all seven colonies at each of the three condo sites. These plots display the data from a late season (October 29) sample day when some of the colonies had already become broodless. By comparison, similar data for two months earlier at Canal Creek (August 27) exhibit the impressive thermoregulatory ability of the set of integrated behaviors and physiological devices employed by colonies to produce heat through metabolism, curtail unwanted loss of heat to the environment, and prevent overheating.









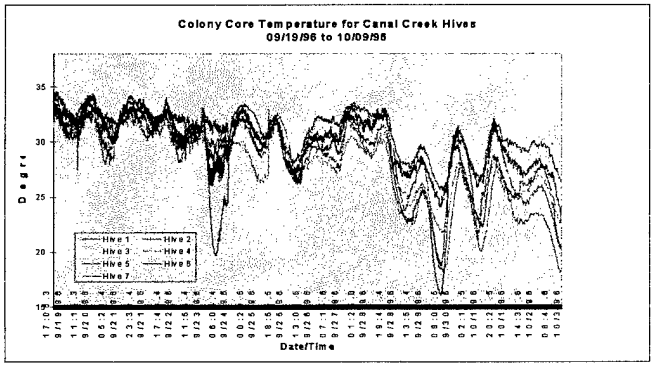
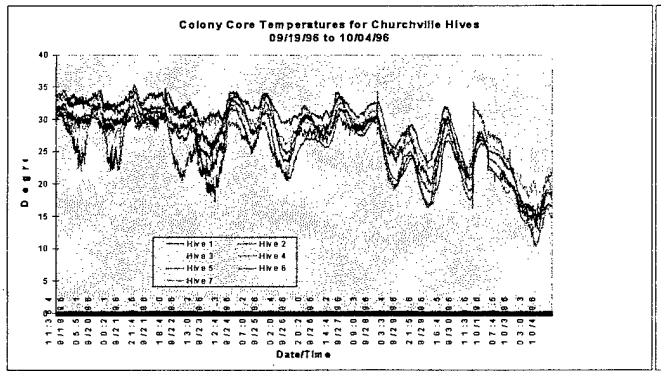
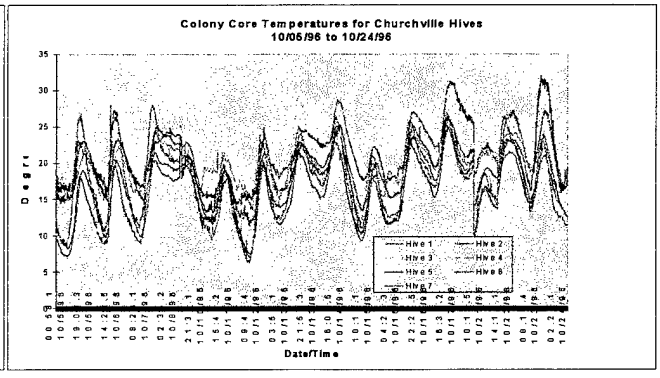


Figure 3.3 Colony Core Temperature for Canal Creek hives throughout the season (upper charts) and for Churchville hives near the end of the sampling period as thermoregulation decreases (bottom two charts). Temperature was monitored using thermal transistors placed in the hive.





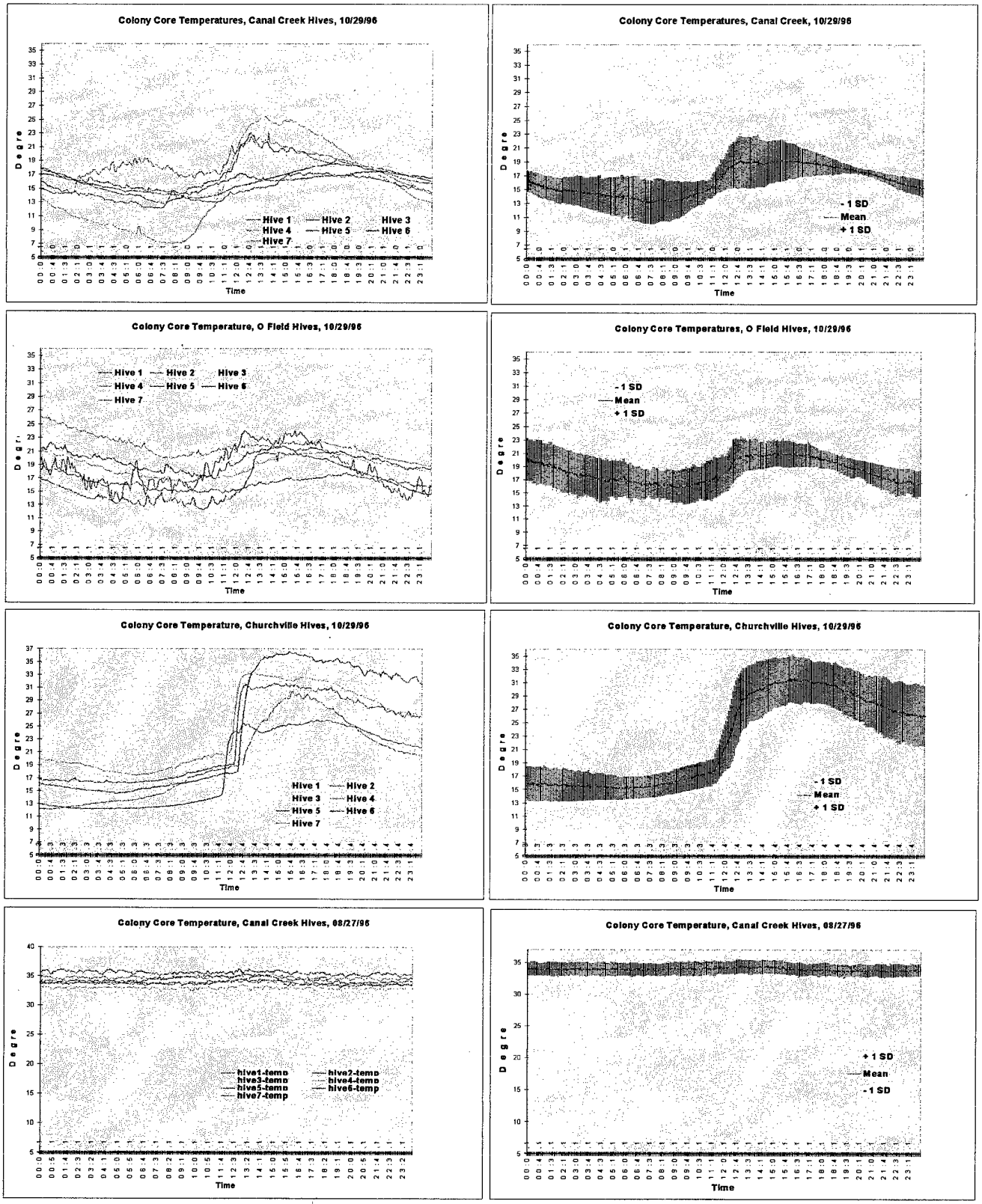


Figure 3.4 Mean colony core temperatures ± 1 standard deviation. First three rows depict temperatures in colonies during the fall when most colonies have suspended brood rearing. Bottom row illustrates temperature constancy in brood nest. Note that maximum Y axis value varies by 2 degrees.

This thermoregulation behavior is further exemplified by Fig. 3.5. By September 9, the colonies in hives 2, 5, and 6 were queenless. Colonies 1, 3, 4, and 7 contained brood and each had a laying queen. These colonies also were tightly thermoregulating. The queenless colonies were progressively declining with respect to temperature control. Colonies 5 and 6 were re-queened late in the afternoon of September 15. Additional bees and capped brood were added to colony 6, which contained a small bee population. Colony 5 was larger and received only a queen. Colony 2 was left queenless. The effect of requeening was immediate. Core temperatures in hives 5 and 6 increased within a few hours, while those for hive 2 declined and became even more variable. For a few days after requeening, temperatures in colonies 5 and 6 continued to be more variable than those of colonies 1, 3, 4, and 7, probably because of an absence of much uncapped brood in the requeened colonies.

Core relative humidity vacillated between 55% and 85%, usually remaining at about 60% to 70% as illustrated by an example data set from West Branch Canal Creek (Fig. 3.6). By comparison, relative humidity of the ambient air varied from about 30 to 90%. These results are consistent with reports of conditions inside the hive ranging from 40 to 70% or more (6).

Overall, core relative humidity did not appear to be useful for assessing colony condition. As shown by Fig. 3.6, although less variable, humidity inside the hive tracked daily levels in the ambient air. In addition, the RH probes proved to be relatively costly and somewhat difficult to maintain calibration. For example, hive 5 was deleted from the Fig. 3.6 data set because of highly variable responses. Also, we suspect that the flatter response exhibited by hive 2 reflects a lack of probe sensitivity rather than a real colony response. Because extremely low relative humidity may affect brood viability (6), this parameter may be an important indicator of colony condition in dry climates.

Core temperature plus flight activity provided good indications of colony condition. By using these two parameters, we could reliably predict overall colony, queen and brood rearing status, even from Montana. In all cases where both indicators were depressed, the colonies were found to be broodless, queenless, or both. Deviations from the norm for these measurement endpoints were easy to identify during the period when brood should be present. These could be readily differentiated both by visual examination of the data or by simple statistically based methods such as those typically used for quality control charts.

How best to utilize these assessments of colony condition during the broodless period remains to be determined. Although flight activity can be expected to decline or even cease during cold periods, colonies have to maintain a core cluster temperature of about 18 °C or higher in order to survive. When the air surrounding the bees reaches 14 °C, bee clusters become well defined. Thus, temperature should continue to be a useful monitor of the proper functioning of the integrated behaviors necessary for heat retention and clustering. Based on our findings to date, we suspect somewhat lessened sensitivity during broodless periods extending from late autumn through mid-winter.

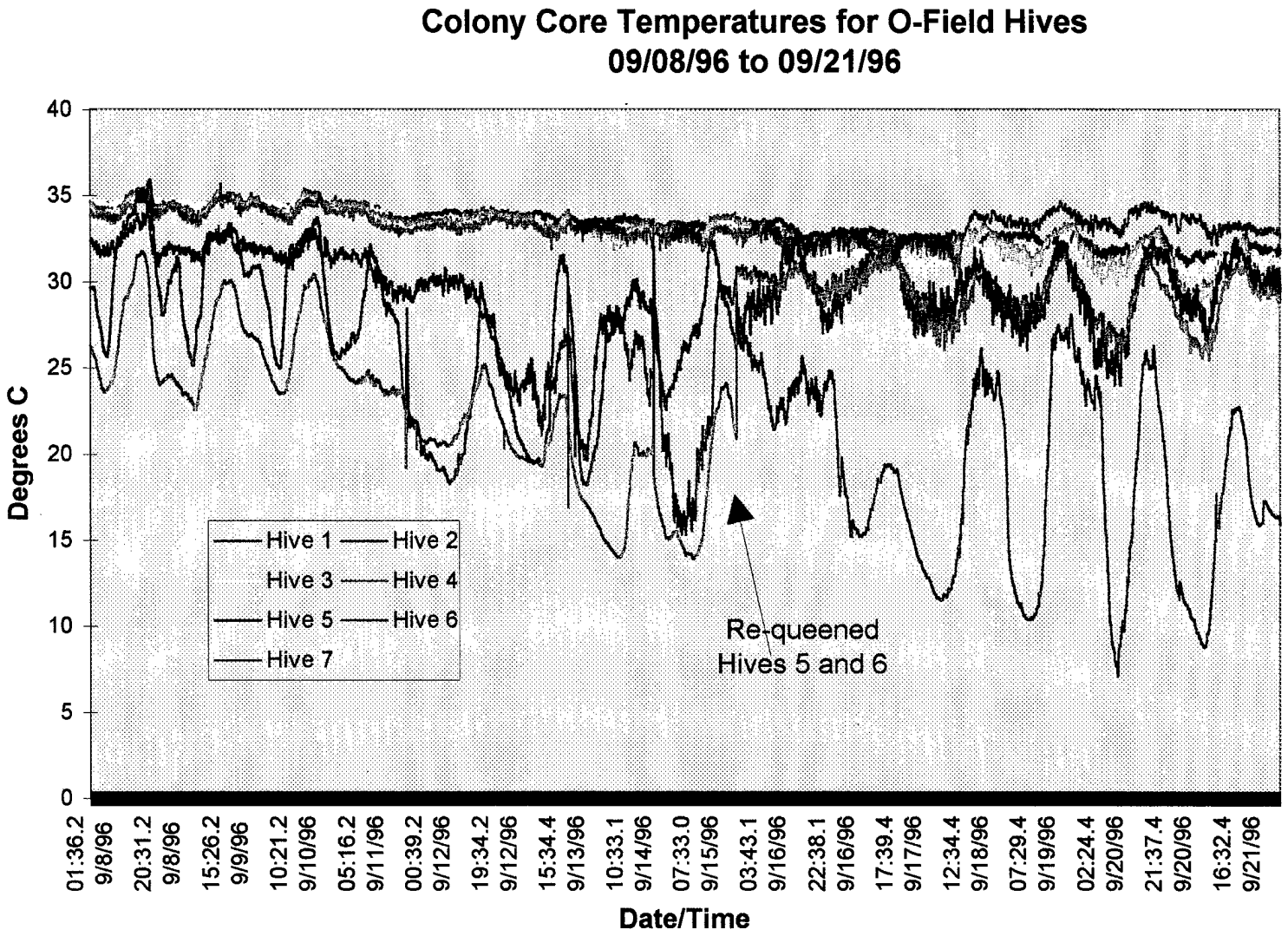
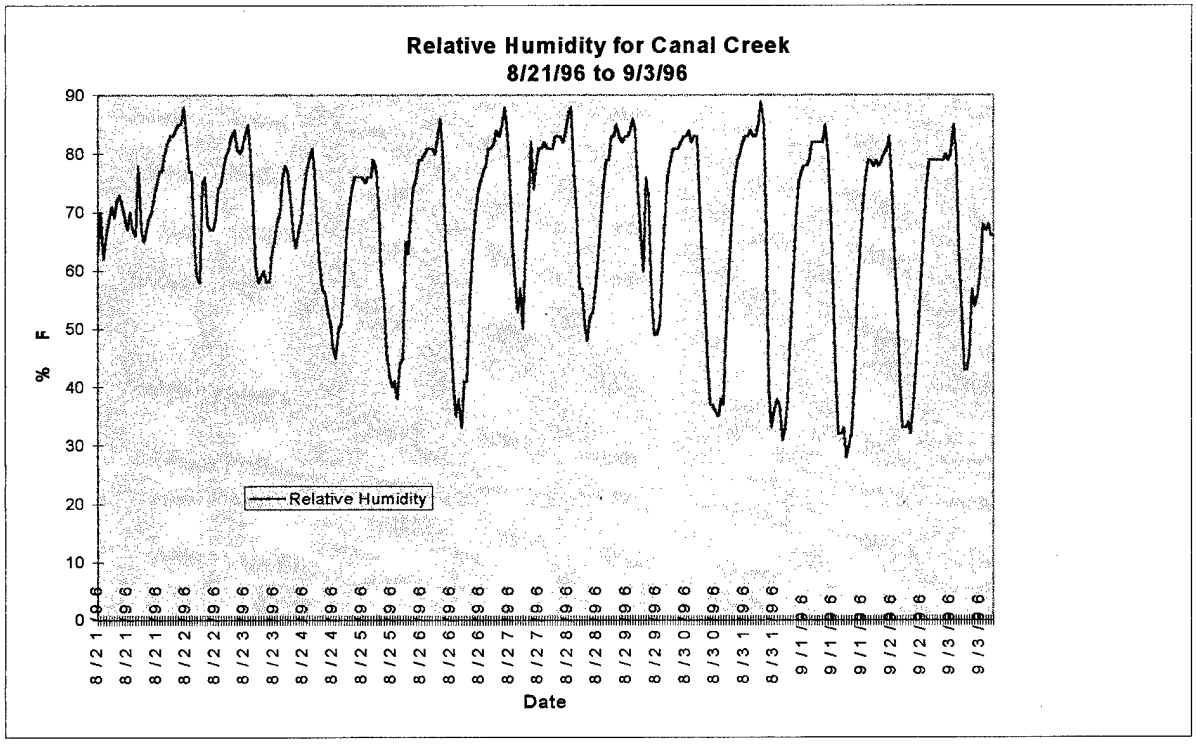


Figure 3.5 Colonies 1, 3, 4, and 7 had laying queens; colonies 2, 5, and 6 were queenless. Colonies 5 and 6 were re-queened on 9/15/96. Colony 2 was left queenless. This chart presents 28,000 data points.



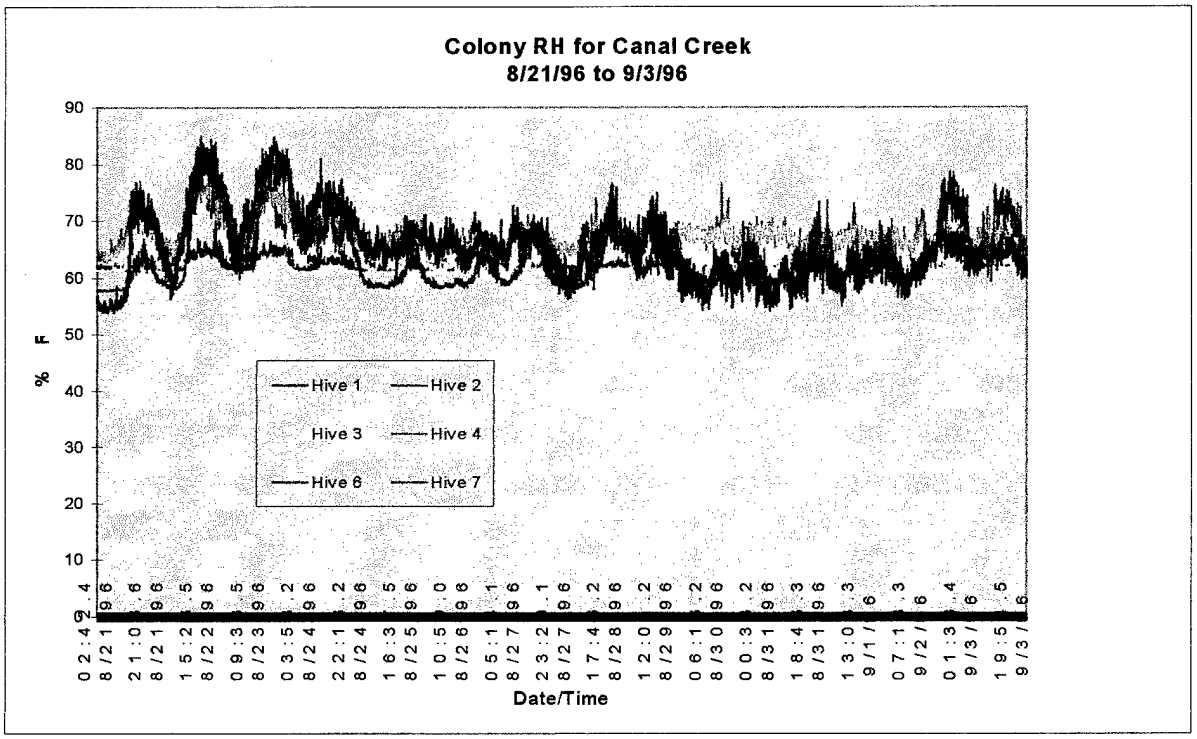


Figure 3.6 Ambient and Colony Core Humidity for the Canal Creek hives. Hive RH tends to track ambient levels with less extremes. Colony 5 humidity readings were removed due to highly variable reponses.

3.3.6 Induced Flight Activity and Bee Losses in the Field

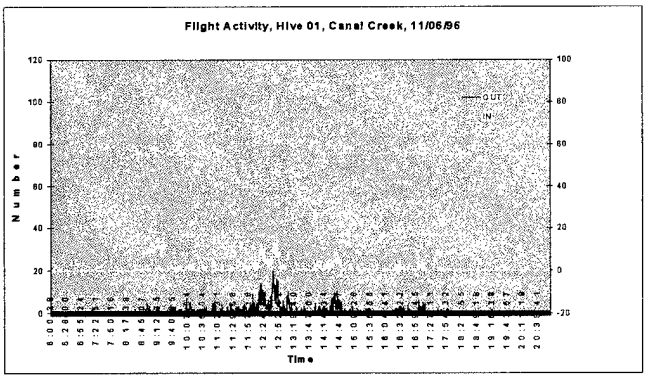
Honey bees must maintain a body temperature above 27 °C in order to fly. During winter, when floral resources are absent, bees continue to fly at quite low temperatures to gather water to dilute or rehydrate crystallized honey. The ability of colonies to fly can be tested on warm days by providing an external feeder. Fig. 3.7 depicts the flight activity of Canal Creek colonies on November 6, 1966. Very few bees foraged. Some flight activity from hives 4 and 7 was observed during mid-afternoon. An open feeder was set out after most of the bees had returned to the hive for the day. Fig. 3.8 shows the affect of providing a rich food source during a period when little forage was available. Flight activity increased six-fold. Some trapping of bees occurred at the feeder. As indicated by the counters, about 6% of the forager bees failed to return to the hive. This same experiment was repeated with the colonies at Old O Field. Colonies at this site had continued to fly somewhat more than those at Canal Creek. The feeder increased overall flight activity by 1.6, with approximately 3% of the bees unable to return to the hive.

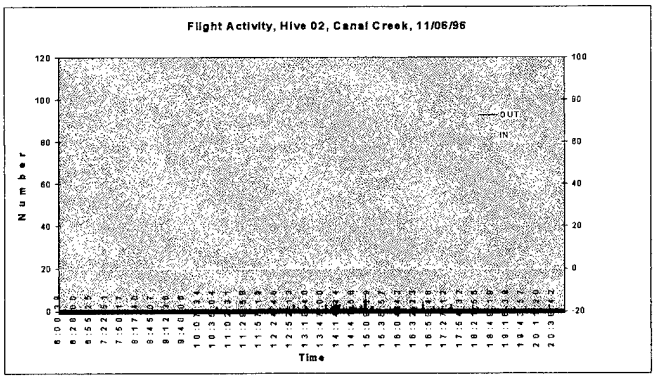
Statistically, the observed increase in foraging at Canal Creek was highly significant ($P=0.000578$) as indicated by an analysis of variance using square root transformed values to normalize the count data. The 6% reduction in returns was not significant ($P=0.0593$). At Old O Field, neither the increase nor the reduction in returns of bees to the hives were significant at the 0.05 level.

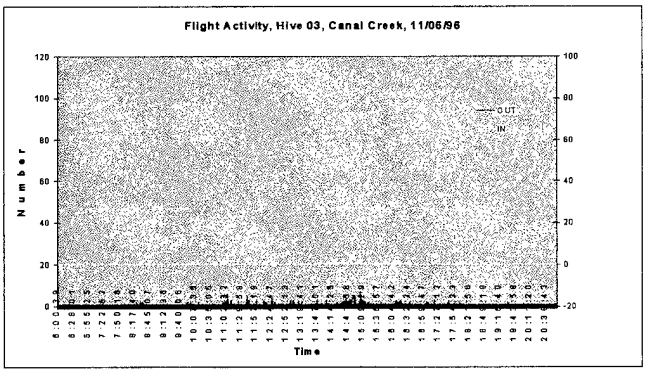
Keeping in mind that colony strength and flight activity were more variable in late autumn than during mid-summer, we suspect that the counters should be able to identify statistically significant differences of 6% or less between numbers of incoming and outgoing bees.

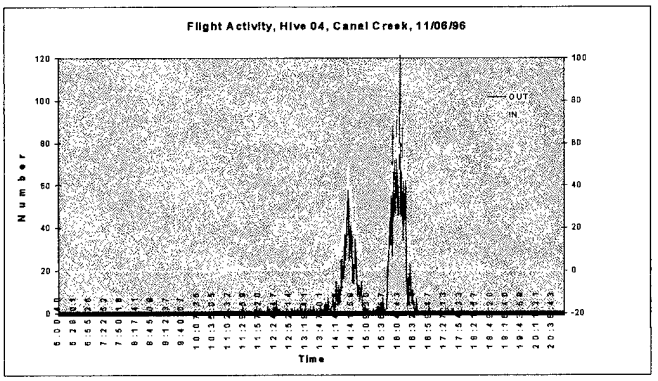
3.3.7 Flight Activity and Multiple Weather Factors

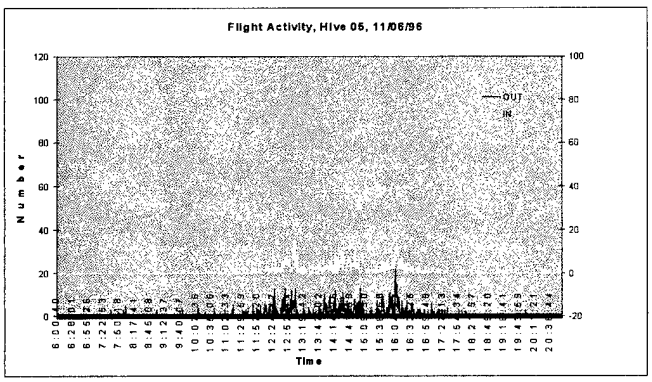
Fig. 3.9 illustrates colony responses to the approach of a thunderstorm at Canal Creek on August 27, 1996 (see also Section 2). As discussed earlier, bees anticipate the approach of storms and often return to the hive. Most beekeepers believe that bees respond to decreases in barometric pressure. As shown by Fig. 3.9, the number of incoming bees increased markedly almost an hour before the onset of rain. In this example, barometric pressure did drop, reaching a low shortly after most of the bees had returned to the hive. Because rainstorms are common during mid- to late summer at APG, we had many opportunities to observe the influence of storm events on colony flight. From a preliminary review of the data, it appears that bees may respond to an array of meteorological changes, not simply barometric pressure. In fact, barometric pressure did not always drop before the commencement of a storm.

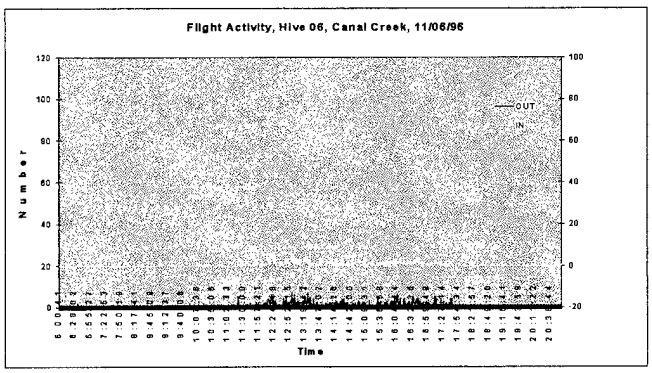












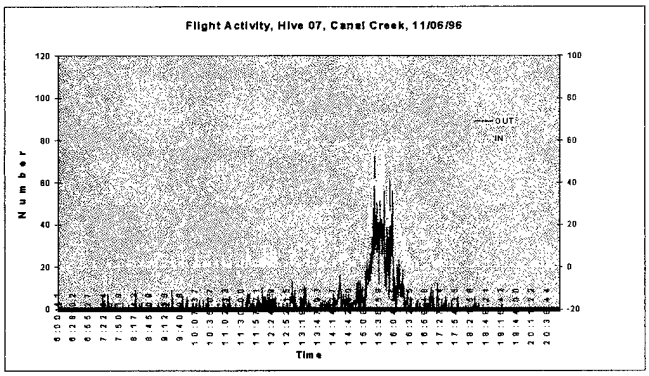
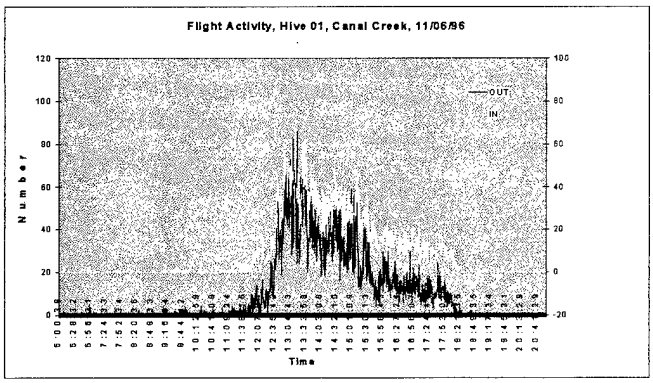
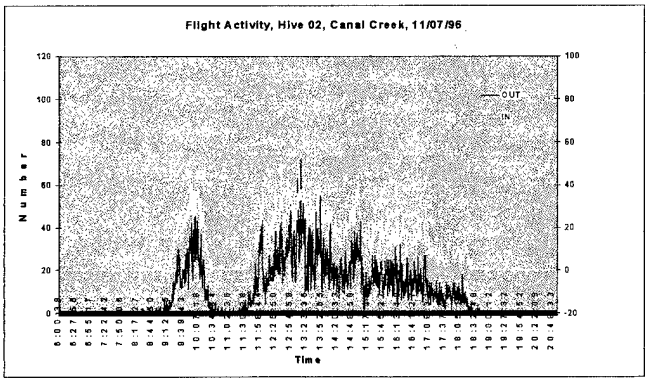
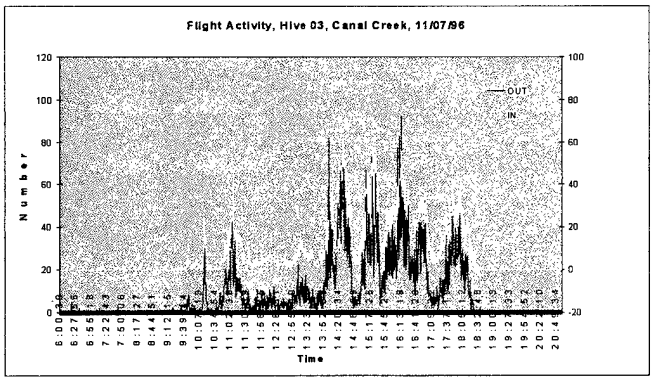
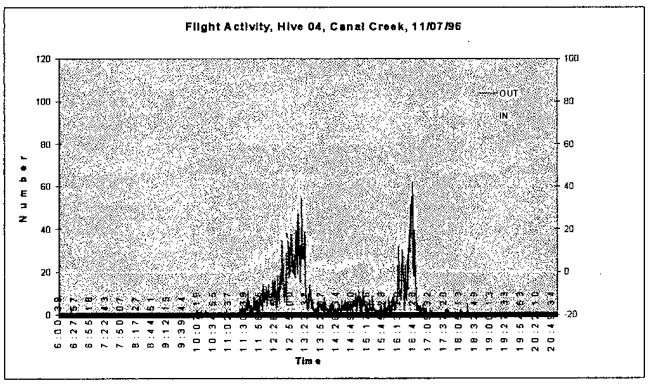


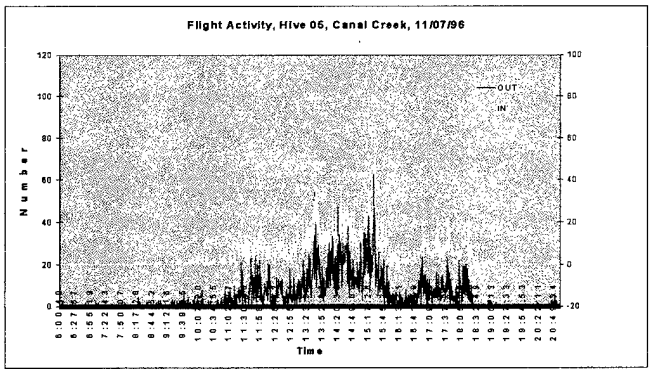
Figure 3.7 Sugar syrup made available to all hives at 1700 hr in an open, external feeder after most flight acitivity had ceased. Only hives 04 and 07 actively foraged during the day. Black series indicates number of outgoing bees; white series incoming bees.

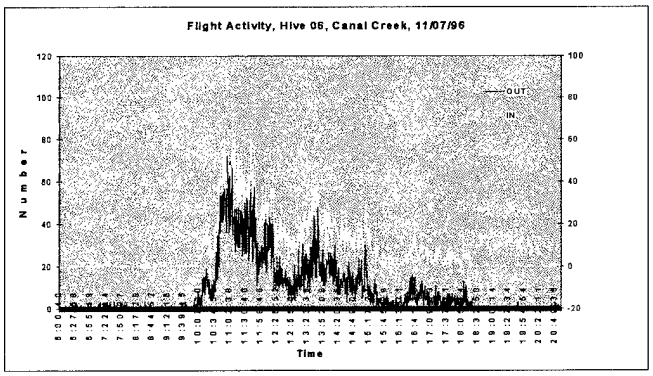












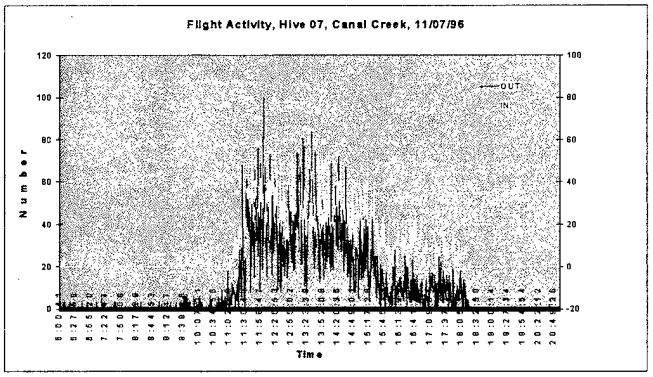
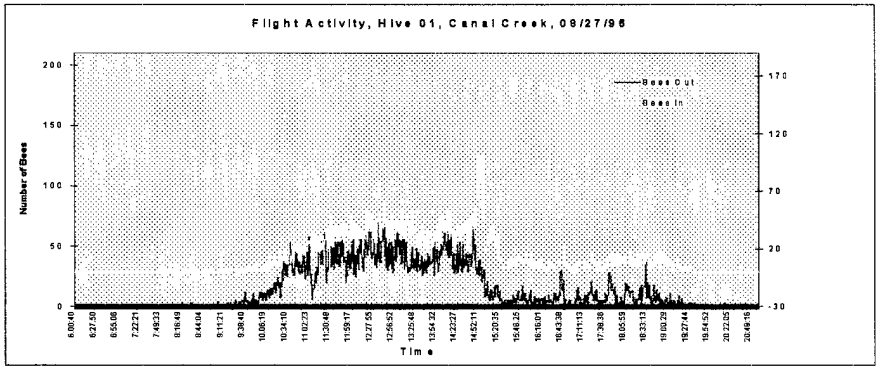
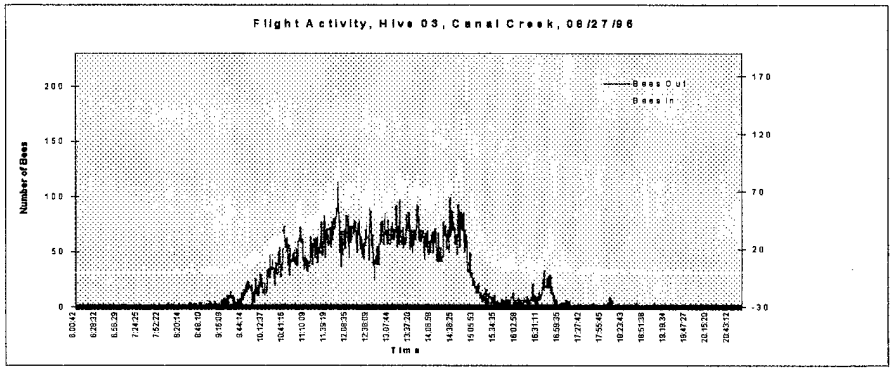
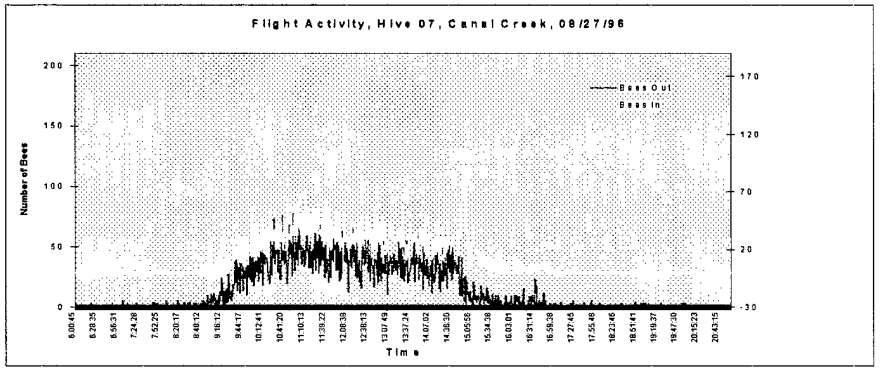
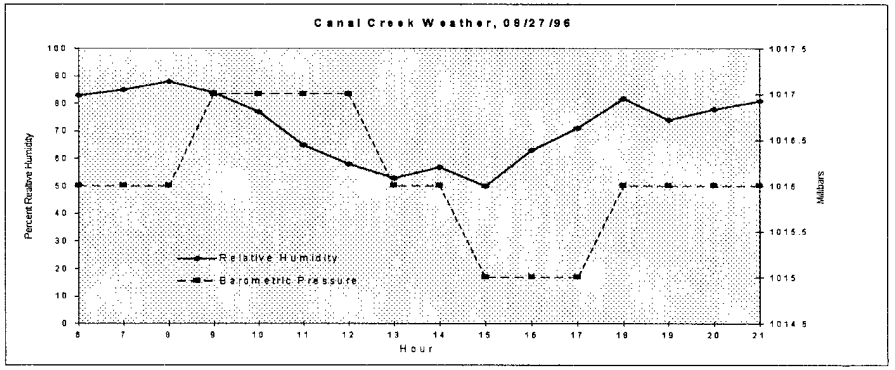


Figure 3.8 Sugar syrup available throughout the day to all hives in an open, external feeder. Flight activity increased by a factor of six compared to previous day. Trapping of about 6% of the bees occurred at the feeder as shown by greater numbers of outgoing bees (black series) than incoming bees (white).









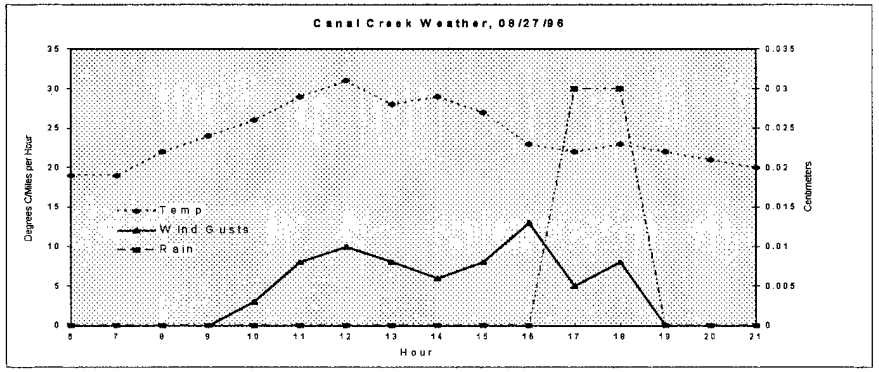


Figure 3.9 Flight activity for three of seven hives at Canal Creek illustrating return of bees to the hives before a thunderstorm. Black series indicates numbers of outgoing bees; white incoming bees. Weather data was averaged over hourly periods. Rainfall occurred between 1600 and 1900 hr. Bees returned to hives about 1500 hr.

3.4 Advanced Data Processing

We suspended field activities on November 14, 1996. Given the hundreds of megabytes of data produced during the field season, this report presents a preliminary evaluation of the data. We document colony behaviors and chemical exposures clearly evident from visual examination of the data set and have included preliminary summary statistics, such as repeated measures ANOVA.

Post-field applications have focused on preparing the colony behavior and meteorological data in combination with the chemistry exposure data for the next stage of data processing—advanced statistical, Artificial Neural Network Analysis (ANN), and modeling. For example, our initial analysis of colony data will first determine whether the 30 second intervals can be collapsed into larger time intervals to facilitate analysis without sacrificing sensitivity to colony responses. Then we will submit daily and seasonal activity records to time series analysis with the intent of determining whether there are any temporal patterns in colony activity. We will also determine whether there is any fundamental consistency in daily and seasonal activity patterns among sites and how much variation exists around the mean activity pattern. From this we can build a model of expected activity which can be used to examine the observed patterns for suspect departures from the predicted activity schedule. We expect to be able to use residuals between observed and expected activity schedules in correlation analyses with weather data and chemical data to determine which factors are associated with departures from expected activity patterns.

Our objective has been to develop techniques through which subtle colony responses to chemical exposures can be quickly detected and differentiated from those produced by natural cycles or other stressors such as food resource availability and weather. The goal is to be able to flag those times when chemical sampling should be initiated, rather than continuously or periodically performing costly chemical procedures. These colony responses can provide both a characterization of exposure (i.e., distribution or pattern of change) and a characterization of ecological effects (i.e., population responses in the context of ecosystem attributes that affect the temporal and spatial distribution and nature of the stressor). Although the full array of responses that can be utilized in this context has yet to be identified, and the degree to which these responses provide reliable information is still being investigated, our initial studies have provided three endpoints that demonstrate consider promise as indicators of environmental change—net daily loss of forager bees, coefficients of variation for flight activity between the colonies at each site, and colony core temperatures. Additional discussion of how these endpoints could be applied to an environmental assessment, as well as limitations of a honey bee biomonitoring system, appears in Section 5 of this report.

3.5 Artificial Neural Network Analysis and A Honey Bee Warning System

Because honey bee colony population dynamics reflect complex behaviors that are affected by a number of external and internal stressors, any decision-making computer program used to flag departures from normal behavior would have to be able to evaluate specific behaviors in the context of total colony functioning. These programs also would have to take into account critical driving variables such as weather conditions and food resource availability. For example, flight activity would be expected to be altered or suppressed on rainy, stormy days. However, a sudden depression in flight activity or the numbers of returning foragers on calm, sunny days, when food resources are plentiful, should trigger a warning.

In the simplest case, time series statistical programs can provide real-time monitoring action thresholds, analogous to the warning and out-of-control limits used to monitor quality assurance. This approach can be applied to a metric such as a net loss of returning foragers. However, to take into account co-variables such as weather, colony condition, and food resource availability, a more sophisticated approach is required. A recently developed software tool that has demonstrated consider utility for evaluating complex data sets is the artificial neural network (ANN).

Unlike expert systems that reach a conclusion by applying a rule at each stage and selecting the next step based on the results of the previous step, ANNs are a form of artificial analysis that learn by example or training. We have successfully applied this tool to the chemical fingerprinting for the hives (see Section 4.4.3). Given any chemical analysis data set, the ANN reliably identified the hive from which the air sample was derived. Development of a network capable of recognizing the appearance or increase of contaminants in the hives is ongoing. Similarly, ANNs are being trained to recognized colony populations dynamics as altered by internal and external factors ranging from chemicals in the hive, to hive manipulation, weather conditions, and chemicals outside the hive.

The first step in using a neural network to process data collected on honey bee colony dynamics is to reduce the volume of the data used. Currently, a full day's worth of data from each site consists of over 54,000 data points. Unless the input is scaled down, the neural network could have more than 270,000 input nodes, and as many hidden nodes. Our objective is to reduce the amount of data so that development and testing of a neural network can begin.

The colony dynamics neural network is being developed using the Stuttgart Neural Network Simulator (SNNS), a Unix-based software package which includes all the tools necessary to design, develop, and test various configurations of networks. After the neural network is properly trained, we can experiment with the configuration (perform "brain surgery") and help determine which input signals are critical to the network, and which signals have little or no significance. This will allow us to concentrate on the input signals which play the greatest role in classifying the colony dynamics.

Once an appropriate network or networks is developed, a DOS or Windows-based, stand-alone version can be written. This can easily be integrated into the existing data-acquisition system, providing immediate (real-time) or overnight analysis of current colony dynamics.

Ultimately, we hope to be able to use advanced statistics and artificial intelligence tools to establish rules or patterns for rapidly identifying and classifying colony responses to environmental stressors. Given the large amounts of data being continuously generated by the electronic hives and weather stations, we anticipate passing the information from the data gathering computers to another computer that serves as a decision tree. For example, flight activity, weather data, and colony core temperatures and relative humidity are all saved at intervals of less than five minutes. Changes in flight activity would be referenced to meteorological conditions by the decision-making system. On warm, sunny days, with food available (as indicated by pollen collection), foraging should be high. Cold, wind, rain or lack of nectar or pollen would explain reduced foraging. However, when foraging conditions are optimal, departures from normal flight patterns would raise a warning.

Acute events should be discernible within a few minutes. Chronic events could be looked for at the end of the day or week, depending on the parameter and the appropriate time frame. For example, the data smoothing followed by derivatives (illustrated in Fig. 2.8d) would then become just one more step in the data processing.

Although we expect the ANN to be a primary tool for these alerts, advanced statistics (such as Repeated Measures Analyses of Variance, and even simpler statistical methods should be applicable for parameters that are more stable or constant. For example, when brood is present, a control chart approach using the expected mean temperature and deviations from the mean could be used to provide warning and control data limits. Following this approach, a single colony out of control, a systematic positive or negative drift for one or more colonies, or a group of colonies exceeding the warning limits would warrant inspection and if appropriate, chemical sampling.

Our data collection procedures already accomplish real-time measures of colony condition and activity. Real-time warnings of departures from normal colony structure or performance should be feasible using the decision tree approach. By connecting the decision-making computer to a phone line or satellite uplink, it is feasible to send an immediate system alert to another computer anywhere in the world, providing true real-time monitoring capability of colony activities and internal conditions.

Development of an ANN for use in discriminating colony responses to contaminant exposure and to the presence of contaminants in the hives is an ongoing activity. Dose-response and chemical uptake and depuration tests are planned for the summer of 1997 to determine colony responses to the industrial solvents detected in the hive atmospheres of Maryland bee colonies. Because many of these chemicals were not found inside hives in the

Missoula valley, but occurred at all of the Maryland locations, Montana offers a better control location for this testing.

Whether an ANN, advanced statistics, a combination of these approaches, or another technology such as a decision tree offers the best discrimination tools for linking colony responses to specific stressors such as specific chemical agents or mixtures remains to be seen. However, these technologies represent the current state-of-the-art and have been shown to be useful for evaluating other complex data sets.

ANNs are being used by Montana's Petroleum Reservoir Characterization research team to process diverse, complex geophysical and petroleum engineering data. The objective is to be able to incorporate multi-disciplinary and extremely large data sets into an integrated data set that can be used to better characterize and enhance the productivity of existing oil fields. Similarly, this honey bee project has already demonstrated that an ANN can reliably identify a specific hive based on its chemical fingerprint, consisting of the chromatograms for over 200 chemicals. Given the complexity of a data set comprised of peaks and retention times for multiple chemicals, it would be impossible for a person to identify a hive from the raw chromatograms. Currently, an ANN has been able to learn to identify some of the behavioral characteristics of specific hives deployed at APG and the Churchville reference site during 1996. At this time, the ANN can reliably identify a hive based on its unique behavioral characteristics. The degree to which an ANN can further discriminate complex behaviors in the context of multiple stressors is a primary focus of our ongoing investigations.

3.6 References

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SECTION 4

MONITORING OF EXPOSURES TO BIOAVAILABLE CONTAMINANTS

4.1 Introduction to Chemical Sampling and Analysis

Chemical sampling and analysis for this project have been guided by two overall objectives: 1) Establish the biomonitoring relationship between measured behavioral endpoints in honey bees and acute exposures to specific chemical agents; and 2) Assess the bioavailability of chemical agents to honey bees for site-to-site comparisons with respect to chronic ecosystem exposures.

Colonies of honey bees were monitored for volatile and semi-volatile organic compound residues by pulling hive atmospheres through chemical traps and, subsequently, thermally desorbing them into a gas chromatograph/mass spectrometer (TD/GC/MS). Heavy Metals and other inorganic elements of concern (Be, As and Se) were assayed by digesting whole bees or pollen samples for analysis by inductively coupled plasma mass spectrometry (ICP/MS).

A challenging aspect to evaluating the presence of organic contaminants was identifying them against the extremely complex background of organic compounds normally found inside bee hives. Chemical fingerprints were collected for honey bees, hive stores (honey, pollen, wax and propolis) and hive components (wood, paint, vinyl-coated screens, etc.) to account for compounds arising from these sources. In addition, chemical fingerprints were recorded for known breakdown products of mustard gas by pumping on sealed vessels containing them (under a hood) and then analyzing the chemical traps by TD/GC/MS.

Finally, strategies are under development for the real-time or rapid identification of volatile and semi-volatile contaminants encountered by foraging honey bees. Chromatograms and mass spectra are digitally encoded for processing by artificial neural networks (ANN's). Work completed during the past year on results from the 1995 APG pilot study and reported here demonstrates that networks can be trained to unambiguously identify the exact hive from which a sample has been collected. Since a network can be trained to recognize individual hives under normal conditions, it appears feasible to train a network to detect the presence of new compounds that have been assimilated by returning hive foragers.

4.2 Experimental Methods and Materials

4.2.1 Sampling Design/Frequency - 1995 APG Pilot Study

Chemical sampling conducted during the 1995 pilot field study at Aberdeen Proving Ground was confined to the West Branch Canal Creek. Before and during the field demonstration, the Corps of Engineers was performing light clean-up activities in the vicinity - cutting grass and shrubbery on dry ground and some standing plants in the tidal river bottom. Debris from dismantled chemical plants was removed wherever it was encountered.

Three levels on the instrumented hive condos were selected for insertion of the chemical sampling probes: 1) the upper hive story where stores of honey are kept; 2) the lower hive story where the queen bee raises new brood and pollen is stored; and 3) the pollen hopper where incoming bees may lose pollen pellets as they climb through a scraper mesh and dead bees are trapped as workers attempt to remove them through the bee counters. The pollen hopper area was of interest since it contained hive components manufactured from polyvinyl chloride plastics and since it was a region of the hive where high concentrations of dense vapors from chlorinated organics might accumulate.

Samples of hive atmospheres were withdrawn during eight periods between August 24th and August 31st. The schedule of sampling among the six hives in the condo cluster is shown in Table 4.1. Usually, only six samples could be acquired each day as that was the total number of constant flow pumps in the on-site equipment inventory. On one occasion (8/30/95), we used splitting valves to draw air through two traps with a single pump. In retrospect, this sampling technique was not satisfactory as it gave low ion abundances on the GC/MS for samples from the split tubes and adversely affected the limit of detection. Ambient air was pulled through sorption traps on two dates by placing a pump on the ground near Condo 4 with the orifice elevated about 20 cm. Air was also sampled around selected USGS well bundles near the floating docks in the West Branch Canal Creek after USGS personnel reported seeing honey bees drinking from water that adhered to well bundle coupling nuts.

4.2.2 Sampling Design/Frequency - 1996 Missoula Fingerprint Studies

Fingerprinting studies were conducted in Missoula following the APG pilot study. The goal of these experiments was to establish a profile of the volatile and semi-volatile components given off by honey bees, hive stores and hive components. A set of experiments conducted inside the project director's house revealed that indoor air contained a wide range of additional organic contaminants - presumably from materials used in household items like upholstery, rugs, foam, and insulation. There was so much background from the indoor air that it was difficult to unequivocally ascribe compounds to hive materials. Subsequent pumping trials were delayed until the summer of 1996, when they could be conducted outdoors.

TABLE 4.1

1995 APG Sampling Schedule

(X - sample taken, L/R - side-by-side replicates, S - splitting valve used, WB - USGS well bundle)

Hive	Level	Aug 24	Aug 24 eve	Aug 25	Aug 27	Aug 28	Aug 29	Aug 30	Aug 31
1	Upper Lower Hopper	X	X		X		 X		 X
2	Upper Lower Hopper	X	X		X		 X		 X
3	Upper Lower Hopper	X	X		X		 X	S S X	 X
4	Upper Lower Hopper	X	X	L/R X X	X		 X	S S X	 X
5	Upper Lower Hopper	X	X		X		 X		 X
6	Upper Lower Hopper	X	X		X		 X		 X
Other location				WB28		WB24 WB26 WB34 WB37		WB24	
Ambient Air				X				X	

Starting in early July, seven dates of fingerprint experiments were carried out (Table 4.2). A stainless steel cage was fabricated to contain about 2,000 honey bees so that a chemical signature of compounds from their active physiology could be obtained. The top of the cage was outfitted with a syrup bottle to feed the bees during the pumping period. An aluminum foil sun shade was loosely shaped around the syrup bottle and the pump probe to keep the light level reduced inside the cage. Hive stores were evaluated by pumping on a previously occupied upper story box with and without honey frames, plus two samples of propolis from Missoula colonies. Hive components profiled included unpainted wood, painted wood, machined plastic parts, vinyl-coated screen wire and complete condo units. The effect of aging on the loss of volatile and semi-volatile components from hive boxes was assessed by comparing unpainted wood from the 1995 and 1996 lumber inventories. We also compared a condo used during the 1995 season to a newly completed 1996 model. Fingerprinting tests were terminated when constant flow pumps were transported with the bee colonies for the 1996 APG field season.

Several additional experiments were performed as part of our quality assurance practices. Since there were several unrelated projects based in the room housing The University of Montana's TD/GC/MS instrumentation, we sampled laboratory air from four locations to ascertain background levels of volatile and semi-volatile compounds in our storage, preparation and analysis areas.

Finally, in response to input from APG personnel associated with the Old O Field capping project, we performed a TD/GC/MS characterization on reagent grade samples of mustard gas breakdown products that had been detected in groundwater tests - thiodiglycol, 1,4-dithiane and 1,4-oxathiane (Aldrich).

4.2.3 Sampling Design/Frequency - 1996 APG Field Applications

Fabrication of three sets of instrumented condo units (7 colonies each) permitted APG field applications at three sites: 1) West Branch Canal Creek, 2) the Old O Field landfill capping site, and 3) a reference site at a hobbyist beekeeper's farm near Churchville, MD. In addition, 16 free-standing colonies were dispersed at Old O Field and throughout the office, residential and recreational portions of APG's Edgewood area. They were transported from Montana with the instrumented condo colonies. Six other free standing colonies were Maryland bees acquired from local, private beekeepers. These units were sampled at locations near Jarretsville and Churchville, then five of the six were placed at Old O Field in June.

Sampling of hive atmospheres took place on 19 dates during the Field Applications (Table 4.3). Several sample sets were collected in spring and summer at the residences of the private beekeepers to establish baseline levels of colony chemistry prior to moving them onto potentially contaminated sites at APG. Sample sizes on any one date were constrained to 13, the number of constant flow pumps in our 1996 equipment inventory. Sites with samples missing on a given date were generally due to tube breakages, wet tubes or pump failures.

TABLE 4.2

1996 Missoula Fingerprint Studies

Category	Sample Dates
Honey bees	7/5, 7/6, 7/7
Hive stores	
Unoccupied 1995 hive box (no bees or frames)	7/5, 7/6, 7/7
Unoccupied hive 56 (no bees with frames)	7/11
Propolis A	7/19, 7/20
Propolis B	7/19, 7/20
Hive Materials	
Unpainted 1995 wood	7/5. 7/6, 7/7
Unpainted 1996 wood	7/5, 7/6, 7/7
Painted 1996 box	7/5, 7/6, 7/7
Old plastic parts	7/11
New plastic parts	7/11
Vinyl-coated screen	7/19, 7/20
Old condo	7/19, 7/20
New condo	7/19, 7/20
Clock drive assembly	7/13
Aluminum foil	7/13
Ambient air (with 300 and/or 400 tubes)	7/5, 7/6, 7/7, 7/11(2), 7/19, 7/20

TABLE 4.3

1996 APG Field Application Samples
(through September 29, 1996)

Site/Colony ID	Sampling Dates
Old O Field	
JZ1N	5/13*, 6/16*, 6/19, 6/25, 7/3, 8/9, 9/2, 9/21
JZ2N	5/13*, 6/16*, 6/19, 7/3, 8/9, 8/20, 9/2
JZ3S	5/13*, 6/16*, 6/19, 6/25, 7/3, 8/9, 8/20, 9/21
DS1S	6/16@, 6/19, 6/25, 8/9, 8/20
DS2N	6/16@, 6/19, 6/25, 8/9, 8/20, 9/2, 9/21
OF1 (#102)	9/2, 9/21
OF2 (#105)	8/9, 8/20, 9/2, 9/21
OF4 (#154)	9/2, 9/21
OF5 (#137)	9/2, 9/21
OF6 (#119)	8/9, 8/20, 9/2, 9/21
OF7 (#175)	8/9, 8/20, 9/2, 9/21
OF Air	5/13*, 6/16*, 6/25, 7/3, 8/9, 9/2, 9/21
West Branch Canal Creek	
CC1	8/14, 8/18, 9/20
CC2	8/14, 8/18, 9/20
CC3	8/14, 8/18, 9/20
CC4	8/14, 8/18, 9/20
CC5	8/14, 8/18, 9/20
CC6	8/14, 9/20
CC7	8/14, 8/18, 9/20
CC Air	8/14, 8/18, 9/20
APG Edgewood Area	
G Street 1 (#136)	8/18, 8/23, 9/25
G Street 2 (#149)	8/18, 9/25
G Street Air	8/23, 9/25
Lauderick Creek 1 (#118)	8/18, 9/20
Lauderick Creek 2 (#153)	8/18, 9/20
Lauderick Creek Air	9/20
National Guard 1 (#126)	8/20, 9/25
National Guard 2 (#185)	8/20, 9/25
National Guard Air	8/20, 9/25

TABLE 4.3 (Cont'd.)

1996 APG Field Application Samples

Site(Colony ID)	Sampling Dates
APG Edgewood Area	
Beach Point 1 (#160)	8/23, 9/29
Beach Point 2 (#186)	8/23, 9/29
Beach Point Air	8/23, 9/29
Youth Center 1 (#147)	8/23, 9/24
Youth Center 2 (#148)	8/23, 9/24
Youth Center Air	8/23, 9/24
East Branch Canal Creek 1 (#107)	8/23, 9/24
East Branch Canal Creek 2 (#142)	8/23, 9/24
East Branch Canal Creek Air	8/23, 9/24
Churchville	
CV1	8/22, 8/25, 9/26
CV2	8/22, 8/25, 9/26
CV3	8/22, 8/25, 9/26
CV4	8/22, 8/25, 9/26
CV5	8/22, 8/25, 9/26
CV6	8/22, 8/25, 9/26
CV7	8/22, 8/25, 9/26
CV Air	8/22, 8/25, 9/26

*Measurements performed at Jarretsville site of private beekeeper
@Measurements performed at Churchville site of private beekeeper

Each time that the condo clusters were sampled, an ambient air sample was collected in the same vicinity and over the same duration. At West Branch Canal Creek, the ambient air sample pump was place 1.5 meters off the ground, attached to a tree trunk 2 meters in front of Condo 7. At Old O-Field, the ambient air sample was collected by placing the cassette pump on a metal housing about 10 meters from Condo 1 and about 1.5 meters above ground level. At Churchville, the air sample was pumped positioned on the metal frame of an abandoned farm implement about 8 meters behind the condo cluster. Ambient air samples from the free-standing Edgewood colonies were gathered by placing the pump on the ground within a few feet of the hives, with the copper inlet aimed horizontally about 20 cm off the ground. Because of the limited number of pumps, we were not able to collect an ambient air sample at each Edgewood colony on every sample date. The dates on which colony ambient air was sampled are noted in Table 4.3.

Trip blanks were collected from each site on every sampling date. In each case a thermal desorption tube, sealed in its individual glass storage vial, was selected from the same batch as those pumped, left at the site aside the cassette pumps during the duration of the sampling period, and stored and transported with sample tubes until analysis time. Trip blanks were thermally desorbed into the GC/MS and analyzed in the same batch as the sample tubes they accompanied.

4.2.4 Air Samples

Air samples were initially collected on 11.5 cm x 6 mm OD x 4 mm ID Carbotrap 300 thermal desorption tubes (Supelco) with three phases:

300 mg of 20/40 Carbotrap C - graphitized carbon black with 10 m^2/gram surface area for trapping and efficiently releasing molecules in the C9 to C30 range;

200 mg of 20/40 Carbotrap B - graphitized carbon black with 100 m^2/gram surface area for trapping and releasing molecules starting at the C4 to C5 range; and

125 mg of 60/80 Carbosieve S-III spherical carbon molecular sieve with 820 m^2/gram surface area for trapping small airborne molecules, such as chloromethane.

After some sampling during the summer of 1995 was plagued by moisture lodging in the sorption bed, we added four-phase Carbotrap 400 tubes to our inventory in 1996. These tubes, designed to perform better under humid conditions, contained a slightly different sorbent mixture:

150 mg of 20/40 Carbotrap F - graphitized carbon black with 5 m^2/gram surface area for trapping and efficiently releasing molecules in the C20 to C30 range;

150 mg of 20/40 Carbotrap C - graphitized carbon black with 10 m^2/gram surface area

for trapping and efficiently releasing molecules in the C9 to C30 range;

125 mg of 20/40 Carbotrap B - graphitized carbon black with 100 m^2/gram surface area for trapping and releasing molecules starting at the C4 to C5 range; and

125 mg of 20/45 Carboxen-569 - a highly hydrophobic carbon molecular sieve that is useful in high humidity to trap small airborne organics.

Desorption tubes were connected with Tygon tubing to low flow sample pumps (SKC, Inc models 222-3 and 222-4). Pump flows were adjusted to 22.5 ml/min and 60 ml/min for the 1995 pilot study. Since this rate did not seem to saturate many sorption sites on these early samples, flows were eventually increased to around 100 ml/min on most pumps for the 1996 samples. This allowed larger volumes of air to be sampled without having to pump during the dew point hours. We achieved far better limits of detection in this manner and, thus, were much more successful in seeing a variety of contaminants. We also lost fewer samples to wet tubes.

The distal end of the sorption tube was protected from bee interferences and dirt by attaching a copper tube with a brass compression screw and vespel/graphite ferrule. The copper tube was then inserted directly into the hive interior, usually between the wooden frames that support the wax combs. Total volume pumped was obtained by multiplying factory-calibrated cycle volumes for each pump by the number of cycles registered on each pump's digital counter. Pumping periods varied from 8 hours to 24 hours.

Sample tubes were sealed in individual vials and stored in a refrigerated chamber to enhance retention of volatile contaminants. Samples from APG were air expressed, with trip blanks, to our University of Montana labs. Once in Missoula, they were stored in a dedicated 4 °C sample refrigerator.

4.2.5 Thermal Desorption GC/MS Analysis

Air samples were analyzed by thermal desorption GC/mass spectrometry. Sample tubes were placed in an 8-station thermal desorption unit (Dynatherm MTDU 910). After a five-minute helium purge (Liquid Air, ultra high purity grade) at 46 °C, tubes were subjected to a 10-minute desorption cycle at 300 °C. A final 5-minute cooling flush was used to remove residual contaminants trapped in the sorbent bed and transfer line. All phases of the desorption utilized a helium flow rate of 35 ml/min.

As the project progressed, system blanks were employed to a greater extent. Samples from 1995 that contained sorbed moisture gave unsatisfactory separations. Wet samples yielded a broad initial peak followed by low level noise for the remainder of the chromatogram. Furthermore, there was significant carry-over into subsequent chromatograms. With 1996 samples, a system blank or a trip blank was inserted after every two samples. Previously,

only one blank was included in each 8-station run. The frequent blanks provided continual assurance that peaks appearing in a sample's chromatogram were real, not laboratory artifacts or carry-over from earlier sample tubes.

Thermally desorbed contaminants from the sample were captured by a 6" Vocarb 3000 trap from Supelco (10 cm Carbopack B graphitized carbon, 6 cm Carboxen 1000 molecular sieve and 1 cm 1001 molecular sieve) installed in a Tekmar LSC2000 Liquid Sample Concentrator. From there, the sample was introduced into the gas chromatograph by heating the Vocarb 3000 trap to 260 °C and flushing it with 40 ml/min of ultra high purity helium. The entire helium flow from the trap entered the GC for 15 seconds and was split 1:50 thereafter.

Chromatographic separations were accomplished on a Hewlett Packard GCD instrument containing a 60 m x .32 mm ID Restek RTX-502.2 capillary column (phenylmethyl polysiloxane, 1.8 mm coating). Helium flow was 1 ml/min and the total time for an analysis was 48 minutes (5 min initial temperature 40 °C, ramp 5 °C/min to 220 °C, 7 minute hold time at 220 °C). Detection of the mass spectrum covered a range of 35 to 260 m/z, although some runs were extended to 435 m/z.

Prior to any analyses, we performed a manual calibration tune of the mass spectrum detector to match previous performance characteristics. 1 uL of a methanol solution containing 25 ng of bromofluorobenzene (BFB) was injected and the resulting total ion chromatogram and mass spectrum examined. The mass detector was adjusted until the absolute abundance of the BFB peak on the total ion chromatogram was 11,000 $\pm$ 10% and the relative abundances on the accompanying mass spectrum were:

m/z	% relative abundance
50	20.1$\pm$ 10%
75	42.4$\pm$ 10%
95	100.0 (base peak)
96	8.2$\pm$ 10%
174	74.8$\pm$ 10%
175	7.4$\pm$ 10%
176	96.8$\pm$ 10%
177	7.7$\pm$ 10%

If the instrument did not pass on all eight tests (TIC and seven relative abundances), the mass detector was adjusted and a new BFB injection made. Given these procedures, relative sample concentrations, expressed as characteristic ion abundance/liter of air pumped, were reproducible with a maximum error of 10%.

4.2.6 Metal analysis

Two rounds of whole bees and pollen were analyzed for their heavy metal and inorganic content. Live bee samples with 100 to 200 individuals were collected via a hand-held vacuum with a PVC nozzle that trapped returning forager bees in a plastic bag (Whirl-Pak). Plastic bags were sealed and immediately placed on ice. Dead bee samples were collected in Whirl Pak bags from the dead bee traps at the bottom of the entrance hopper. Hive workers would normally drag dead bees out the hive entrance, but the bidirectional bee counters precluded this in condo colonies. Pollen also was gathered from plastic trays at the bottom of the entrance hopper. Pollen pellets from the tray were transferred into glass scintillation vials (Wheaton), sealed and stored on ice. Both bee and pollen samples were kept continuously on ice until delivered to the analytical chemistry personnel in the US Army Biomedical Research and Development Laboratory at Ft. Detrick.

Whole bees and pollen samples were oven dried in covered, glass beakers for 24 hours at 105 °C. One-half gram of oven-dried material was placed in a pressure-controlled Teflon digestion bomb (CEM Corp) with 10 ml of trace metal grade concentrated nitric acid. Samples were digested in a controlled pressure CEM MDS-2000 microwave to achieve a transparent, yellow solution. Pressure regulation during digestion was as follows: ramp at 20% power to 20 psi over 10 minutes, hold 5 minutes, ramp at 50% power to 40 psi over 10 minutes, hold 5 minutes, ramp at 50% power to 80 psi over 10 minutes, hold 5 minutes, ramp at 50% power to 100 psi over 10 minutes, hold for 10 minutes.

Following digestion, samples were diluted with 50 ml of de-ionized water. Metal (Ba, Bi, Cd, Co, Cs, Cr, Cu, Ga, Mn, Ni, Pb, Rb, Sr, Tl, U, V and Zn) and inorganic element (Be, As and Se) concentrations were quantified on a Hewlett Packard 4500 inductively coupled plasma mass spectrometer.

4.3 Chemical Results and Discussion

4.3.1 Volatile and Semi-Volatile Organic Contaminants

Our analytical methodology for volatile and organic contaminants was designed to be a semi-quantitative, general survey since no guidance on potential contaminants, beyond mustard breakdown products, was suggested. Because it was infeasible to anticipate the exact contaminants that would be bioavailable to bees in measurable quantities, we chose a relative concentration metric that would allow us to see spatial or temporal distributions patterns and draw site-to-site comparisons.

The air inside beehives placed at APG contained measurable quantities of organic contaminants commonly associated with industrial sites. Among the volatile and semi-volatile contaminants found, seven have been systematically quantified throughout the 1996 Field

Application data set (through September 29th) -- five chlorinated hydrocarbons, a polycyclic aromatic hydrocarbon (PAH) and a ketone. Several contaminants detected in the hives were of particular interest since their presence had previously been reported in ground and/or surface water at West Branch Canal Creek (1), at Beach Point (1) and at the Old O Field landfill (2) areas.

The seven contaminants quantified in hive atmospheres were: perchloroethylene (PCE), trichloroethylene (TCE), tetrachloromethane (TCM), hexachloroethane (PCA), dichlorobenzene (DCB), naphthalene and acetophenone. A typical total ion chromatogram for a hive atmosphere sample appears in Fig. 4.1. A complete listing of the contaminant levels found in 1996 field samples through September 29th is provided in Table 4.4. The sections that follow discuss the confirmation and analysis of the organic contaminants. The pattern of each contaminant's occurrence is illustrated with plots that group results by site and date. Finally, an overall, comparative picture of the organic contaminants is drawn.

Because of the complex nature of the hive samples, chromatographic isolation of all contaminant peaks was rarely achieved. Fig. 4.2, for example, shows a group of three overlapping peaks in the September 2nd sample from O Field Condo 5. TCE is contained in the shoulder that appears in the upper panel of Fig. 4.2 at a retention time of 18.55 minutes. A mass spectrum with a TCE fingerprint superimposed on other mass peaks appears in the lower panel. While single ion mass spectrometry (SIMS) techniques could be used to accurately follow a single compound in the presence of co-eluting interferants, we chose instead to use a traditional scan mode to capture data on a full mass range. Then, in a strategy similar to the SIMS technique, we quantified individual contaminants in the presence of co-eluents by noting the abundance of a mass spectral ion characteristic of each compound. This was often, though not always, the molecular ion.

To quantify a compound in a sample we: 1) searched the total ion chromatogram at the appropriate retention time of the contaminant for a peak; and 2) reviewed individual mass spectra for each time slice in the peak until the maximum abundance of the characteristic ion was located. In this manner, our SIMS-like technique could be used to simultaneously quantify the relative concentrations for a suite of compounds. Levels reported for all seven contaminants in Table 4.4 have been determined in this fashion and carry units of characteristic reference ion abundance per liter of air sampled (ipl), a semi-quantitative value suitable for site-to-site comparisons and gradient analysis.

4.3.2 Perchloroethylene (PCE)

Perchloroethylene or tetrachloroethene, $Cl_2C=CCl_2$, was the first of the seven organic contaminants recognized in hive atmosphere samples from APG. Samples drawn from several Old O Field hives on September 2nd had sharply elevated levels of PCE. Its identity was unmistakable since the isotopic fingerprint of its molecular ion with four chlorine atoms is easily recognized. The fingerprint arises from the probability of finding the chlorine-35

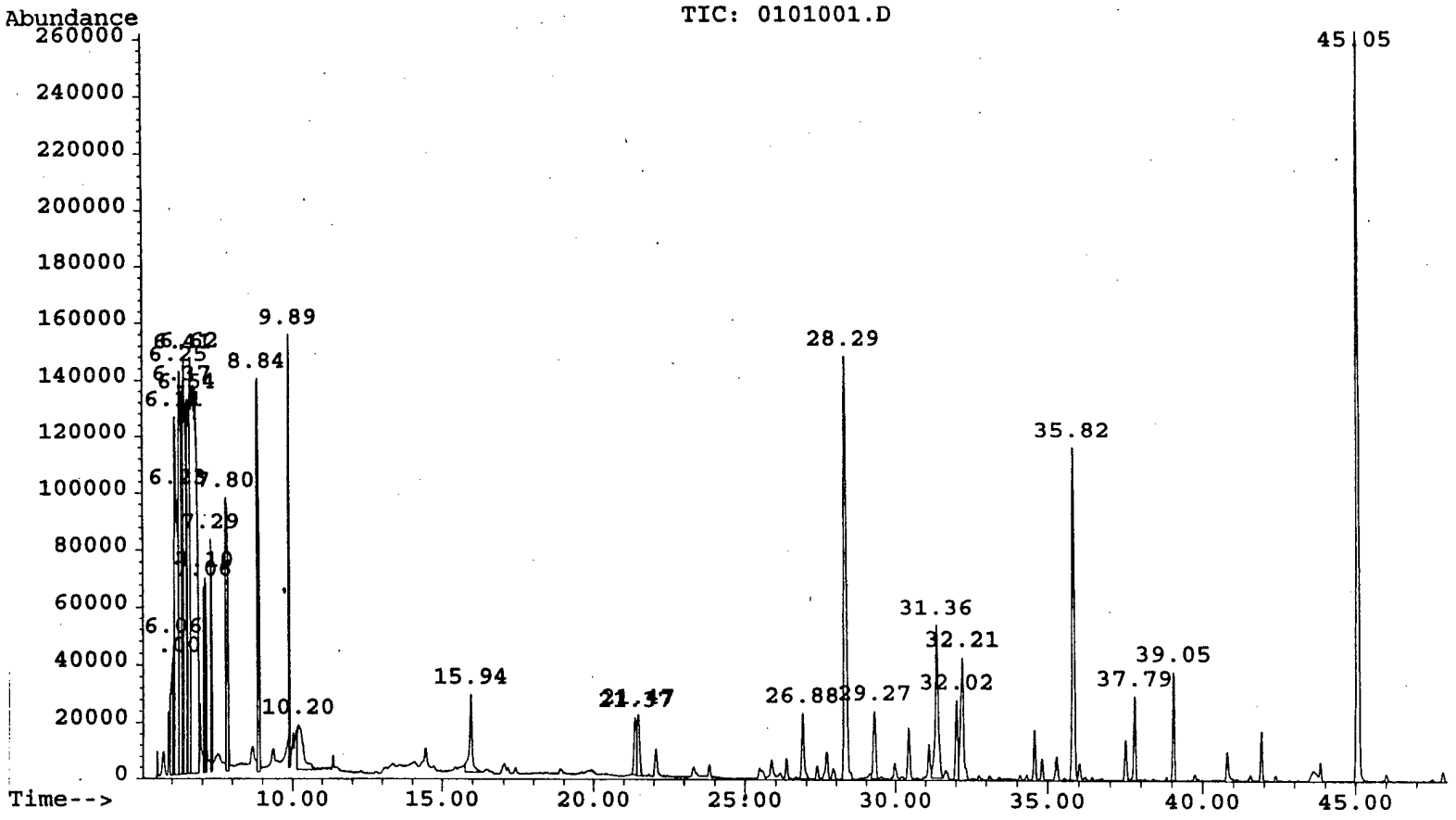


Figure 4.1. Typical Total Ion Chromatogram (TIC) for a Hive Atmosphere Sample. This TIC is from Old O Field Condo 1, September 2, 1996

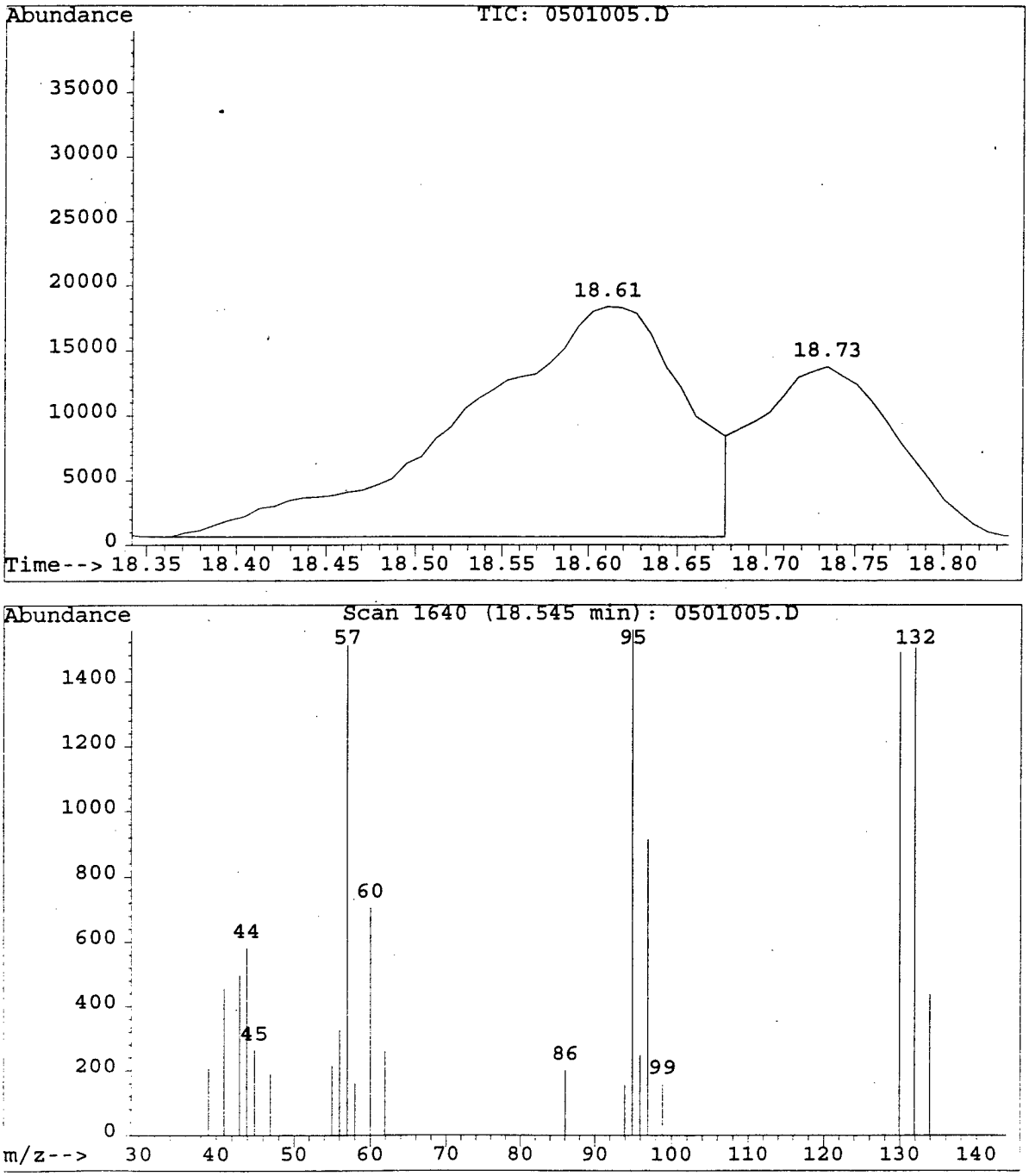


FIGURE 4.2. Total Ion Chromatogram Peak from September 2nd Sample, O Field Condo 5 Demonstrating Co-elution of Trace Level TCE at 18.55 Minutes and an Interferant Compound at 18.61 Minutes

TABLE 4.4
Organic Data for 1996 APG Field Applications

Colony locations: O Field = DS, JZ or OF; Canal Creek = CC; Lauderick Creek = LC; G Street = GS; National Guard Site = NS; Churchville = CV; Beach Point = BP; Youth Center = YC; East Branch Canal Creek = EB. Contaminant abbreviations: perchloroethylene = PCE; trichloroethene = TCE; tetrachloromethane = TCM; hexachloroethane = PCA; 1,4-dichlorobenzene = DCB; naphthalene = Naph; acetophenone = AcPh. Values listed are abundance of characteristic ion / liter of air sampled ± 10%.

Site ID	Date	PCE	TCE	TCM	PCA	DCB	Naph	AcPh
DS1	6/19	5.2	0.0	5.2	0.0	3.5	25.9	5.2
DS2	6/19	4.9	0.0	4.9	0.0	4.9	6.5	0.0
JZ1	6/19	0.0	0.0	0.0	0.0	0.0	0.0	0.0
JZ2	6/19	0.0	0.0	0.0	0.0	0.0	0.0	0.0
JZ3	6/19	0.0	0.0	0.0	0.0	0.0	1.3	0.0
DS1	6/25	5.2	0.0	0.0	0.0	3.5	0.0	10.4
DS2	6/25	8.8	0.0	8.8	0.0	0.0	0.0	17.6
OF air	6/25	5.4	0.0	0.0	0.0	0.0	0.0	0.0
JZ1	6/25	13.6	0.0	13.6	0.0	13.6	0.0	31.7
JZ3	6/25	13.7	0.0	22.8	0.0	9.1	9.1	27.4
JZ1	7/3	0.0	0.0	0.0	0.0	0.0	24.3	0.0
JZ2	7/3	10.6	0.0	0.0	0.0	7.6	6.1	12.1

Site ID	Date	PCE	TCE	TCM	PCA	DCB	Naph	AcPh
JZ3	7/3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
OF air	7/3	7.9	0.0	0.0	0.0	0.0	3.2	0.0
JZ1	8/9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
JZ2	8/9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
JZ3	8/9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DS1	8/9	3.9	0.0	0.0	0.0	0.0	0.0	0.0
DS2	8/9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
OF2	8/9	9.8	0.0	5.9	0.0	0.0	5.9	0.0
OF6	8/9	0.0.	0.0	0.0	0.0	0.0	0.0	0.0
OF7	8/9	32.9	4.4	17.5	0.0	4.4	4.4	0.0
OF air	8/9	10.1	0.0	7.6	0.0	0.0	6.3	0.0
CC1	8/14	8.8	1.8	5.3	0.0	1.8	1.8	0.0
CC2	8/14	3.9	0.0	5.2	0.0	0.0	2.6	3.9
CC3	8/14	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CC4	8/14	8.1	0.0	3.2	0.0	2.4	6.5	0.0
CC5	8/14	10.4	0.0	3.9	0.0	5.2	15.6	5.2
CC6	8/14	6.4	0.0	6.4	0.0	0.0	4.8	4.8
CC7	8/14	4.5	0.0	3.6	0.0	0.0	3.6	3.6
CC air	8/14	3.4	0.0	4.3	0.0	0.0	0.0	0.0

Site ID	Date	PCE	TCE	TCM	PCA	DCB	Naph	AcPh
CC1	8/18	0.0	0.0	0.0	0.0	0.0	3.5	4.4
CC2	8/18	2.6	0.0	2.6	0.0	0.0	5.3	7.9
CC4	8/18	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CC5	8/18	1.6	0.0	8.1	0.0	0.0	4.4	1.6
CC7	8/18	2.5	2.5	2.5	0.0	0.0	2.5	7.5
CC air	8/18	2.4	0.0	2.4	0.0	1.6	4.1	2.4
LC1	8/18	12.7	0.0	0.0	0.0	8.4	0.0	16.9
LC2	8/18	0.0	0.0	0.0	0.0	8.8	0.0	8.8
GS1	8/18	0.0	0.0	0.0	0.0	0.0	0.0	0.0
GS2	8/18	0.0	0.0	4.0	0.0	0.0	0.0	4.0
NS1	8/20	0.0	0.0	0.0	0.0	0.0	0.0	7.7
NS2	8/20	0.0	0.0	0.0	0.0	0.0	0.0	15.6
NS air	8/20	0.0	0.0	8.2	0.0	0.0	0.0	0.0
DS1	8/20	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DS2	8/20	0.0	0.0	0.0	0.0	0.0	0.0	0.0
OF2	8/20	11.4	1.1	2.9	0.0	1.7	2.3	2.9
OF6	8/20	9.4	0.6	1.3	0.0	1.6	0.0	1.9
OF7	8/20	2.0	0.0	0.0	0.0	1.7	1.2	4.3
JZ2	8/20	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Site ID	Date	PCE	TCE	TCM	PCA	DCB	Naph	AcPh
JZ3	8/20	0.0	0.0	0.0	0.0	0.0	0.0	11.5
OF air	8/20	11.0	3.1	9.4	0.0	2.3	0.0	3.9
CV1	8/22	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CV2	8/22	3.0	0.0	2.0	0.0	0.0	0.0	0.0
CV3	8/22	10.1	21.7	7.2	0.0	0.0	20.2	0.0
CV4	8/22	10.7	0.0	5.4	0.0	5.4	5.4	0.0
CV5	8/22	5.6	11.2	6.6	0.0	2.8	7.5	6.6
CV6	8/22	8.4	6.7	5.1	0.0	3.4	5.1	11.8
CV7	8/22	8.6	5.2	5.2	0.0	4.3	0.0	0.0
CV air	8/22	8.2	0.0	11.4	0.0	4.9	3.3	8.2
GS1	8/23	50.9	0.0	6.4	0.0	10.2	17.8	38.2
GS air	8/23	21.4	0.0	17.1	0.0	6.4	42.8	15.0
BP1	8/23	32.8	0.0	21.9	0.0	16.4	10.9.	32.8
BP2	8/23	31.3	10.4	26.0	0.0	10.4	26.0	15.6
BP air	8/23	32.0	5.1	25.6	0.0	7.7	6.4	7.7
YC1	8/23	37.5	74.9	9.4	0.0	9.4	26.2	28.1
YC2	8/23	0.0	4.2	0.0	0.0	0.0	0.0	0.0
YC air	8/23	35.3	14.1	11.8	0.0	8.2	8.2	7.1
EB1	8/23	0.0	22.7	0.0	0.0	0.0	39.8	0.0

Site ID	Date	PCE	TCE	TCM	PCA	DCB	Naph	AcPh
EB2	8/23	27.2	16.3	16.3	0.0	10.9	38.0	43.5
EB air	8/23	24.3	0.0	2.4	0.0	7.3	8.5	2.4
CV2	8/25	16.7	543.9	20.9	0.0	0.0	251.0	29.3
CV3	8/25	0.0	0.0	6.0	0.0	0.0	3.0	6.0
CV4	8/25	3.7	140.6	9.4	0.0	9.4	56.2	9.4
CV5	8/25	2.1	0.0	6.2	0.0	0.0	4.1	8.3
CV6	8/25	1.9	1.9	4.8	0.0	1.9	0.0	2.9
CV7	8/25	2.7	0.0	9.4	0.0	2.7	2.7	4.0
CV air	8/25	1.8	0.0	1.8	0.0	1.8	0.0	3.7
OF1	9/2	5.3	1.2	0.0	0.0	1.2	8.8	14.7
OF2	9/2	116.3	21.8	20.3	4.4	10.2	14.5	145.3
OF4	9/2	2.9	0.0	0.0	0.0	0.0	10.8	7.2
OF5	9/2	111.2	5.6	11.1	2.6	13.0	11.9	7.4
OF6	9/2	297.6	39.7	29.8	5.0	11.9	29.8	14.9
JZ1	9/2	669.7	15.2	10.7	15.2	12.2	7.6	15.2
JZ2	9/2	26.3	8.2	13.2	0.0	0.0	6.6	6.6
DS2	9/2	39.5	1.7	0.0	2.3	6.8	141.2	2.8
OF air	9/2	4.2	0.0	0.0	0.0	0.0	4.2	1.1
CC1	9/20	0.0	7.2	0.0	0.0	0.0	7.2	7.2

Site ID	Date	PCE	TCE	TCM	PCA	DCB	Naph	AcPh
CC2	9/20	0.0	3.2	0.0	0.0	0.0	3.2	3.2
CC3	9/20	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CC4	9/20	1.9	0.0	1.9	0.0	0.0	0.0	0.0
CC5	9/20	3.4	0.0	1.7	0.0	1.7	3.4	5.1
CC6	9/20	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CC7	9/20	4.2	1.7	3.4	0.0	1.7	1.7	2.5
CC air	9/20	4.2	2.5	12.5	0.0	0.0	1.7	1.7
LC1	9/20	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LC2	9/20	13.4	13.4	0.0	0.0	0.0	100.7	94.0
LC air	9/20	6.5	0.0	0.0	0.0	0.0	0.0	0.0
OF1	9/21	12.8	0.0	12.8	0.0	0.0	12.8	12.8
OF2	9/21	13.6	0.0	20.4	0.0	6.8	8.5	5.1
OF3	9/21	13.1	4.9	6.5	0.0	8.2	48.9	40.8
OF4	9/21	0.0	0.0	0.0	0.0	0.0	0.0	0.0
OF5	9/21	17.3	8.7	21.6	0.0	8..7	108.2	26.0
OF6	9/21	9.8	2.0	4.9	0.0	3.9	3.9	5.9
OF7	9/21	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DS2	9/21	10.1	2.0	3.0	0.0	4.0	3.0	40.3
JZ1	9/21	11.8	2.9	5.9	0.0	2.9	22.1	10.3

Site ID	Date	PCE	TCE	TCM	PCA	DCB	Naph	AcPh
JZ3	9/21	23.7	0.0	23.7	0.0	9.5	66.4	23.7
OF air	9/21	11.8	2.0	9.8	0.0	2.0	0.0	0.0
YC1	9/24	7.3	0.0	18.2	0.0	3.6	3.6	3.6
YC2	9/24	5.8	2.3	14.0	0.0	2.3	2.3	3.5
YC air	9/24	4.6	2.3	13.9	0.0	2.3	7.0	2.3
EB1	9/24	2.2	0.0	3.3	0.0	1.1	1.1	1.1
EB2	9/24	2.6	2.6	0.0	0.0	2.6	0.0	0.0
EB air	9/24	3.9	0.0	15.7	0.0	0.0	5.9	0.0
GS1	9/25	0.0	0.0	0.0	0.0	0.0	5.5	2.2
GS2	9/25	4.4	0.0	7.3	0.0	0.0	7.3	5.8
GS air	9/25	3.6	1.8	7.2	0.0	0.0	7.2	1.8
NS1	9/25	3.7	0.0	1.9	0.0	3.7	18.7	18.7
NS2	9/25	3.7	1.8	2.7	0.0	1.8	1.8	5.5
NS air	9/25	3.1	0.0	14.1	0.0	0.0	4.7	4.7
CV2	9/26	0.0	0.0	0.0	0.0	0.0	8.8	0.0
CV3	9/26	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CV4	9/26	4.4	2.2	4.4	0.0	0.0	0.0	3.3
CV5	9/26	0.0	0.0	0.0	0.0	6.4	0.0	3.2
CV6	9/26	4.4	0.0	0.0	0.0	2.9.	0.0	0.0

Site ID	Date	PCE	TCE	TCM	PCA	DCB	Naph	AcPh
CV7	9/26	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CV air	9/26	8.6	3.5	13.8	0.0	0.0	0.0	5.2
CV porch	9/26	5.8	3.9	14.5	0.0	3.9	6.8	0.0
BP1	9/29	0.0	0.0	3.9	0.0	0.0	5.9	3.9
BP2	9/29	0.0	0.0	17.9	0.0	0.0	0.0	3.6
BP air	9/29	2.4	0.0	14.4	0.0	0.0	2.4	2.4

isotope (75.53% natural abundance) versus the chlorine-37 isotope (24.47% natural abundance) in the four substituted positions. Fig. 4.3 compares the mass spectrum of PCE as found at a retention time of about 23.7 minutes in the September 2nd chromatogram from Old O Field Condo 5 (top panel) to that from the National Bureau of Standards (NBS) spectral library (lower panel). The cluster of peaks near mass 166 represents the molecular ion. The peak clusters at 129/131 m/z, 94 m/z and 59 m/z represent loss of one, two and three chlorines, respectively. The match quality between the two spectra was 97%.

Perchloroethylene was detected at all three instrumented colony settings (Fig 4.4). Levels occurring in some Old O Field hive atmospheres were dramatically higher, however, for samples collected during the September 2nd sampling period. Note that an expanded y-axis range was required to accommodate the Old O Field data within the graph panel.

Old O Field colonies JZ1, OF2, OF5 and OF6 had PCE values well above any others encountered throughout the rest of the summer -- 669.7, 116.2, 111.2 and 297.6 ions per liter, respectively. Most others were below 10 ipl. Ambient air collected during the same period at Old O Field registered a PCE level of 4.2 ipl. Clearly, honey bee foragers encountered PCE somewhere in the vicinity and brought it back inside the hives.

PCE that made its way into the sorption tube could have been released from contaminated water for evaporative cooling, contaminated nectar, contaminated pollen, contaminated resins (for propolis), or contaminated forager bees themselves (e.g., in or on body tissues or contents such as unextruded beeswax in their wax glands). The large differential between the ambient air and hive levels suggests that PCE was being transported to the hive from a condensed phase source. Honey bees prefer to gather water from standing pools rather than from riverbanks where the water is in motion. Open storage vessels or pools in areas of contaminated surface soil could give rise to an oily film containing a concentrated source of PCE and other lipophilic contaminants. If honey bees had to walk through or penetrate a contaminant film, PCE adhering to the exoskeleton could easily volatilize once back in the warm hive.

Ground water studies that preceded capping activities at the Old O Field site have documented a PCE presence in wells installed in both the upper confined aquifer and the water table aquifer (2). For example, a water sample drawn on November 22, 1993, from Well EX-8 in the water table aquifer at Old O Field showed a PCE concentration of 1030 ug/L.

We suspect that the colonies where high PCE levels were measured were subjected to a chemical stress. Queen loss was observed in 50% of the colonies at Old O Field. When these hives were later opened for inspection, no queens were evident. It is unknown whether the queens abandoned the hive box due to "unpleasant" chemical odors or perished. Hive abandonment is distinctly possible; one queen from the Old O Field free-standing hives was found outside in August, crawling on the ground. When the hives were ranked on the basis of relative PCE levels, queen loss was associated with colonies hiving high PCE levels:

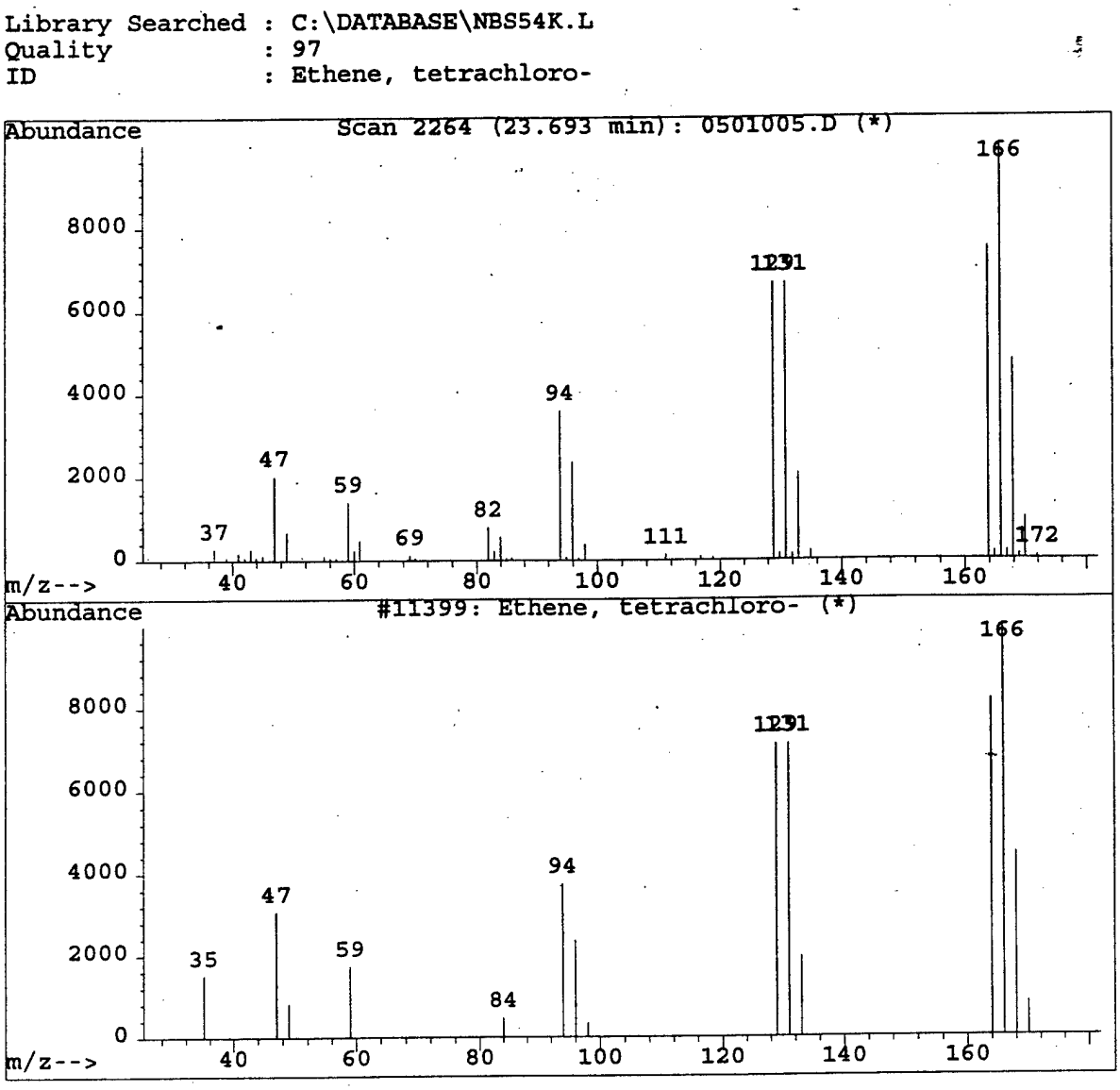


FIGURE 4.3. Comparison of Mass Spectrum for Perchloroethylene in Old O Field Condo 5 on September 2nd to NBS Library Spectrum

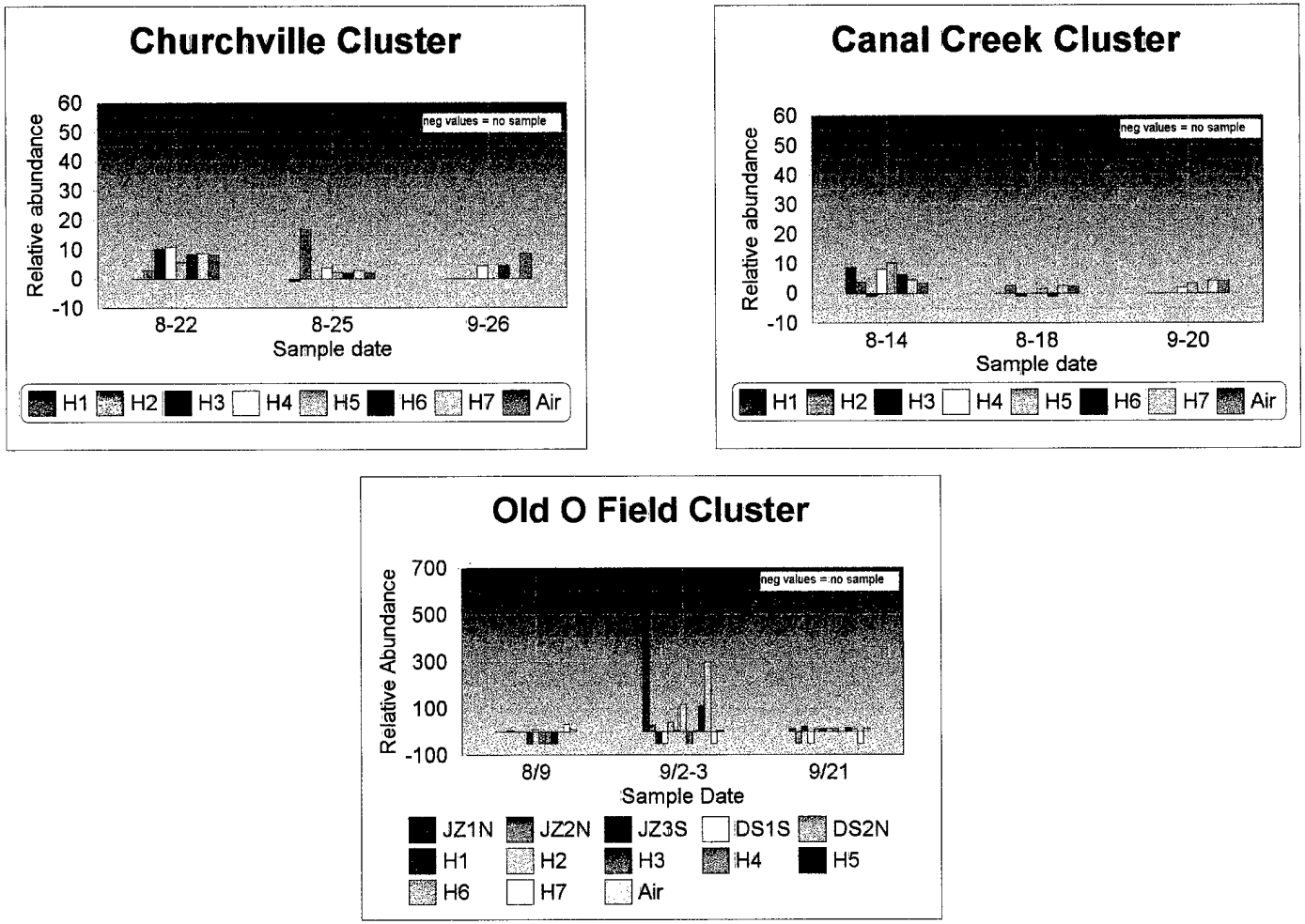


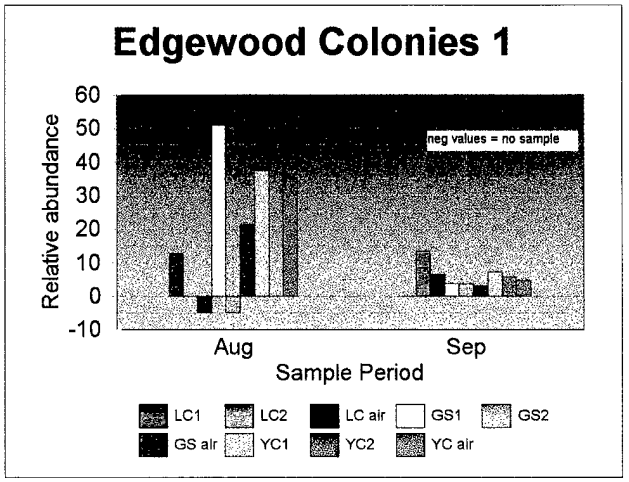
Figure 4.4. Perchloroethylene (PCE) Levels in 1996 Condo Cluster Samples

Hive Sampled	PCE level (ipl on 2-Sep-96)	Queen Present?
JZ1	669.7	No
OF6	297.6	No
OF2	116.3	No
OF5	111.2	No
DS	239.5	Yes
JZ2	26.3	No
OF1	5.3	Yes
OF4	2.9	Yes

PCE levels at O Field on September 2nd illustrate how variable contaminant exposures can be with foraging honey bees. Hives adjacent to those showing the anomalously high readings had PCE results in the 3 to 6 ipl range. Large fluctuations between hive levels indicate that the PCE sources are probably "hot spots" scattered about in the foraging area rather than a uniform blanket of contamination over the entire terrestrial setting. Only some of the hives had forager populations that spent time at the hot spots. Others did not find them or avoided them.

PCE was also found at most sites in the residential/recreational/office areas of Edgewood (Fig. 4.5). The frequency of detection in the more residential areas was not surprising as PCE is practically ubiquitous in the environment. Its use as the principal dry cleaning fluid and as a popular degreasing agent have led to its wide dispersal in the environment, even in home settings. In most instances, the levels found in hive atmospheres were not greatly different from that registered in the ambient air immediately outside the hive boxes. Thus, hive PCE levels were often simply a reflection of outside air. The highest ambient air level of PCE (35.3 ipl) was associated with the August sampling near the Youth Center. Perhaps PCE was off-gassing from some material used in the remodeling of the adjacent Post Exchange. Higher ambient levels of PCE were also noted at G Street (50.9 ipl), Beach Point (32.0 ipl) and the East Branch of Canal Creek (24.3 ipl). The proximity of maintenance buildings and former operations to these areas provide probable historical sources.

Ambient and hive PCE levels for all sites were depressed in the late September sampling periods, consistent with the seasonal temperature fluctuations that would reduce PCE's soil vapor pressure and solubility. The high levels of PCE seen on September 2nd in the Old O Field hives were gone by September 21st; hive readings were essentially the same as the ambient air. The influx of new PCE-contaminated media must have been outweighed by offgassing from the hive, die-off of contaminated bee tissue and consumption of hive stores. In addition, re-queening and replacement of some of the most severely exposed colonies may have offset earlier PCE build-up.



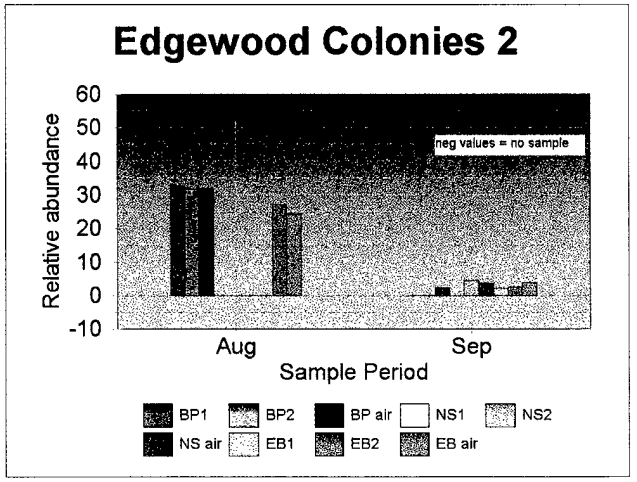


Figure 4.5. Perchloroethylene (PCE) Levels in 1996 Edgewood Colonies

4.3.3 Trichloroethylene (TCE)

Trichloroethylene or trichloroethene, $Cl_2C=CHCl$, saw use at APG in manufacturing plants, munitions-filling plants, machine and maintenance shops (1). Often waste TCE was disposed of through sewer systems. Castleton Air Force Base in the Central Valley of California furnishes a noted example where 100 gallons of TCE disposed into a sewer system over 25 years contaminated an entire aquifer (3). The presence of TCE was confirmed through spectral matching such as that shown in Fig. 4.6 where the mass spectrum of TCE in hive OF6 (top panel) is compared to the NBS library spectrum for TCE (lower panel) at a 95% match quality. The molecular ion cluster at 130 m/z was used for quantification.

TCE made its first strong appearance in hive atmospheres sampled in the latter third of August (Figs. 4.7 and 4.8). Interestingly, the two highest readings, 543.9 and 140.6 ipl, were recorded at the Churchville reference site on August 25th in hives CV2 and CV4, respectively. TCE was also seen at elevated readings in the August 23rd sample from the Youth Center YC1 hive (74.9 ipl) and in the September 2nd sample from Old O Field hive OF6 (39.7 ipl). Both East Branch Canal Creek hives showed evidence of bioavailable TCE in the August sampling period. Their levels were in relatively close agreement at 22.7 and 16.3 ipl, respectively.

Once again, the high TCE hits were orders of magnitude greater than the ambient air level suggesting a condensed phase source of contamination. The ambient air at Churchville showed no measurable TCE. The bees must have found a contaminated medium sufficiently distant from the ambient air sampler that it was not picked up in the near the condo cluster. Evidence of robbing behavior by the Churchville bees suggests that they might have picked up the TCE in an area where the owner stored unused hive boxes for his personal colonies. Storage in proximity to cleaning agents could give rise to the observed results.

If the TCE source was not being brought into the hive in a contaminated condensed phase (water, nectar, pollen, etc.), but was being continually bioaccumulated over time, one would expect to see a monotonic rise in hive atmosphere values. That was not observed. As was true for PCE, the TCE levels did not increase on the next sampling date, but returned to background levels. This, too, suggests that foragers were collecting at hotspots on an episodic basis.

4.3.4 Tetrachloromethane (TCM)

Tetrachloromethane, CCl_4, better recognized under the name of carbon tetrachloride, was used in a number of historical activities at APG - as a decontaminating and cleaning agent and as a raw material for impregnite and chloroacetophenone. The molecular ion for TCM should exhibit an isotopic signature at 152 m/z. With electron impact sources (EI), such as our MS instrument uses, the largest mass seen is typically a CCl_3 fragment at 117/119 m/z. This was

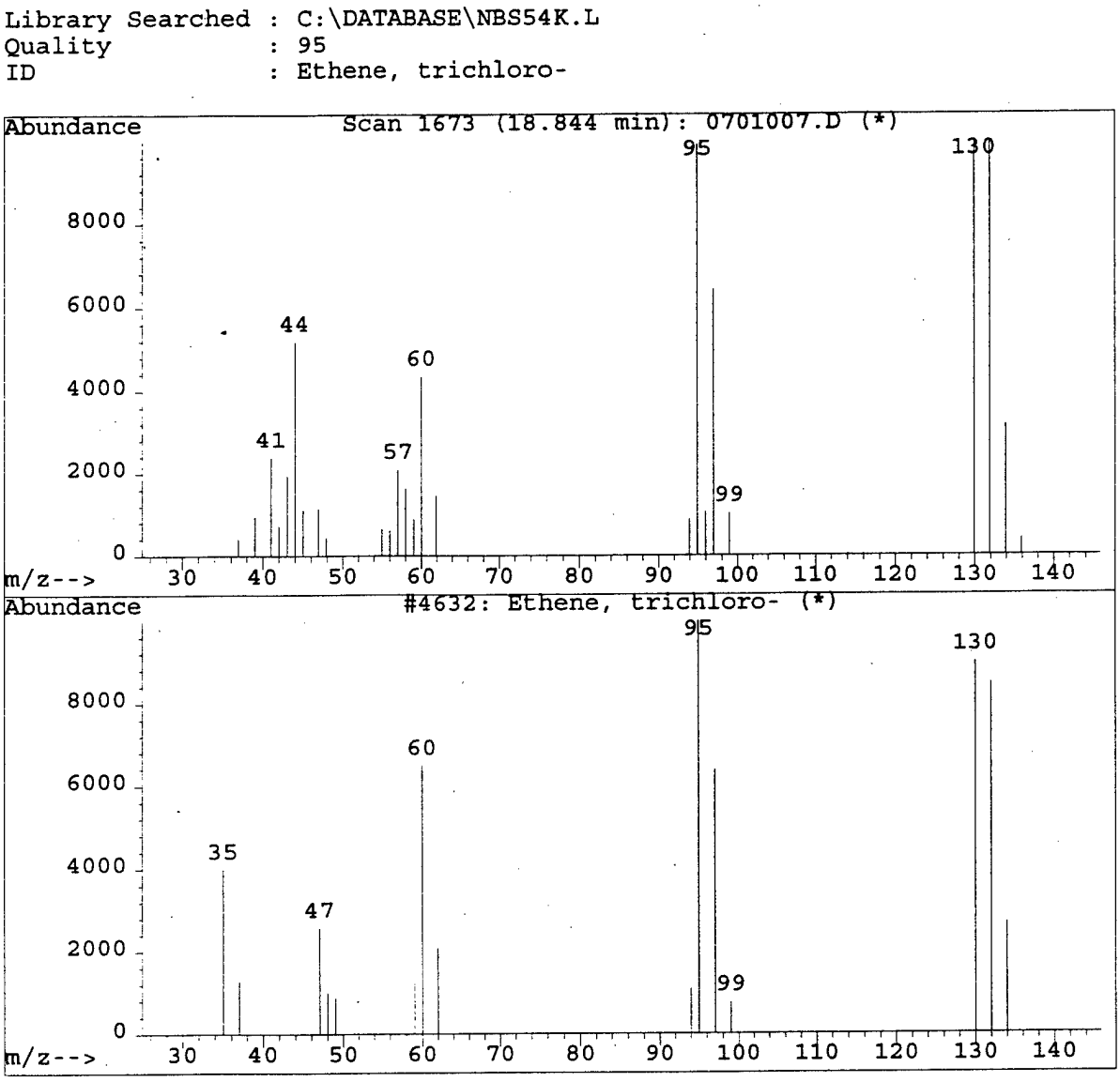
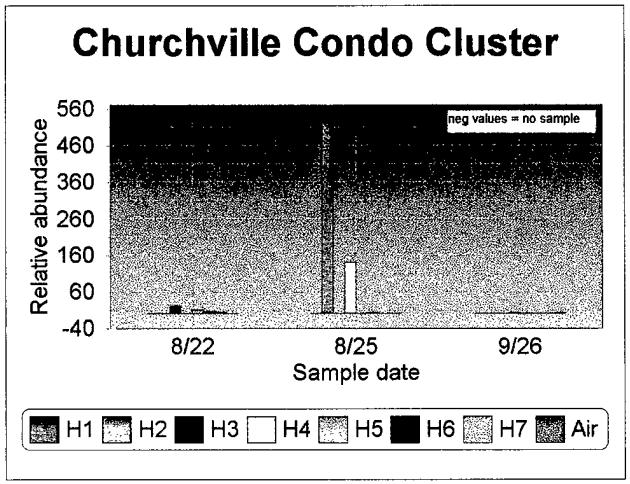
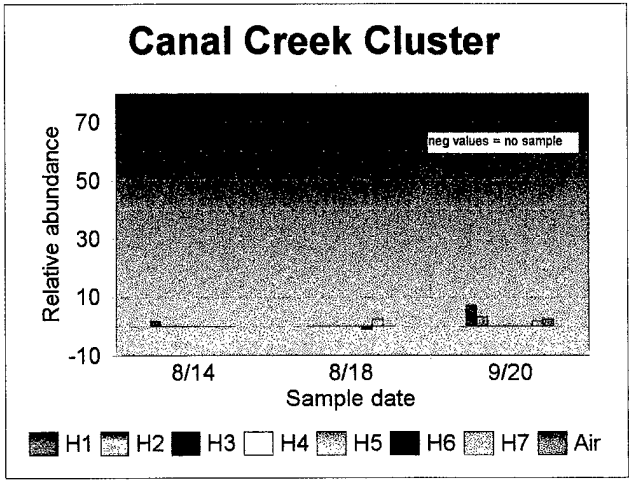


FIGURE 4.6. TCE in Old O Field Condo 6 (top panel) Compared to NBS Library Spectrum (bottom panel)





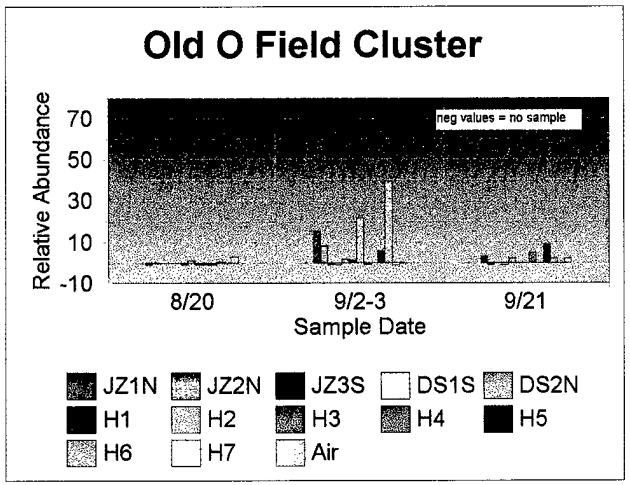
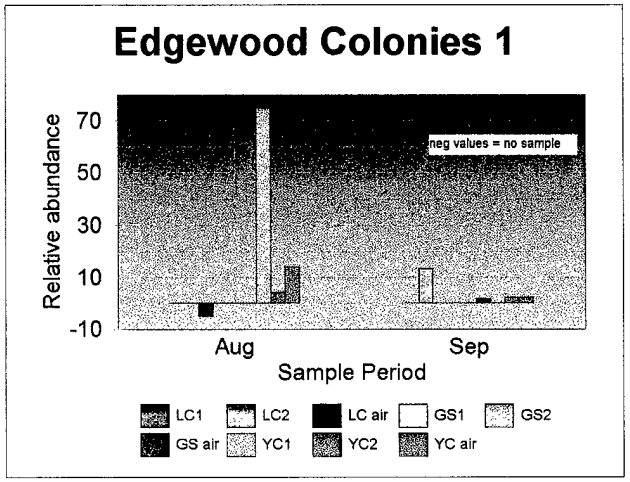


Figure 4.7. Trichloroethylene (TCE) Levels in 1996 Condo Cluster Samples



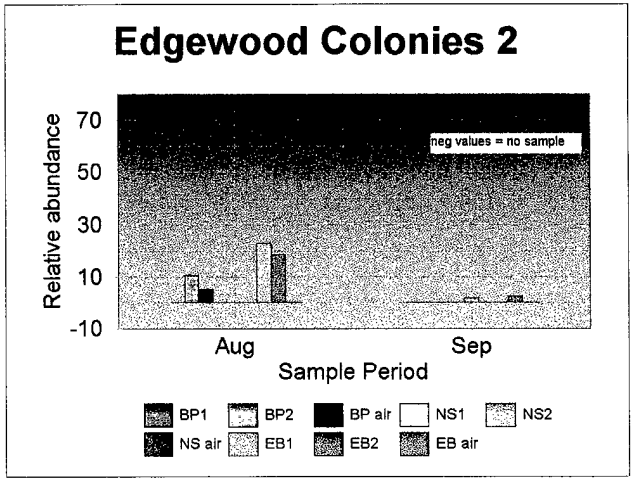


Figure 4.8. Trichloroethylene (TCE) Levels in 1996 Edgewood Colonies

observed at a retention time of about 16.6 minutes on our column. Fig. 4.9 demonstrates a 74% quality match achieved between a September 29th ambient air sample from Beach Point and the NBS spectrum for TCM. TCM always co-eluted with other compounds in the sample matrix so we used the abundance of the 117/119 m/z pair for quantification purposes.

While the mass spectral match seems reasonably good for TCM, it should be taken with some caution because there is a second compound of concern at APG that gives an almost identical mass spectral signature on our instrument, namely, trichloronitromethane or chloropicrin, CCl_3NO_2. Chloropicrin was used in formulating the CNS tear gas mixture -- 23% chloroacetophenone, 38.5% chloroform and 38.5% chloropicrin (1). It gives a fragmentation practically identical to TCM; the nitro group separates from the carbon leaving a CCl_3 fragment trio at 117/119/121 m/z and a CCl_2 fragment pair at 82/84 m/z with congruent relative intensities. The only significant difference in the two mass spectra as seen by our MS instrument is a small peak at 59 m/z for TCM (a Cl-C-C recombination) vs. a slightly larger peak at 61 m/z for chloropicrin (that represents a Cl-C-N fragment). Given the levels at which we capture trace level contaminants in hive atmospheres and the low relative abundance of the distinguishing mass peaks, our MS is blind to the difference between these two compounds. It assigns equal match qualities to both. TCM was selected as the more likely candidate for our peaks since: 1) TCM exhibited more widespread use in past APG activities, 2) the retention time on the GC column is consistent for TCM relative to other chlorinated hydrocarbon homologs, and 3) there was no evidence of a peak at 61 m/z as would be seen for chloropicrin. A soft ion source, such as chemical ionization, could easily distinguish TCM from chloropicrin on the basis of molecular ions should resolution of this uncertainty be of concern.

TCM was detected at all honey bee colony placements (Figs. 4.10 and 4.11) except Lauderick Creek. There are, however, several qualitative differences between the pattern shown for TCM occurrence compared to that for perchloroethylene and trichloroethylene. First, none of the hives showed TCM levels that exceeded typical ambient air readings by several orders of magnitude. Note that all five graph panels for TCM in Figs. 4.10 and 4.11 use the same y-axis scaling; no scale extensions were needed for large TCM hits. Second, note that the ambient air samples are fairly prominent throughout the data set, an indication that TCM is regularly found in the gas phase. Third, the seasonal reduction that was noted for PCE and TCE is not necessarily seen with tetrachloromethane. The values found in September samples are comparable in level to those seen in earlier sample periods at all locations except the Churchville reference site. Thus, TCM appears to represent a more constant contaminant from the perspective of chronic exposure.

The highest reading noted for TCM was 29.8 IPL from Old O Field Condo 6 on September 2nd. On the next sampling date, September 21st, most of the Old O Field hives showed significant levels of TCM still present. Thus, TCM did not disappear from the hive environment as rapidly as PCE and TCE did.

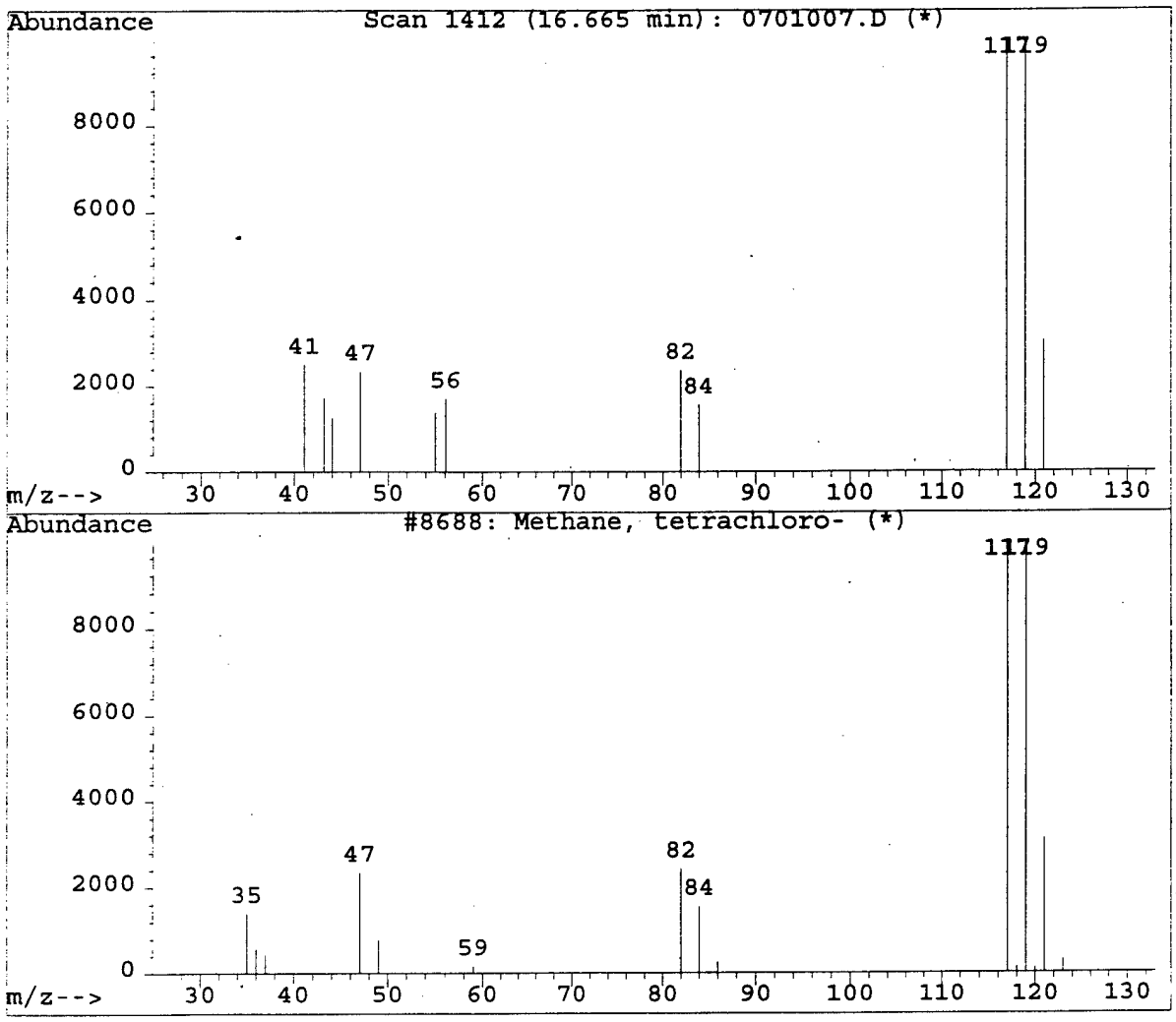


FIGURE 4.9. TCM in September 29, 1996, Beach Point Ambient Air (top panel) Compared to NBS Library Spectrum (bottom panel)

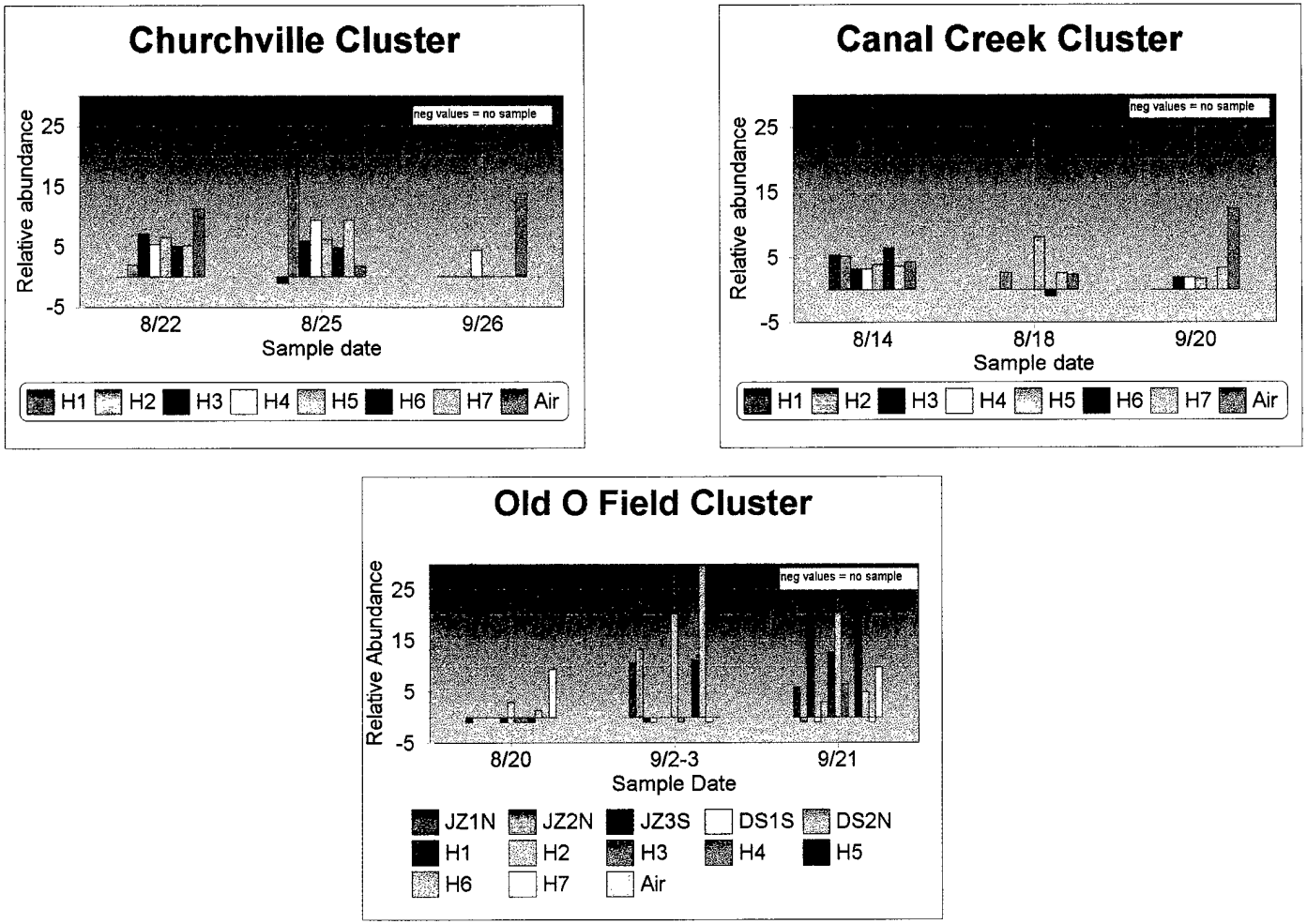
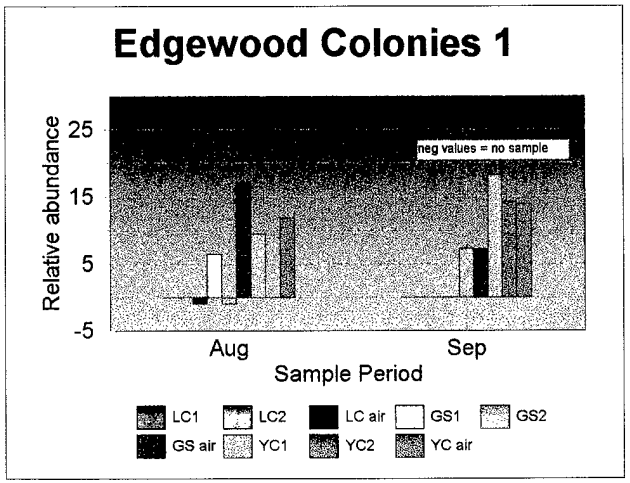


Figure 4.10. Tetrachloromethane (TCM) Levels in 1996 Condo Cluster Samples



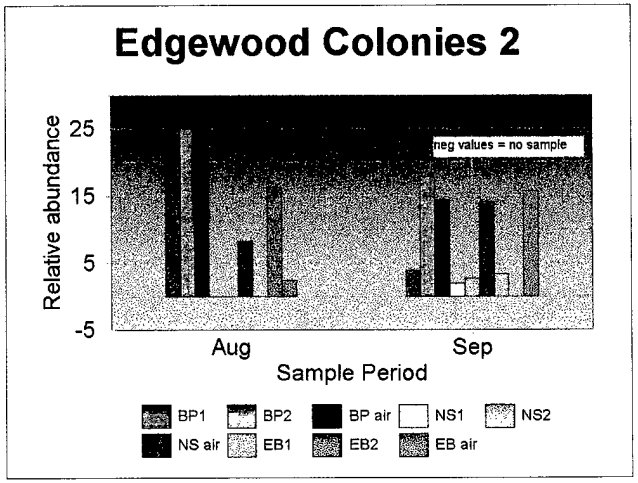


Figure 4.11. Tetrachloromethane (TCM) Levels in 1996 Edgewood Colonies

TCM was the most uniformly observed contaminant among the stand alone colonies distributed throughout the Edgewood area. Beach Point had a strong TCM presence during the August sampling period, all three samples recording levels above 20 ipl. Ambient air levels near 15 ipl were recorded at G Street, the Youth Center, the National Guard site and the East Branch Canal Creek sites. Given the consistent levels observed and noting that some of these sites are not located near water courses, soil vapor seems a likely source of TCM in many APG areas.

4.3.5 Hexachloroethane (PCA)

Hexachloroethane or perchloroethane, Cl_3CCCl_3, is a completely chlorinated alkane. Being significantly higher in molecular weight (236.74 g/mol), it is slower to elute from our GC column, appearing at about 35 minutes. Like TCM, the molecular ion is not present in an EI source mass spectrum. The largest fragment observed is an isotopic cluster at 201 m/z which represents the molecule with one chlorine atom missing. Confirmation of its presence in hive atmospheres is illustrated with Fig. 4.12 which compares its appearance in the September 2nd Old O Field Condo 5 to that in the NBS spectral library. The peak abundance at 201 m/z was used for quantification.

PCA only appeared at the Old O Field site, and even there it was at fairly low levels on a single date (Figs. 4.13 and 4.14). This result is consistent with ground water studies done at APG in that PCA is not reported near the surface in the Canal Creek study (1) but does appear in the Old O Field ground water (2) in wells MW-4-1A (25-Feb-94), OF-6A(R) (20-May-93), EX-3A (9-Sep-93), EX-4A (15-Apr-93), EX-5 (13-Sep-93), and EX-8 (3-Sep-93). PCA is not as widely used as a solvent as other chlorinated hydrocarbons because its volatility means longer drying times after use. The Merck Index (4), however, cites its use in explosives, and Army staff noted its use in military screening smoke, which may explain its presence in the Old O Field area. PCA was detected in the JZ1, DS2, OF2, OF5 and OF6 Old O Field colonies on September 2nd. The highest reading of 15.2 ipl was registered in JZ1. Because the ambient air sample collected at the same time showed no detectable PCA, it is reasonable to assume that PCA became bioavailable to the bees through a source of contaminated water, was picked up in nectar or pollen foraging, or encountered in a transient plume.

4.3.6 1,4-Dichlorobenzene (DCB)

1,4-Dichlorobenzene or *para*-dichlorobenzene, $C_6H_4Cl_2$, has seen use as a solid insecticide for years. One trade name for DCB is Paramoth, testimony to its efficacy in killing moth larvae that attack furs, woolen goods and rugs. DCB was detected in hive atmospheres even before any hives were emplaced at APG for the 1996 field season. It appeared in stand-alone hives at Churchville in early June while we were collecting pre-APG chemical baseline data. When questioned about pesticide use on the Churchville site, David Simmons, the hobbyist beekeeper/owner, indicated he had treated unoccupied hive boxes in January with DCB for

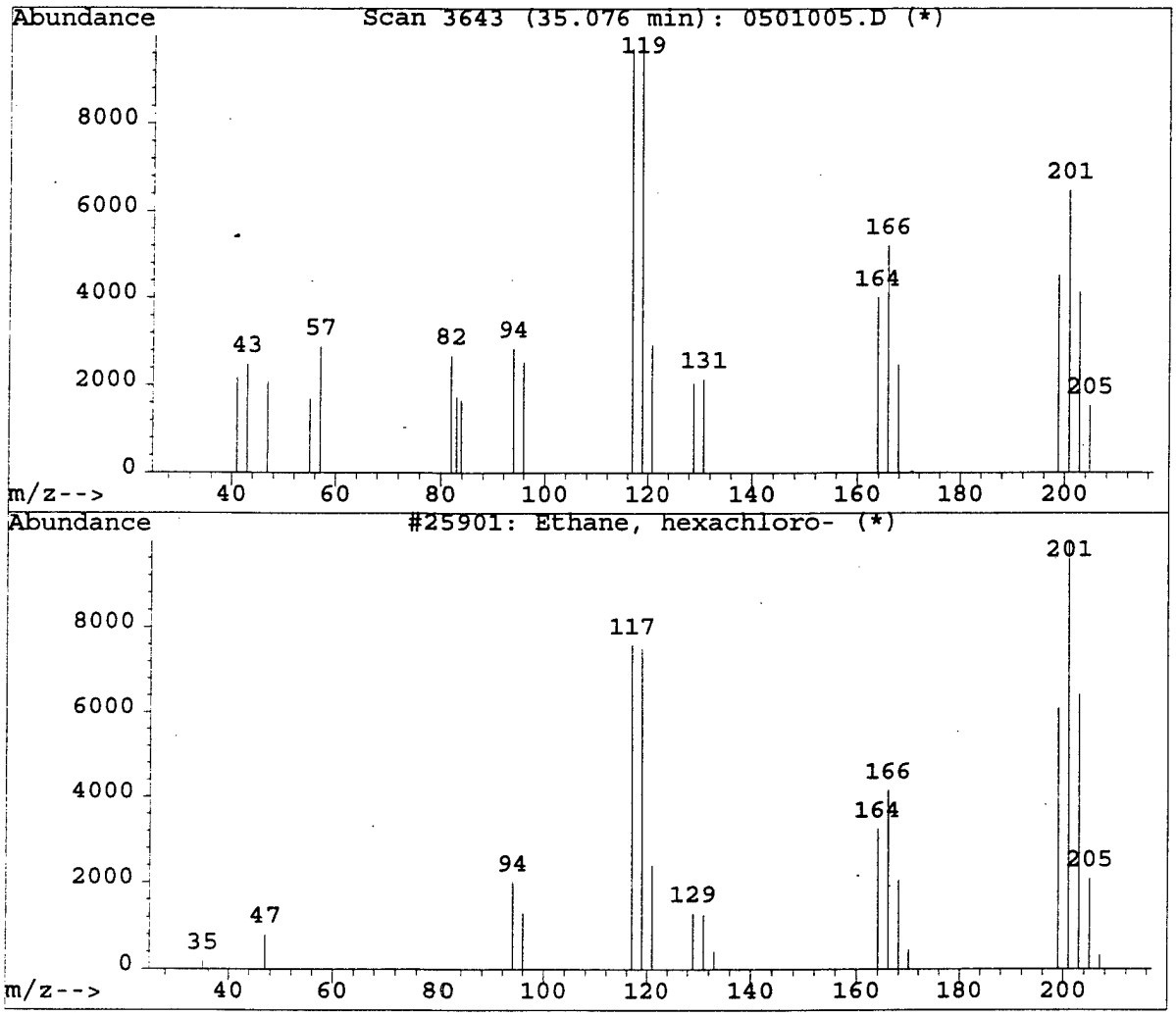


FIGURE 4.12. PCA in September 2, 1996, Old O Field Condo 5 (top panel) Compared to NBS Library Spectrum (bottom panel)

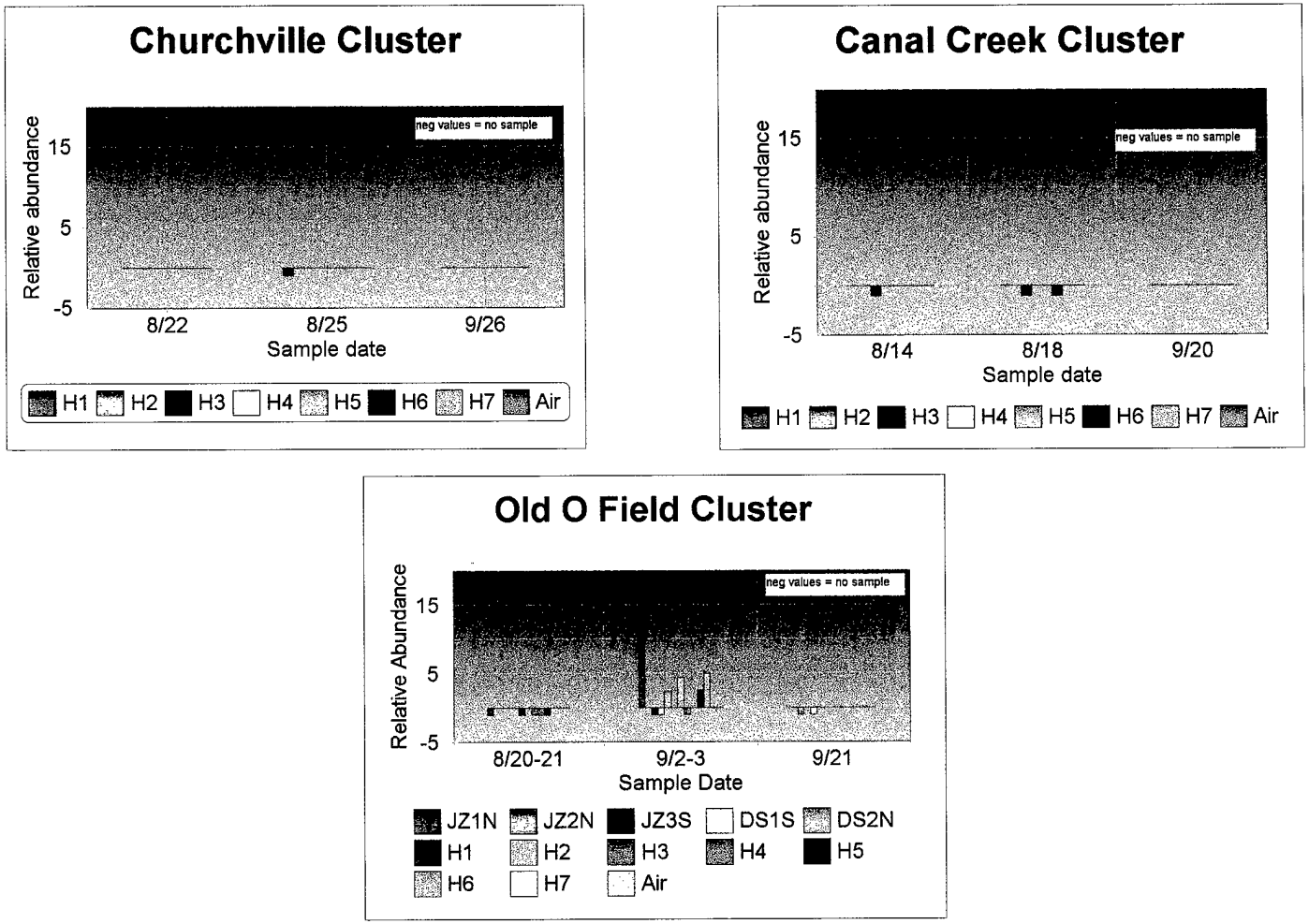


Figure 4.13. Hexachloroethane (PCA) Levels in 1996 Condo Cluster Samples

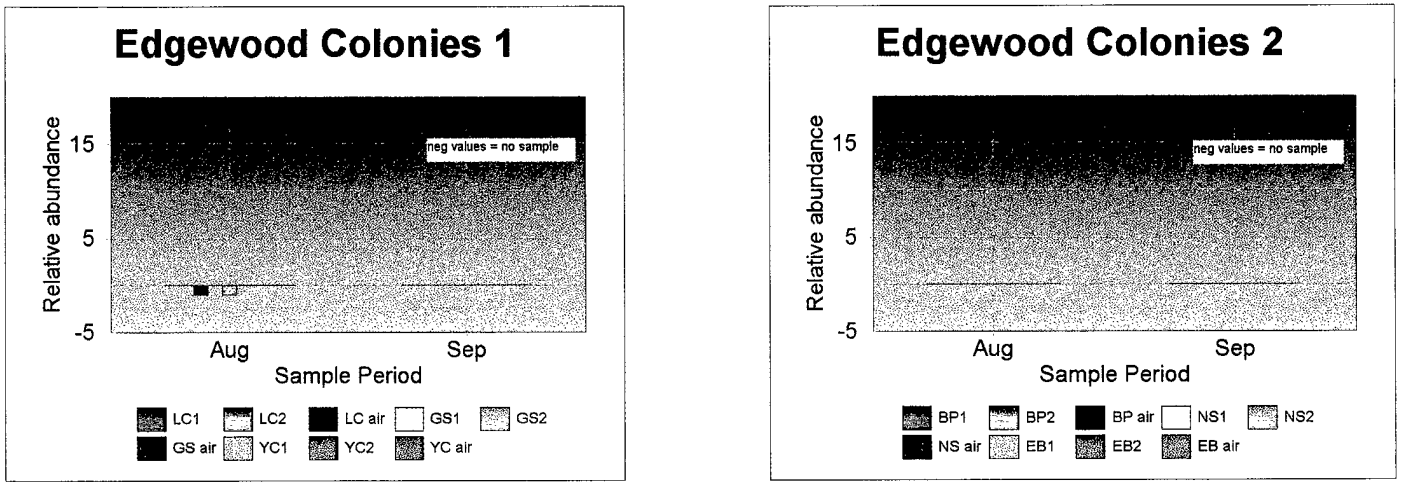


Figure 4.14. Hexachloroethane (PCA) Levels in 1996 Edgewood Colonies

wax moths. Evidently bees from active colonies subsequently robbed his empty boxes for hive stores and picked up a dosage of DCB.

DCB gives a stable molecular ion cluster at 146 m/z and displays a retention time in our GC column of 32.9 minutes. Bioavailability of DCB at APG is confirmed in Fig. 4.15 which compares its mass spectrum in the September 2nd Old O Field Condo 5 atmosphere to the NBS library spectrum. This was scored as a 95% match quality. The 146 m/z was used for quantification purposes.

DCB was present at low levels at all 1996 field sites (Figs. 4.16 and 4.17). In all cases, hive levels were slightly higher than ambient air values, so some short term bioconcentration may have taken place. Easily detectable ambient air levels of DCB were recorded at G Street, the Youth Center, Beach Point and the East Branch Canal Creek. Only a trace amount was seen in air samples from the West Branch Canal Creek site.

At Old O Field and Churchville, DCB concentrations inside the hives persisted into the late September sampling period. The levels decreased at all other sites over the same period. This suggests that there are condensed phase sources of DCB for O Field and Churchville that provide ongoing exposure. The use of DCB to treat wax moths at Churchville has already been noted, but none of the hives brought from Montana had been treated with DCB. The presence of DCB in ground water at Old O Field has been documented in wells OF-6A(R), EX-5, EX-6A and EX-9 (2).

4.3.7 Naphthalene (Naph)

Naphthalene, $C_{10}H_8$, is the smallest member of the polycyclic aromatic hydrocarbons (PAH's). It can be purchased over the counter in pure form as moth balls. It is also found in petroleum fuels; naphthalene comprises about 7% of typical diesel fuel residuals from spills (5). Naphthalene is also produced during incomplete combustion of organic fuels. Burning burlap in the smoker used for management of honey bees is probably a good naphthalene source. For this reason, we refrained from sampling hives on the same day we worked them with smoke. Given this rich variety of potential non-military naphthalene sources, any contamination in hives arising from naphthalene must be carefully evaluated.

While naphthalene has a high melting point (218 °C), it volatilizes sufficiently at room temperatures to be considered a semi-volatile organic contaminant. It is a very stable molecule that survives electron impact ion sources intact. The naphthalene molecular ion can be seen in Fig. 4.18 at 128 m/z. Since there are no chlorine atoms in naphthalene, the isotopic finger print is rather simple - the fundamental peak at 128 and a 10% peak at 129 M/Z for molecules containing one carbon-13 isotope. This figure demonstrates a 95% quality match between the naphthalene signature in the Old O Field DS2 hive on September 2nd to the NBS library spectrum. The molecular ion peak at 128 m/z formed the quantitation basis.

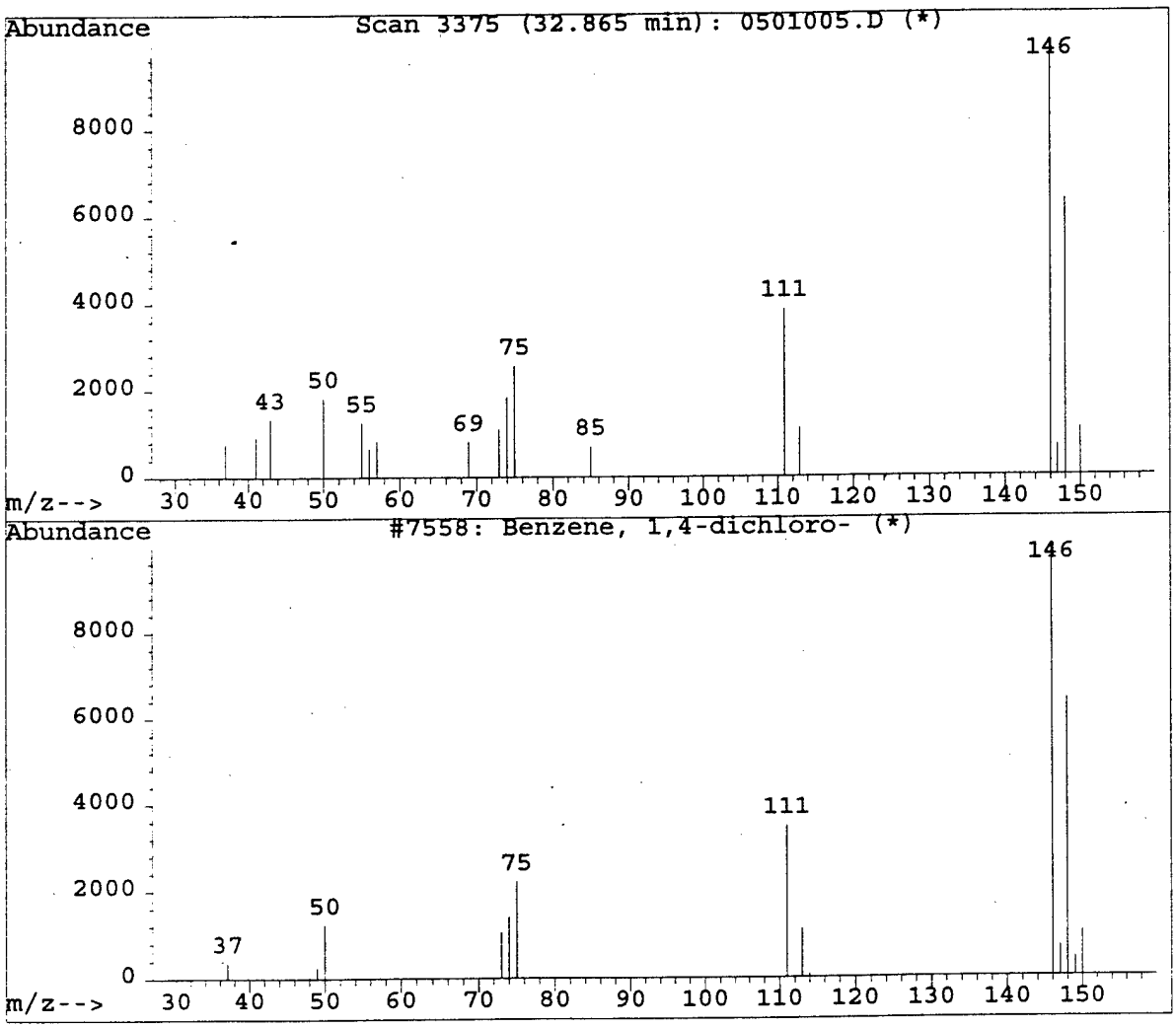


FIGURE 4.15. DCB in September 2, 1996, Old O Field Condo 5 (top panel) Compared to NBS Library Spectrum (bottom panel)

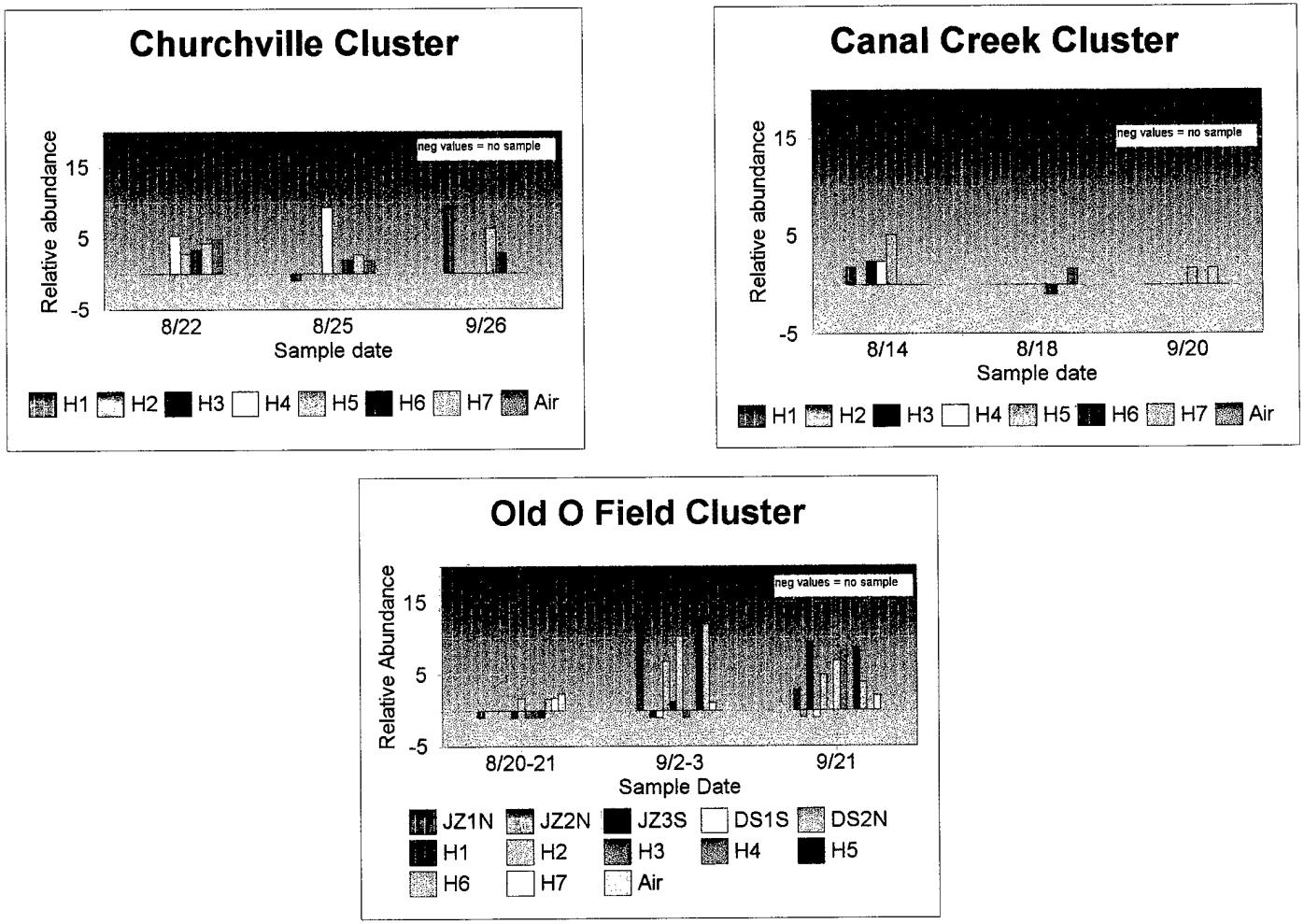
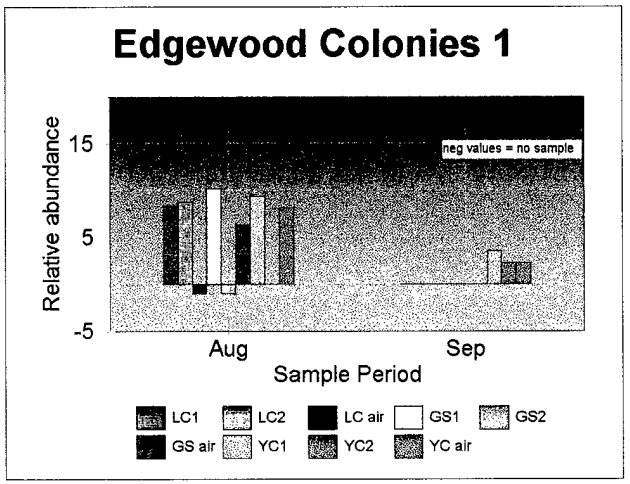


Figure 4.16. Dichlorobenzene Levels in 1996 Condo Cluster Samples



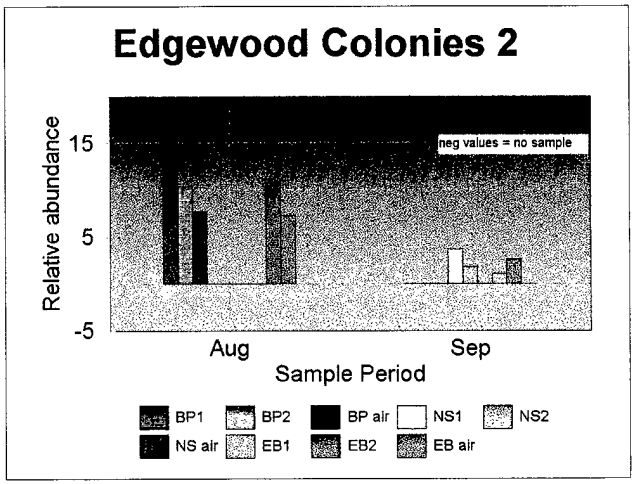


Figure 4.17. Dichlorobenzene Levels in 1996 Edgewood Colonies

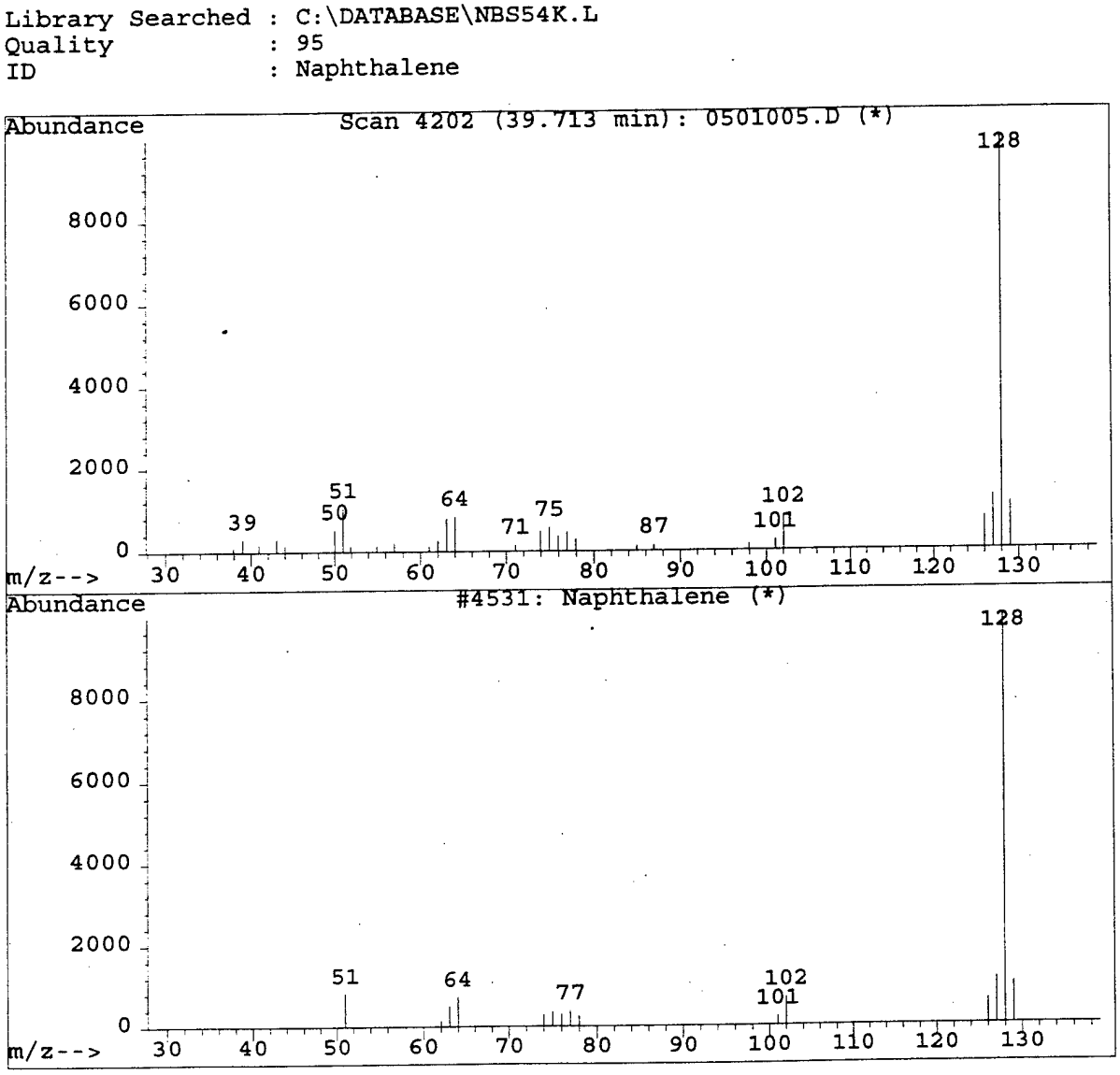


FIGURE 4.18. Naphthalene in September 2, 1996, Old O Field DS2 Hive (top panel) Compared to NBS Library Spectrum (bottom panel)

Naphthalene was detected at all APG field sites (Figs. 4.19 and 4.20). Levels were fairly low, however, at the West Branch Canal Creek condo cluster, G Street, the Youth Center, Beach Point, the National Guard site and East Branch Canal Creek. A small degree of bioconcentration may be evident in the Youth Center hives, the Beach Point hives and the East Branch Canal Creek hives. The most consistent APG ambient air readings for naphthalene were associated with G Street (42.8 ipl), the Youth Center (8.2 ipl) and East Branch Canal Creek (8.5 ipl).

Churchville showed a dramatic naphthalene spike of 251.0 ions per liter in Hive 2 when sampled on September 25th . Two hives away, the colony in Hive 4 registered 56.2 ipl. The remaining five colonies and the ambient air held only very low levels. On other sampling dates, the entire Churchville condo cluster showed negligible naphthalene presence. The two spikes have yet to be explained. The most logical cause would seem diesel contaminated water used by these two hives for evaporative cooling. The magnitude of the hits suggests a condensed phase source. The same set of observations and arguments applies to Lauderick Creek where Colony 2 recorded a single hit with a hive atmosphere level of 100.7 ipl on September 20th.

The site bearing evidence for the most widespread naphthalene contamination was Old O Field. While no naphthalene was noted on the initial sampling period in August, the frequency and magnitude of hits became more significant in September. The DS2 and OF6 colonies exhibited levels of 141.2 and 29.8 ipl, respectively, on September 2nd. The JZ1, JZ3, OF3 and OF5 hives registered values of 22.1, 66.4, 48.9 and 108.2 ipl on September 21st. Since ambient air levels were low on both of those occasions, a condensed phase source of naphthalene is probable. Previous ground water analysis has shown low level naphthalene contamination in wells MW-4-1A, OF-6A(R), EX-5, EX-6A and EX-9 (2). A more plausible source of naphthalene could be standing pools of surface water contaminated with diesel from the capping equipment. Note that the JZ3 colony was one of two stand alone hives placed in the swale to the south of the water treatment plant. Thus, the contamination source was sufficiently extensive to be bioavailable to both the condo cluster on the northeast side of the Old O Field landfill and the hives behind the water treatment plant.

The residence time of naphthalene in the hives appears less than 18 days because the large September 2nd hit in DS2 has essentially subsided back down to ambient air levels by the September 21st sampling date.

4.3.8 Acetophenone (AcPh)

We followed acetophenone, $C_6H_5COCH_3$, in APG samples because it was a possible starting material for the production of the tear gas CN (2-chloroacetophenone or $C_6H_5COCH_2Cl$) and is mentioned as a contaminant of concern in the West Branch Canal Creek Marsh Study Area (6). A name more descriptive of its chemical structure is methyl

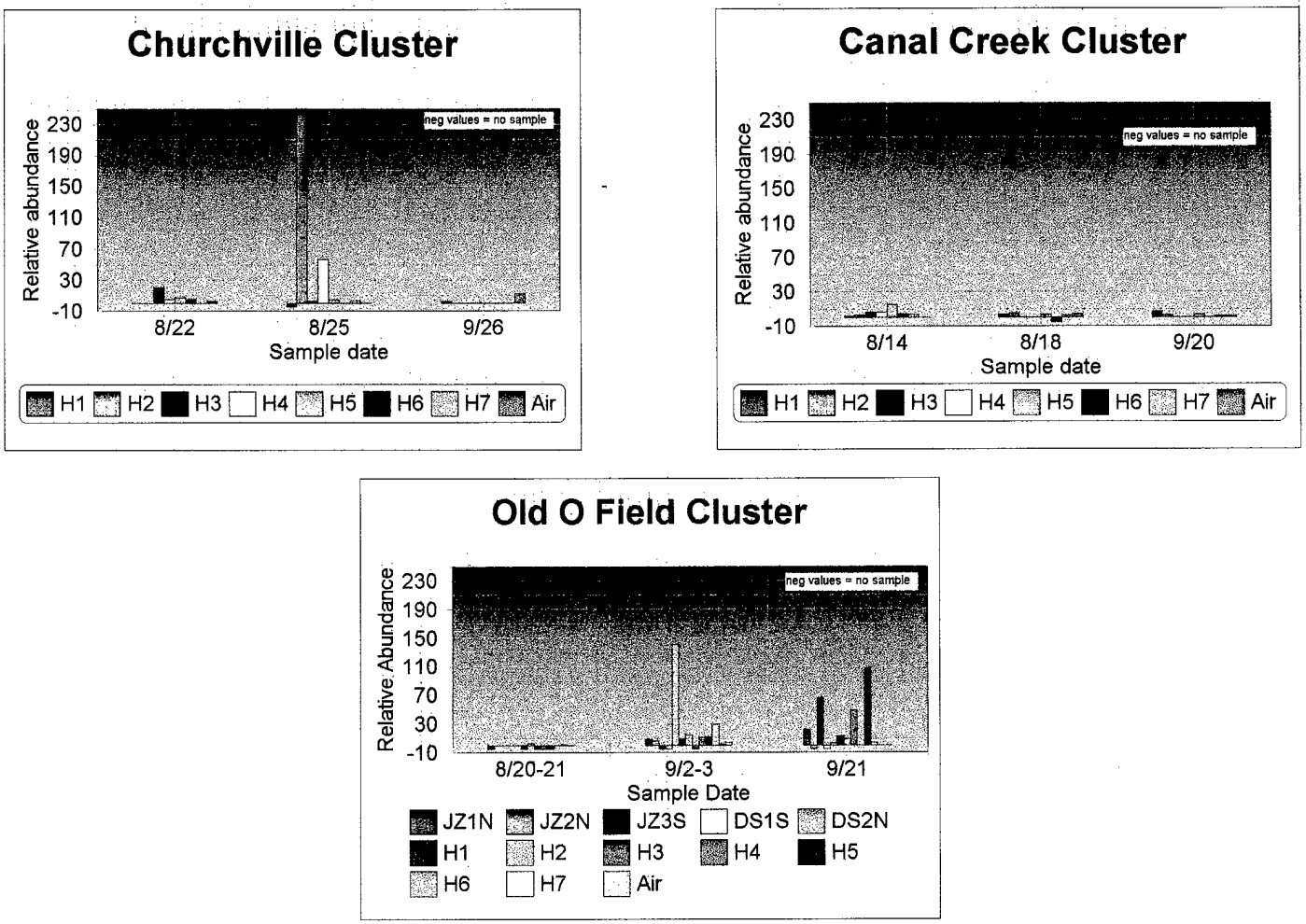
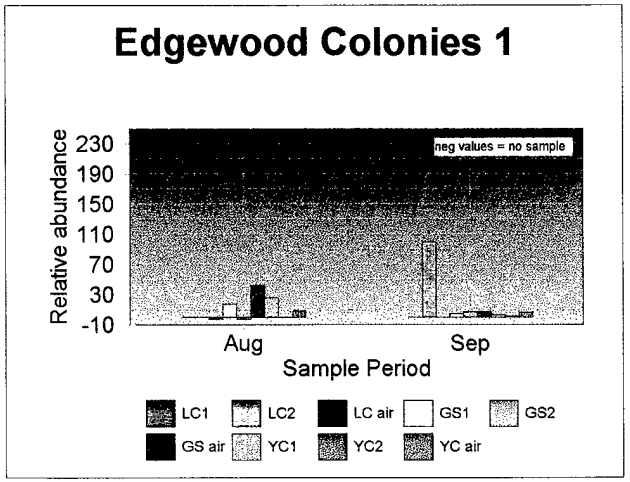


Figure 4.19. Naphthalene (Naph) Levels in 1996 Condo Cluster Samples



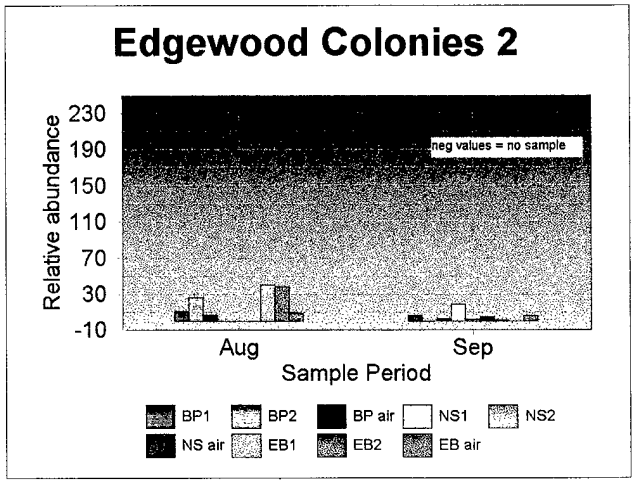


Figure 4.20. Naphthalene (Naph) Levels in 1996 Edgewood Colonies

phenyl ketone. A well known synthetic route by which CN can be prepared involves the chlorination of acetophenone in various media (4). Confirmation of acetophenone is illustrated in the spectral matching of Fig. 4.21. Here, the mass spectrum of the G Street Hive 1 (GS1) sample pumped on August 23rd is matched with 95% quality against the NBS library spectrum. Even though the molecular ion at 120 m/z is not the most prominent mass peak, we still employed it in our quantifications.

The environmental behavior of acetophenone is not as well known as the other six contaminants discussed above; its use and occurrence have not generated the body of case studies that chlorinated solvents and PAH's have. From the Missoula fingerprint studies performed in July, we do know that trace levels are found in non-military settings. There were measurable levels in propolis gathered from Montana colonies in July. Industrially, it has been employed as a catalyst in the polymerization of olefins like polyethylene (4). Acetophenone was detected in hive atmospheres at all 1996 field application sites (Figs. 4.22 and 4.23). The West Branch Canal Creek condo cluster yielded consistently low levels, with a high of 7.9 ipl on August 18th. The maximum at Churchville's cluster was 29.3 ipl on August 25th in the CV2 colony. Old O Field contained a dramatic hit (145.3 ipl) in Condo OF2 during the September 2nd sample period. This same sample contained high levels of the other contaminants, too, which suggests the acetophenone is part of a contamination plume leaching from the body of the landfill or available in a standing water source in the vicinity.

Reasonably strong concentrations of acetophenone were also noted at G Street (38.2 ipl), the Youth Center (28.1 ipl), Beach Point (32.8 ipl) and East Branch Canal Creek (43.5 ipl) on August 23rd, and Lauderick Creek (94.0) on September 20th. Further background is needed to properly interpret these results - both with respect to historical use of acetophenone at APG in CN manufacturing and to its occurrence in non-military settings.

4.3.9 Mustard Gas Breakdown Products

Despite the presence of mustard gas breakdown products (7) in ground water samples from Old O Field (2), no evidence was seen for the bioavailability of these compounds in hive atmospheres. Mustard gas, $ClCH_2CH_2SCH_2CH_2Cl$, has an environmental half-life of about 30 minutes when in contact with water (8). The two terminal chlorines are replaced by hydroxyl groups resulting in the appearance of thiodiglycol, $HOCH_2CH_2SCH_2CH_2OH$. Subsequent transformations yield the cyclic thioethers 1,4-dithiane ($C_4H_8S_2$) and 1,4-oxathiane (C_4H_8OS). Reagent grade samples of these three breakdown materials were placed in glass vials, moved into a fume hood and pumped on with Carbotrap sorption tubes in the flow path. The traps were subsequently desorbed into the GC/MS to establish a retention time and mass spectral fingerprint for each of them.

1,4-Oxathiane exhibited a retention time of 28.4 minutes and a molecular ion at 104 m/z (Fig. 4.24). 1,4-Dithiane appeared at 36.4 minutes and had a fundamental molecular ion peak at 120 m/z (Fig. 4.25). Some oxidation of the dithiane occurred in the pumping process,

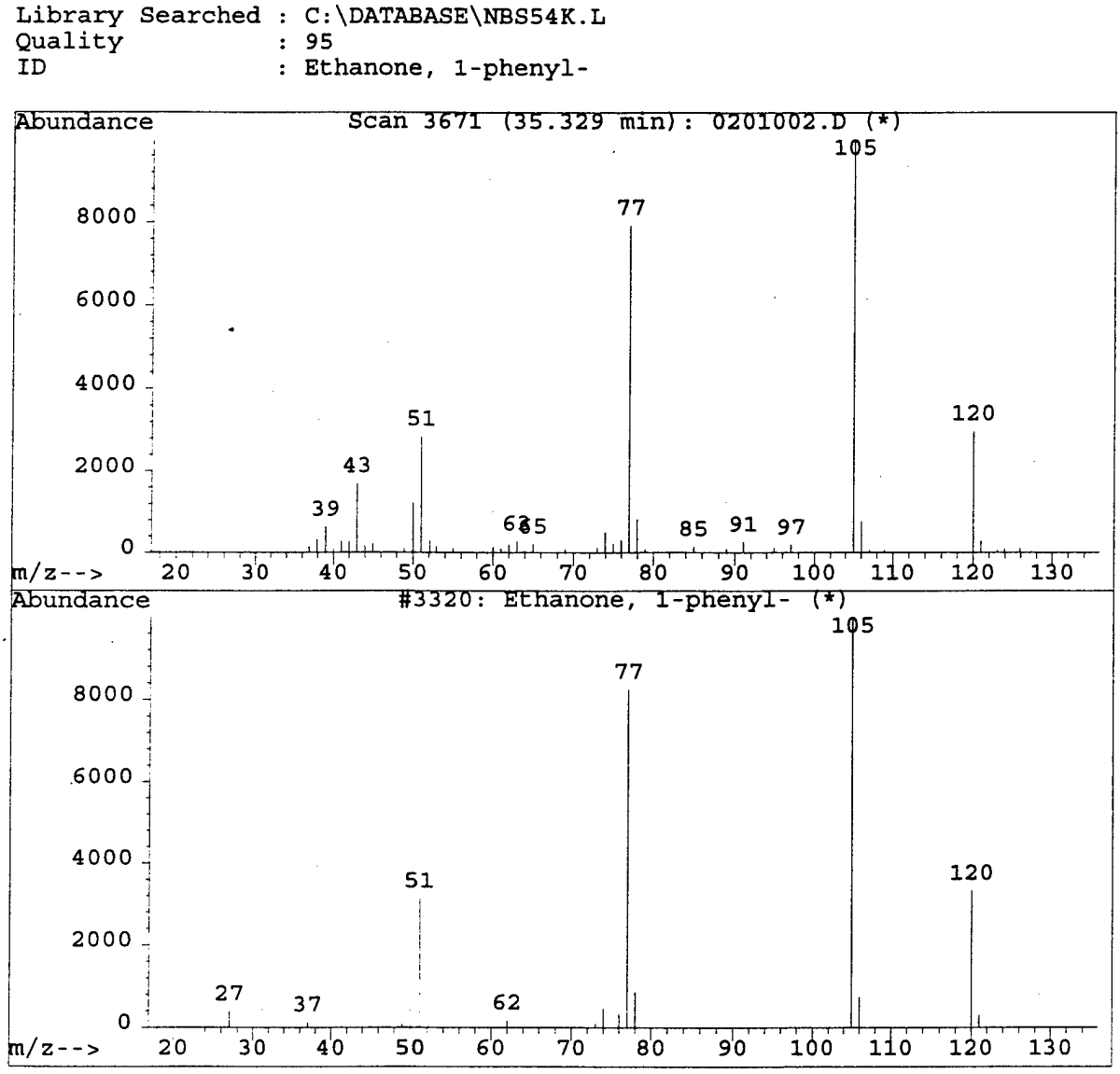


FIGURE 4.21. Acetophenone in G Street GS1 Hive Sample of August 23, 1996 (top panel), compared to NBS Library Spectrum (bottom panel)

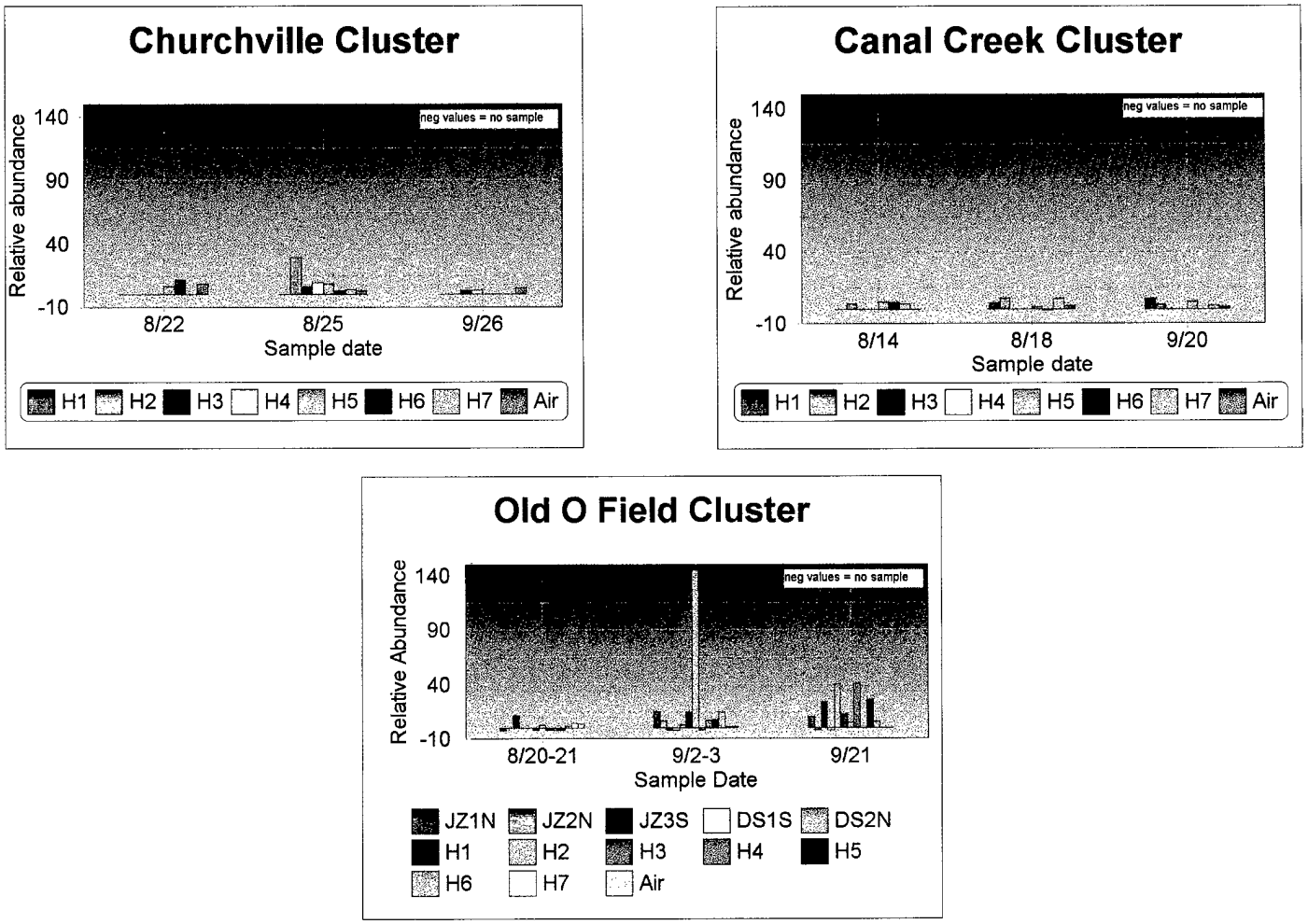
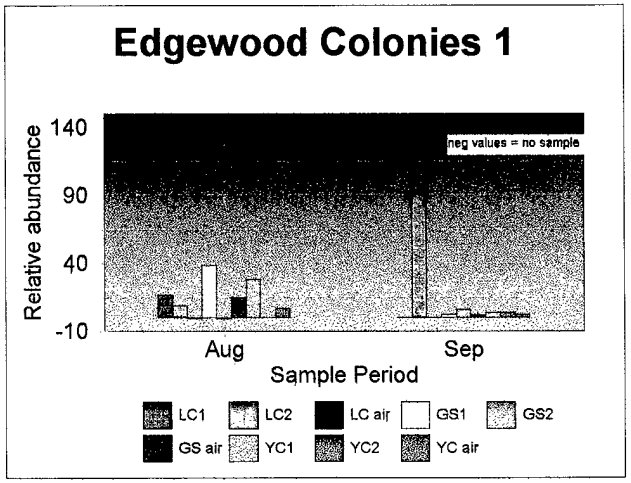


Figure 4.22. Acetophenone (AcPh) Levels in 1996 Condo Cluster Samples



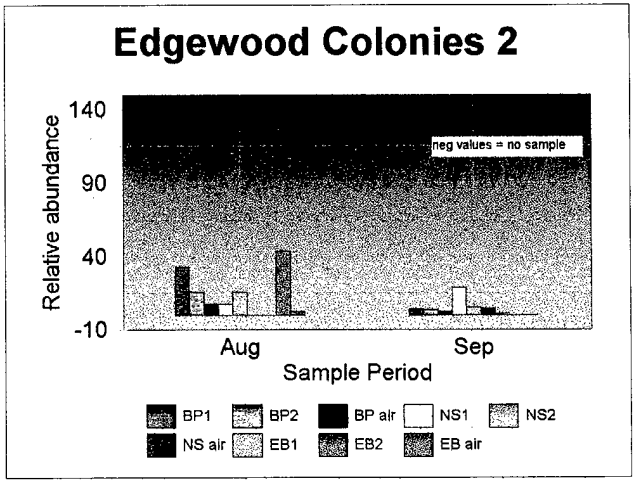


Figure 4.23. Acetophenone (AcPh) Levels in 1996 Edgewood Colonies

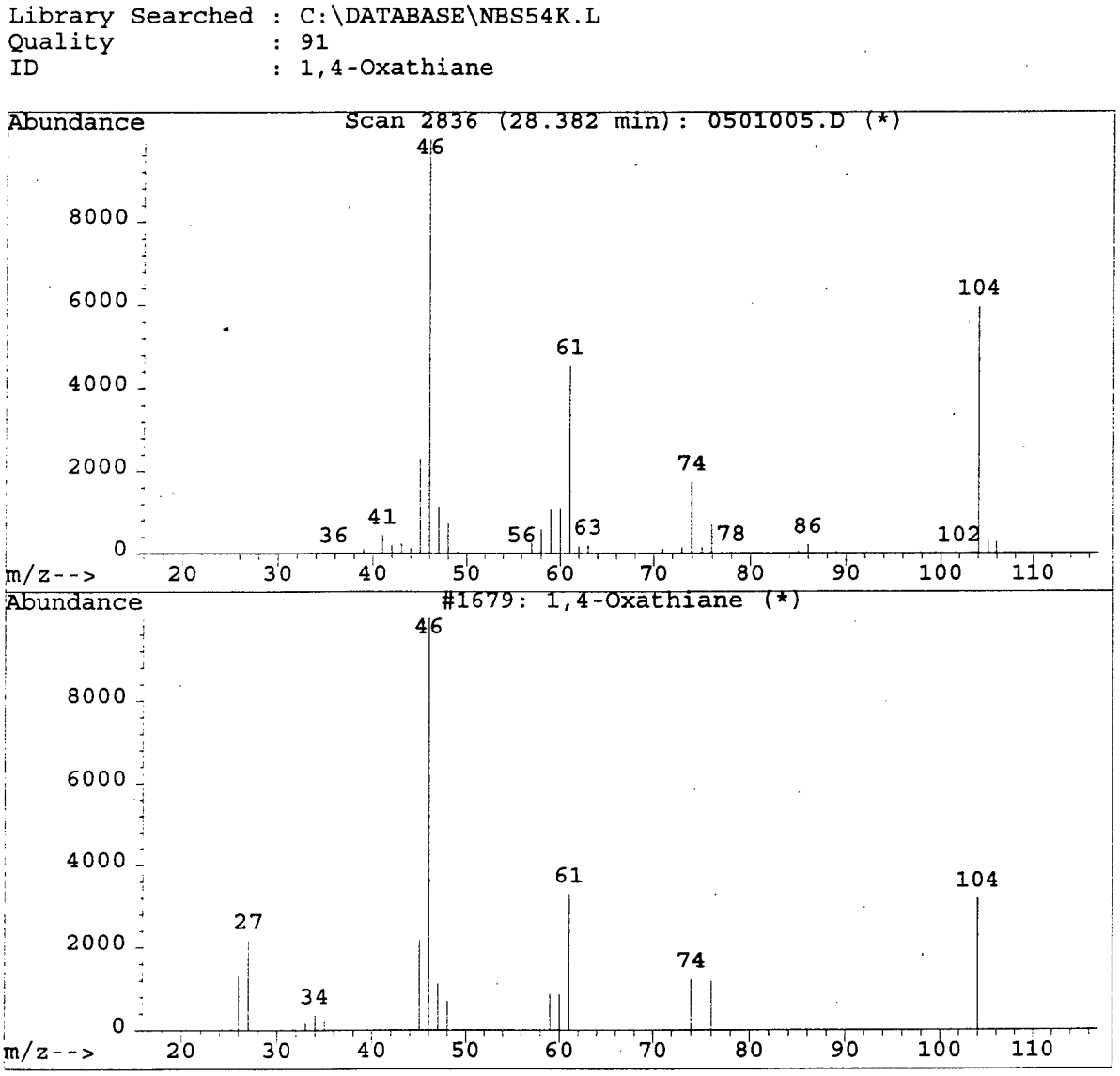


Figure 4.24. Mass Spectral Signature of 1,4-Oxathiane in Thiodiglycol (upper panel) and NBS Library (lower panel)

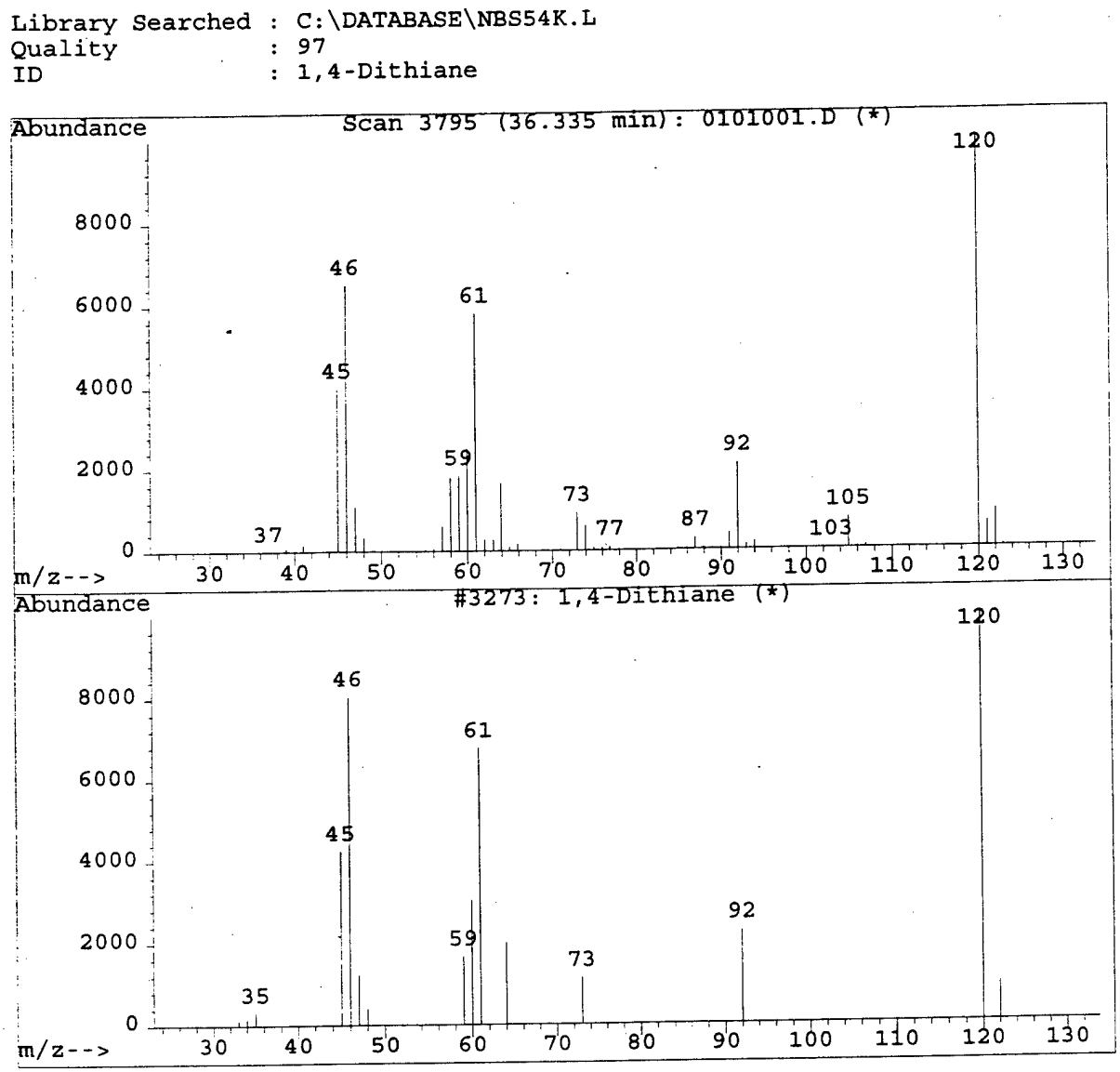


Figure 4.25. Mass Spectral Signature of 1,4-Dithiane as Desorbed (upper panel) and in NBS Library (lower panel)

so both dithiane and oxathiane appear in the chromatogram of Fig. 4.26. Thiodiglycol either: 1) did not volatilize sufficiently at room temperature (vapor pressure = 0.072 at 20 °C (8)) to be trapped on the sorption tube; 2) did not elute from the GC column within the time and temperature range of our method; or 3) air oxidized to 1,4-dithiane over the course of the sample period (Fig. 4.27).

Evidence for mustard products in hive atmospheres consisted of calling up every mass spectral time slice within 1.5 minutes of the observed retention times for 1,4-dioxane and 1,4-dithiane and looking for the appearance of an m/z peak at either 104 or 120 m/z. Neither molecular ion was located in Old O Field samples that had high levels of other contaminants from the landfill.

4.3.10 Menthol

A national outbreak of tracheal mites has necessitated periodic treatment of honey bee colonies with menthol ($C_{10}H_{20}O$, 5-methyl-2-(1-methylethyl)-cyclohexanol). During the course of the 1996 field applications, menthol packets were inserted in three condos at each instrumented cluster to evaluate its movement through the hives and acquire its chemical fingerprint in hive atmospheres. Hives 1, 4 and 7 in each instrumented cluster were dosed with approximately 12 grams of menthol by placing 42 commercial pellets of Mitathol (Mann Lake) in a perforated polyethylene bag atop the upper story ventilation screen. Menthol was emplaced in condos at West Branch Canal Creek on August 18th, at Old O Field on August 20th, and at Churchville on August 22nd. It was removed from the hives in mid-September.

Menthol was easily seen and recognized in hive atmospheres (Fig. 4.28), but substantial concentration differences were seen from hive to hive in condos that received menthol packages (Table 4.5 and Fig. 4.29). The cleanest results came from the West Branch Canal Creek site. No menthol was seen on August 14th (before insertion of the packets) or on September 20 (after removal of the packets). The August 18th sample date shows a strong presence in Condo 1 and an intermediate presence in Condo 7. Only a small amount was detected in Condo 4, which received a packet. More was seen in Condo 2 which did not receive a packet but was adjacent to Condo 1 which did. These results illustrate that even with a known exposure to a contaminant, not all hives will show it to the same extent. Variations in air circulation through the hives can interfere with transport of contaminants into the sample tube orifice. Since the bees were not in physical contact with the menthol, the situation was analogous to the proper positioning of a stationary sampler to intercept a more conventional plume of contaminated air. No menthol was detected at the ambient air sampling tube which was removed from the condo cluster by about 2 meters.

Levels of menthol detected at the Churchville site were generally low. Low levels may indicate colony ventilation was more efficient at this setting; the Churchville condo cluster setting was more open than that at either the West Branch Canal Creek or Old O Field site.

File : C:\HPCHEM\1\DATA\1805\0301003.D
Operator : GA
Acquired : 15 Sep 96 6:17 pm using AcqMethod BTEX
Sample Name: 1,4-Dithiane (S) 9/14/96
Misc Info :
Vial Number: 3

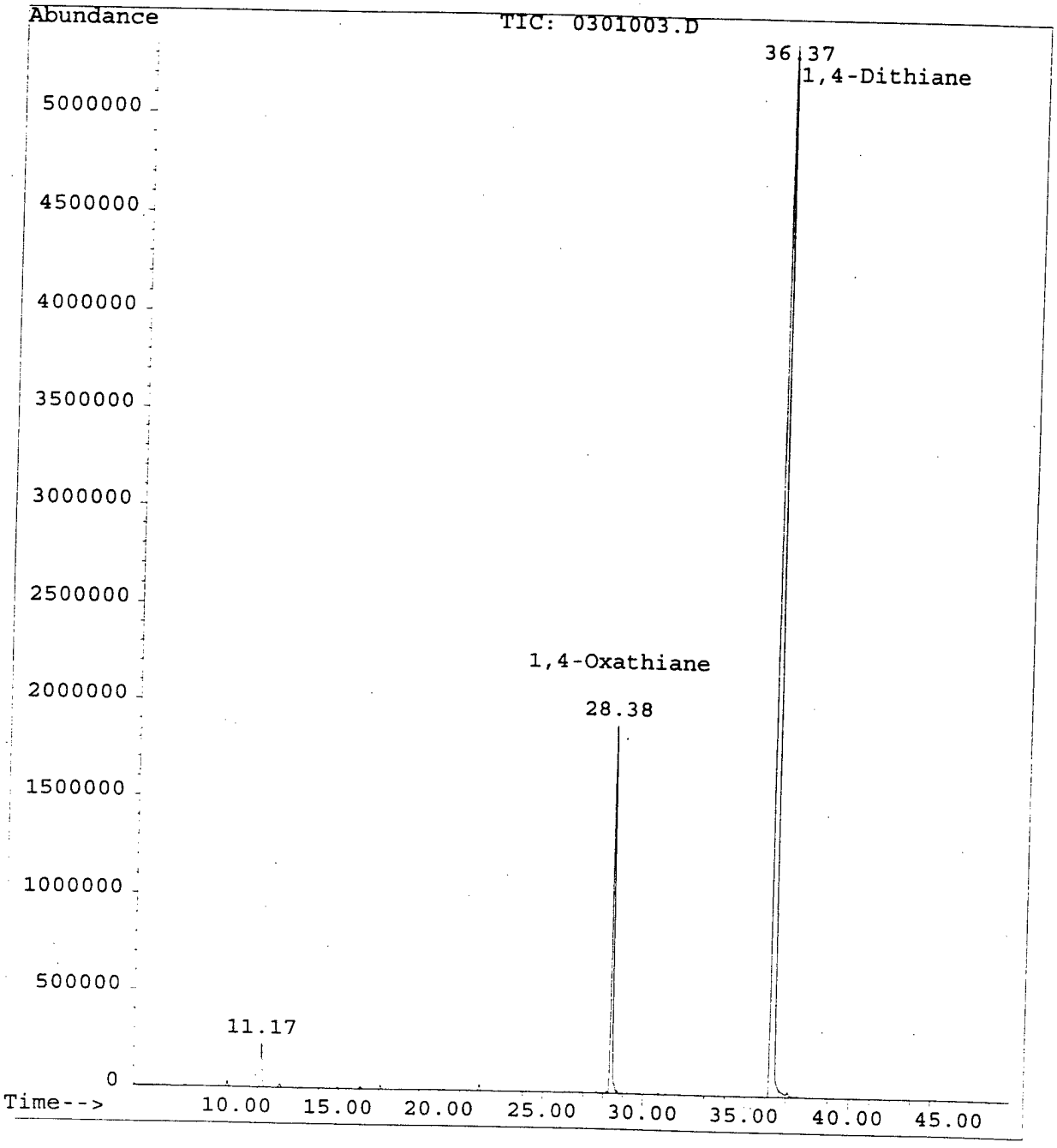


Figure 4.26. Retention Times for 1,4-Oxathiane and 1,4-Dithiane

File : C:\HPCHEM\1\DATA\1805\0501005.D
Operator : GA
Acquired : 15 Sep 96 8:14 pm using AcqMethod BTEX
Sample Name: Thiodiethanol (S) 9/14/96
Misc Info :
Vial Number: 5

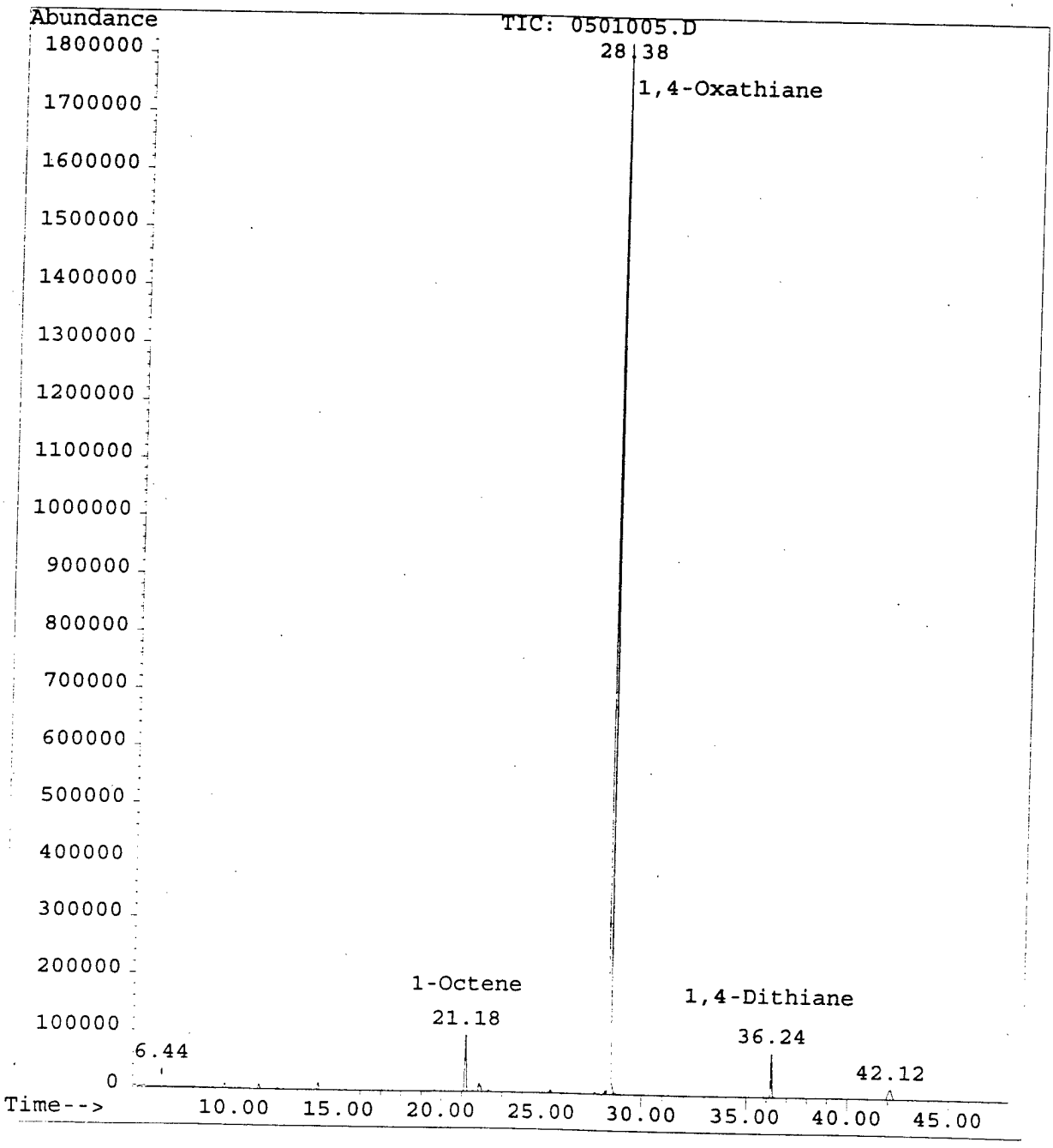


Figure 4.27. Total Ion Chromatogram for Thiodiglycol Sample Showing Degradation Products

Library Searched : C:\DATABASE\NBS54K.L
Quality : 91
ID : Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-

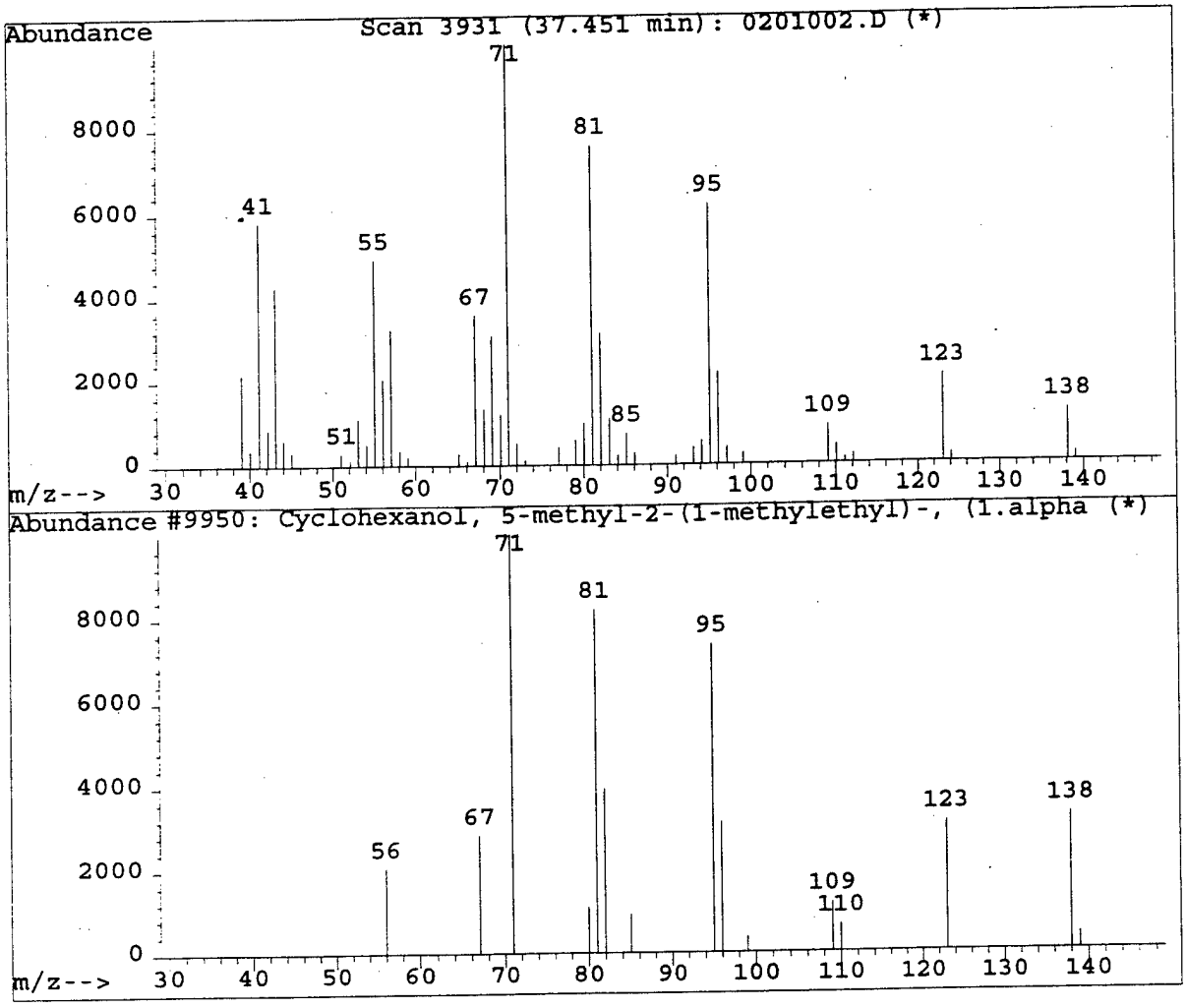


Figure 4.28. Menthol in August 18 Sample from West Branch Canal Creek Condo 2 (upper panel) and NBS Library Spectrum (lower panel)

TABLE 4.5. Menthol in 1996 APG Hive Atmospheres

(Characteristic ion abundance/liter of air sampled; ns = no sample)

West Branch Canal Creek Site

Hive	14-Aug-96	18-Aug-96	20-Sep-96
CC1	0.0	353.4	0.0
CC2	0.0	26.5	0.0
CC3	0.0	ns	0.0
CC4	0.0	3.6	0.0
CC5	0.0	0.0	0.0
CC6	0.0	ns	0.0
CC7	0.0	31.4	0.0
CC air	0.0	0.0	0.0

Old O Field Site

Hive	20-Aug-96	02-Sep-96	21-Sep-96
OF1	ns	2.4	0.0
OF2	0.0	2.9	0.0
OF3	ns	ns	3.3
OF4	ns	0.0	0.0
OF5	ns	0.7	8.7
OF6	0.6	2.0	0.0
OF7	201.7	47.6	0.0
DS1S	0.0	ns	ns
DS2N	0.0	0.0	0.0
JZ1N	ns	60.9	0.0
JZ2N	1.9	4.9	ns
JZ3S	8.6	ns	9.5
OF air	0.0	0.0	0.0

Churchville Reference Site

Hive	22-Aug-96	25-Aug-96	26-Sep-96
CV1	6.3	ns	ns
CV2	7.0	16.7	0.0
CV3	7.2	0.0	0.0
CV4	4.3	11.2	0.0
CV5	3.7	2.1	0.0
CV6	11.8	0.0	0.0
CV7	6.0	20.2	0.0
CV air	0.0	0.0	0.0

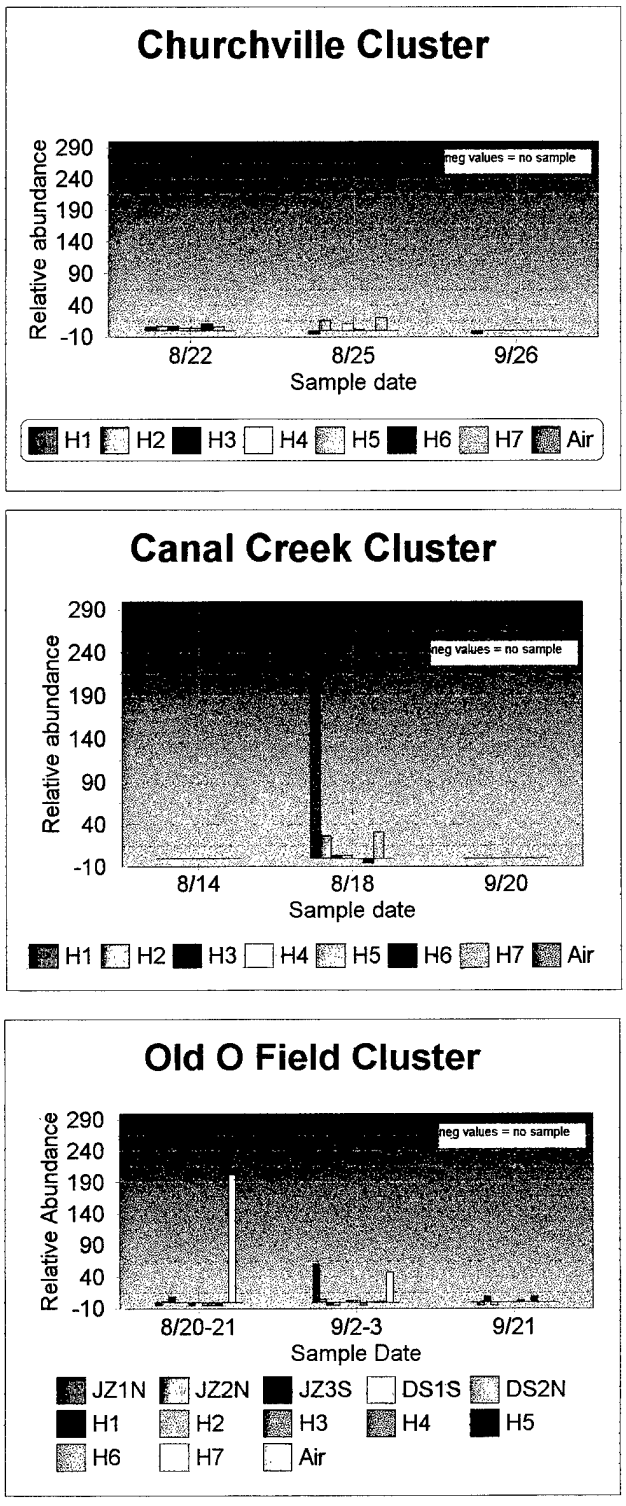


Figure 4.29. Menthol Levels in 1996 Condo Cluster Samples

All Churchville condos showed some menthol present on the date of installation, perhaps a manifestation of having hive doors open when the chemical was dispensed. Samples at Churchville taken on August 25th demonstrate menthol in place in both hives 4 and 7. The sample tube from hive 1 was wet, so no determination could be made. Hive 2 held a concentration of menthol equivalent to that found in hive 4. Carry over from the adjacent hives with menthol could come in the form of ventilation fan downwash or migration of menthol-dosed bees from one colony to another.

The distribution of menthol levels at the Old O Field site is suggestive of natural sources of menthol in the vicinity. The high concentration of menthol seen in OF7 is expected since that condo received one of the pellet packets. The 60.9 ipl menthol reading in the JZ1 stand alone colony is more of a puzzle. Because JZ1 was located in the immediate vicinity of Condo 7, both ventilation downwash and migration of dosed bees are within reason. An interesting result, however, is the menthol measured in the JZ3S colony on both August 20th (8.6 ipl) and September 26th (9.5 ipl). The colonies bearing the S designation were isolated on the opposite side of the landfill from the instrumented condo cluster. Since there was no possibility of capturing menthol emplaced at the "N" colonies, especially after it was removed, the bees in JZ3S assimilated menthol from some other source. Since menthol is found naturally in plants, especially those of the mint family, foragers probably picked it up in the swale area to the south of the water treatment plant. Alternatively, there could have been an anthropogenic source nearby.

4.3.11 Additional Unquantified Organic Contaminants

Several other organic contaminants were identified in hive atmospheres during the 1996 field season but have not yet been fully quantified. We report them here as a means to indicate where future efforts will be directed.

There is a distinct presence of aromatic hydrocarbons throughout the entire set of air samples, especially those seen in gasoline residuals -- the BTEX suite (benzene, toluene, ethyl benzene and xylenes). These compounds are commonly released during vehicle fueling when the saturated vapor phase in tanks is displaced by the incoming fuel fluids. BTEX compounds also comprise a portion of unburned residuals in tailpipe exhaust. In a study which examined contaminants in urban air over the city of Missoula, MT last winter (9), 62 of 64 air sample chromatograms were dominated by BTEX components. With so much vehicular traffic near many of our hive placements, we felt it would be difficult to correctly separate historical sources of these compounds from contemporary auto emissions.

Several chlorinated hydrocarbons in addition to PCE, TCE, PCA, TCM and DCB have been found in hive atmospheres. We are currently performing a systematic quantification of benzoyl chloride. We continue to explore chromatograms for evidence of other common solvents of concern reported in APG ground water, e.g., chloroform, methylene chloride, TCA, etc. (1,2,6,8).

Samples from the Churchville reference site often exhibited strong chromatographic peaks that keyed to derivatives of furfural, $C_5H_4O_2$, a furan ring with an aldehyde moiety on C2. As a result of conversations with the owner of the Churchville site, we discovered that he had supplemented his bee forage with a commercial corn syrup. Beekeepers are familiar with cautions about obtaining corn syrup from suppliers that is high in HMF, hydroxymethyl-furfural. The appearance of HMF in our Churchville bee condos was additional evidence that our bees robbed hive stores from other colonies or unoccupied boxes stored at this site.

4.3.12 Overall Patterns in Organic Contaminants at APG

While the interpretation of results for each specific contaminant has already been attempted, additional insight into possible sources and modes of exposure can be gleaned by combining the individual measurements into a single data set. This has been done both numerically in Table 4.6 and graphically in Fig. 4.30. The levels selected for these compilations generally represent the average of the highest levels we observed in one day's samples from a given site. Availability of pumps precluded sampling all locations on a single day, but the sampling dates fell within the same 2-week period during late August and early September. So all samples were collected under a similar seasonal setting.

The plots reflect relative levels of the seven quantified contaminants at the three instrumented sites. A fourth category combines results from the Edgewood area office/ residential/recreational sites. No standard deviations were computed for these data since the distribution of readings did not follow a normal distribution. In many instances, one hive in a pair had a significant value while the other was at non-detect levels. Since the level of perchloroethylene was so high at Old O Field compared to all other contaminants, we have replotted the same data on two panels. The upper panel displays the PCE value relative to the other six. In the middle panel, the Old O Field PCE peak has been truncated to permit more detail on the remaining contaminants. The ambient air levels, recorded at each site for the same sample date, are plotted together in the bottom panel.

Immediately apparent from an inspection of the two hive contaminant panels is the similar low average levels seen at both the West Branch Canal Creek site and the Churchville reference site. On the other hand, Old O Field and sites scattered around the Edgewood area of the post have readings that are two to three times higher. It is important to realize that exposures to hazardous substances in relatively residential settings can approach those from the more notorious historic waste dump.

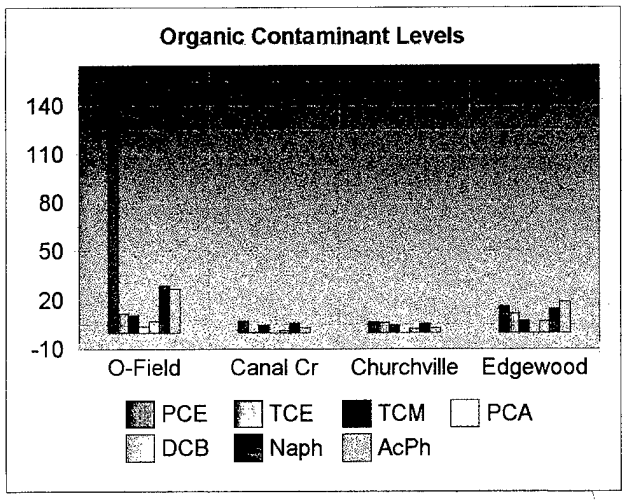
The ambient air panel of Fig. 4.30 suggests that there is a difference in exposure routes to honey bees at West Branch Canal Creek and Old O Field sites compared to those at Churchville and on the Edgewood post. High hive levels accompanied by consistent low ambient air readings across the entire suite of contaminants supports a condensed phase source through which the Old O Field bees receive their dose. The same appears true at the West Branch Canal Creek site; hive levels exceed ambient air levels with the exception of TCM.

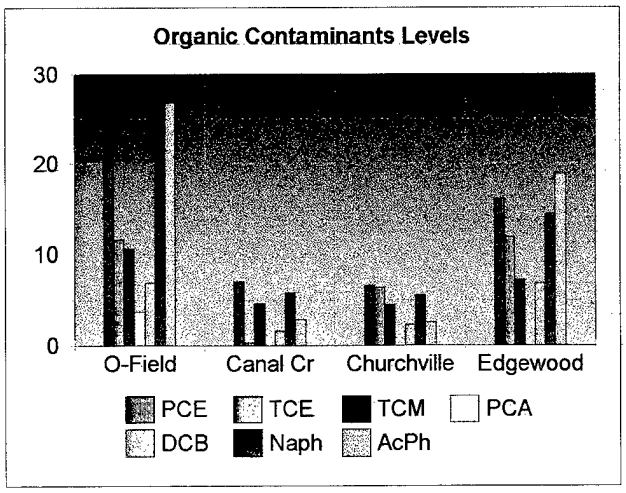
TABLE 4.6

Relative Levels of Organic Contaminants in Hive Atmospheres

All values are average molecular (or characteristic) ion abundance/L of air pumped and generally represent the highest level observed on each site for a single sample date. For comparison, contaminant levels detected in ambient air at each site on the same sample date appear in parentheses immediately below the hive atmosphere values.

Contaminant	O Field	Canal Creek	Churchville	Edgewood
Percholorethylene, PCE	158.7	7.0	6.6	16.1
(ambient air level)	(4.2)	(3.4)	(8.2)	(22.6)
Trichloroethene, TCE	11.6	0.3	6.4	11.9
(ambient air level)	(0.0)	(0.0)	(0.0)	(3.8)
Tetrachloromethane, TCM	10.6	4.6	4.5	7.2
(ambient air level)	(0.0)	(4.3)	(11.4)	(13.0)
Hexachloroethane, PCA	3.7	0.0	0.0	0.0
(ambient air level)	(0.0)	(0.0)	(0.0)	(0.0)
1,4-Dichlorobenzene, DCB	6.9	1.6	2.3	6.8
(ambient air level)	(0.0)	(0.0)	(4.9)	(5.9)
Naphthalene	28.9	5.8	5.5	14.4
(ambient air level)	(4.2)	(0.0)	(3.3)	(13.2)
Acetophenone	26.8	2.9	2.6	18.8
(ambient air level)	(1.1)	(0.0)	(8.2)	(6.4)





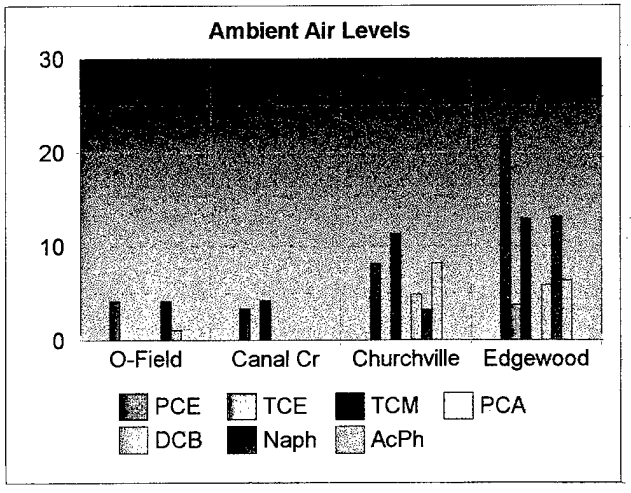


Figure 4.30. Relative Levels of Organic Contaminants in Hive Atmospheres
(characteristic ion abundance/L of air pumped)

Uptake of contaminants would not be expected by organisms who do not forage or drink from these contaminated supplies. Conversely, that the Churchville and the Edgewood colonies harbor levels of the same general magnitude as the ambient air is more troublesome. Birds, animals and humans spending time in these areas are subjected to an exposure similar to what has been measured in the hive. Follow-up work to pinpoint sources that release these emissions is warranted.

4.3.13 Heavy Metal and Elemental Contaminants

Metal and element levels encountered in 1996 APG field samples generally fell within expected ranges from our previous experience with honey bees. Table 4.7 contains a complete listing of concentrations, with uncertainties, that were found for live bee, dead bee and pollen samples. The first round of sampling, in mid-August, focused on live bees and pollen. The seasonal drop-off in foraging activity precluded gathering a complete round of live bees during the September sampling. The September round of samples did include dead bees, however. Location codes in the table ending in "B" represent live bee samples. A "D" at the end of a sample code designates dead bees removed from the condo entrance hoppers and a "P" denotes a sample of pollen gathered from the clock-driven hopper tray. Since the stand-alone hives had no entrance hopper, pollen and dead bee samples were not available from these units.

An overall picture of metal/element distribution in the samples can be gathered from Figs. 4.31 and 4.32 which chart the relative concentrations of all twenty elements by site category. Manganese, zinc, copper, rubidium, barium and strontium show the same order of prominence irrespective of site or sample type although live bee levels are generally higher than dead bee levels, and dead bee levels generally exceed pollen levels. Figs. 4.33 through 4.37 break out individual metals for comparisons between sites.

Bees typically exhibit zinc in the range of 80 to 120 ppm (10). When flown on sites contaminated with smelter or mine wastes, the zinc concentrations tend toward the low end of the range. Some indication of depressed zinc levels is apparent in APG samples from the West Branch Canal Creek (87 ppm) and Old O Field sites (73 ppm).

Levels of copper in honey bees rarely deviate much from the 25 ppm range since honey bees use a copper-based oxygen transport system in their bodies in the same way humans employ iron in hemoglobin. Live bees at all sites demonstrated a normal complement of copper.

Because of the high temperature used to oven dry the bees and pollen for digestion (105 °C), some of the more volatile toxic elements (As, Cd and Pb) may have suffered losses. Complete retention of these elements in future analyses could be assured by spiking some samples prior to the drying and digestion steps. A longer period of drying at a temperature near 45 °C, would also retain of these elements. Volatile losses not withstanding, there appears to be some slight presence of bioavailable arsenic, cadmium and lead in bees that

Table 4.7 Metal Analysis of Bees and Pollen by ICP/MS

(B = live bee sample; P = pollen sample; D = dead bee sample
d = duplicate sample)

		As	Ba	Be	Bi	Cd	Co	Cs
Location	Date	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.
O-Field		± % RSD	± % RSD	± % RSD	± % RSD	± % RSD	± % RSD	± % RSD
OF1B	8/23/96	0.5±4.0	7.7±1.5	BDL	BDL	0.2±0.5	0.3±2.3	BDL
OF2B	8/23/96	0.4±1.7	4.4±1.2	BDL	BDL	0.1±1.1	0.3±4.0	BDL
OF4B	8/23/96	0.3±8.8	5.5±1.2	BDL	BDL	0.2±4.0	0.5±2.8	BDL
OF5B	8/23/96	0.2±7.3	6.1±1.3	BDL	BDL	BDL	0.1±1.7	BDL
OF7B	8/23/96	0.4±28.0	12.1±0.6	BDL	BDL	0.3±31.5	0.6±17.6	0.2±59.9
JZ1B	8/23/96	0.3±6.2	3.6±0.9	BDL	BDL	BDL	0.3±1.3	BDL
JZ2B	8/23/96	0.2±9.1	2.2±0.6	BDL	BDL	BDL	0.2±2.2	BDL
DS2B	8/23/96	0.2±16.5	2.8±2.2	BDL	BDL	BDL	0.3±2.1	BDL
Avg. OFB		**0.3±11.3**	**5.5±1.2**	**BDL**	**BDL**	**0.2±7.6**	**0.3±4.2**	**BDL**
Churchville								
CV1B	8/25/96	BDL	5.8±0.7	BDL	BDL	BDL	0.3±1.3	BDL
CV2B	8/25/96	0.1±7.6	3.3±1.3	BDL	BDL	0.1±5.1	0.4±2.5	BDL
CV3B	8/25/96	BDL	4.0±0.3	BDL	BDL	BDL	0.3±2.2	BDL
CV4B	8/25/96	0.1±8.4	5.4±0.9	BDL	BDL	BDL	0.2±3.7	BDL
CV5B	8/25/96	BDL	5.1±0.5	BDL	BDL	BDL	0.3±3.3	BDL
CV6B	8/25/96	0.1±31.6	4.2±0.8	BDL	BDL	BDL	0.3±3.3	BDL
CV7B	8/25/96	0.3±9.7	8.6±1.4	BDL	BDL	BDL	0.2±5.6	BDL
CV8B(CV1d)	8/25/96	0.3±15.2	4.7±0.8	BDL	BDL	BDL	0.4±0.4	BDL
Avg. CVB		**0.1±18.1**	**5.1±0.8**	**BDL**	**BDL**	**BDL**	**0.3±2.7**	**BDL**
Canal Cr								
CC1B	8/25/96	0.3±4.2	3.4±0.1	BDL	BDL	BDL	0.4±0.8	BDL
CC2B	8/25/96	0.2±9.9	3.0±1.0	BDL	BDL	BDL	0.3±2.3	BDL
CC3B	8/25/96	0.2±12.8	2.3±0.8	BDL	BDL	BDL	0.3±1.5	BDL
CC4B	8/25/96	0.2±17.0	4.1±0.5	BDL	BDL	BDL	0.3±2.1	BDL
CC5B	8/25/96	0.2±14.7	3.3±1.2	BDL	BDL	BDL	0.3±1.2	BDL
CC6B	8/25/96	0.3±13.2	3.2±0.7	BDL	BDL	0.1±23.0	0.4±10.3	BDL
CC7B	8/25/96	0.3±2.6	6.1±0.6	BDL	BDL	0.2±2.2	0.5±0.7	BDL
CC8B(CC3d)	8/25/96	0.3±11.6	1.9±0.9	BDL	BDL	BDL	0.3±0.8	BDL
Avg. CCB		**0.2±10.8**	**3.4±0.7**	**BDL**	**BDL**	**BDL**	**0.3±2.5**	**BDL**
CC2P	8/23/96	0.1±81.9	4.0±1.4	0.1±72.2	BDL	0.1±44.5	0.2±61.6	0.1±99.2
CC5P	8/23/96	0.1±37.4	5.3±1.3	BDL	BDL	BDL	BDL	BDL
CC2P(d)	8/23/96	0.1±13.5	3.3±0.5	BDL	BDL	BDL	BDL	BDL
CC5P(d)	8/23/96	0.2±21.0	3.9±2.5	BDL	BDL	BDL	BDL	BDL
Avg. CCP		**0.1±38.4**	**4.1±1.4**	**BDL**	**BDL**	**BDL**	**BDL**	**BDL**
APG BLANK	8/25/96	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Table 4.7 (Cont.) Metal Analysis of Bees and Pollen by ICP/MS

		Cr	Cu	Ga	Mn	Ni	Pb	Rb
Location	Date	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.
O-Field		± % RSD	± % RSD	± % RSD	± % RSD	± % RSD	± % RSD	± % RSD
OF1B	8/23/96	0.4±2.6	17.7±1.8	0.4±1.5	205.0±1.8	0.2±3.4	0.7±2.4	10.4±1.9
OF2B	8/23/96	0.4±5.0	19.2±0.7	0.2±4.3	157±1.5	0.6±3.3	0.7±3.1	9.2±1.1
OF4B	8/23/96	0.4±3.4	17.9±2.1	0.3±3.0	150.0±0.7	0.6±2.5	0.3±2.3	9.3±0.5
OF5B	8/23/96	0.4±3.0	17.8±1.2	0.3±2.6	92.8±2.6	0.2±3.9	0.5±2.4	5.4±1.1
OF7B	8/23/96	0.6±15.0	20.2±0.7	0.8±14.4	217.0±2.5	0.7±18.0	0.5±17.1	6.3±1.7
JZ1B	8/23/96	0.4±3.1	20.8±1.0	0.2±2.8	168.0±1.0	0.4±1.0	0.2±0.9	7.1±0.6
JZ2B	8/23/96	0.4±4.1	19.9±0.9	BDL	136.0±1.6	0.3±1.7	0.1±2.2	7.7±0.5
DS2B	8/23/96	0.7±2.4	16.4±0.5	0.1±1.0	193.0±1.2	0.5±4.3	0.2±3.1	5.1±1.9
Avg. OFB		**0.4±4.8**	**18.7±1.1**	**0.3±3.8**	**164.8±1.6**	**0.4±4.7**	**0.4±4.2**	**7.5±1.1**
Churchville								
CV1B	8/25/96	0.5±2.4	22.8±1.0	0.3±0.7	223.0±1.7	0.4±2.4	0.4±2.1	9.4±0.8
CV2B	8/25/96	0.5±1.1	22.6±0.5	0.2±1.2	149.0±2.2	0.4±1.9	0.3±2.1	8.4±1.7
CV3B	8/25/96	0.6±2.9	23.4±0.3	0.2±3.2	157.0±1.8	0.5±0.7	0.3±2.9	8.2±0.4
CV4B	8/25/96	0.4±4.2	24.5±0.9	0.3±2.3	91.8±1.9	0.3±3.1	0.5±1.2	7.0±1.3
CV5B	8/25/96	0.5±2.7	21.9±1.2	0.3±4.0	193.0±0.4	0.3±4.8	0.5±0.6	7.8±1.1
CV6B	8/25/96	0.5±4.2	22.2±0.6	0.2±0.4	207.0±2.0	0.3±1.7	0.4±0.3	8.5±-0.7
CV7B	8/25/96	0.4±4.8	23.0±0.9	0.4±4.0	120.0±1.8	0.2±1.5	0.7±0.9	6.2±1.8
CV8B(CV1d)	8/25/96	1.1±2.1	24.0±0.8	0.2±2.6	215.0±0.8	0.5±1.5	0.4±1.0	10.7±0.5
Avg. CVB		**0.5±3.0**	**23.0±0.7**	**0.2±2.3**	**169.4±1.5**	**0.3±2.2**	**0.4±1.4**	**8.2±1.0**
Canal Cr								
CC1B	8/25/96	0.5±0.5	21.2±1.2	0.2±2.7	140.0±1.0	0.4±1.2	0.3±1.8	7.9±1.8
CC2B	8/25/96	0.4±3.9	22.2±0.6	0.1±0.8	143.0±4.0	0.3±2.2	0.3±2.4	7.1±1.2
CC3B	8/25/96	0.6±2.1	20.9±1.7	BDL	134.0±3.3	0.3±2.6	0.5±2.8	7.4±1.1
CC4B	8/25/96	0.4±4.4	21.4±0.3	0.2±4.7	193.0±2.8	0.3±2.0	0.4±2.3	7.4±1.6
CC5B	8/25/96	0.6±2.1	19.6±1.8	0.1±4.2	138.0±1.3	0.5±1.5	0.5±0.7	6.2±1.7
CC6B	8/25/96	0.7±3.3	21.7±0.9	0.2±24.6	178.0±3.7	0.7±6.8	0.3±11.4	5.5±2.1
CC7B	8/25/96	0.6±1.2	19.3±1.7	0.3±5.1	190.0±0.9	0.5±2.8	0.4±0.9	5.4±0.7
CC8B(CC3d)	8/25/96	0.6±3.0	17.2±2.1	BDL	156.0±1.8	0.4±2.3	0.2±1.7	6.0±2.4
Avg. CCB		**0.5±2.5**	**20.4±1.2**	**0.2±6.0**	**159±2.4**	**0.4±2.7**	**0.3±3.0**	**6.6±1.5**
CC2P	8/23/96	0.6±19.8	13.5±1.2	0.3±36.7	32.7±3.3	1.3±10.2	0.2±36.1	13.1±2.2
CC5P	8/23/96	0.4±1.3	10.8±2.7	0.2±3.4	34.6±1.8	0.8±3.0	BDL	12.1±2.1
CC2P(d)	8/23/96	0.5±1.5	13.4±2.0	0.2±0.8	29.9±1.8	0.9±0.7	0.1±1.7	13.0±1.2
CC5P(d)	8/23/96	0.4±3.0	10.9±0.3	0.2±3.6	33.8±2.0	0.8±2.2	BDL	12.0±0.5
Avg. CCP		**0.4±6.4**	**12.1±1.5**	**0.2±11.1**	**32.8±2.2**	**1.0±4.0**	**0.1±10.6**	**12.5±1.5**
APG BLANK	8/25/96	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Table 4.7 (Cont.) Metal Analysis of Bees and Pollen by ICP/MS

		Se	Sr	Tl	U	V	Zn
Location	Date	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.
O-Field		± % RSD	± % RSD	± % RSD	± % RSD	± % RSD	± % RSD
OF1B	8/23/96	0.2±19.5	5.2±1.5	BDL	BDL	0.4±2.1	89.2±0.9
OF2B	8/23/96	0.2±29.6	5.1±1.9	BDL	BDL	0.5±0.9	73.3±1.8
OF4B	8/23/96	0.1±32.8	5.4±1.2	BDL	BDL	0.5±2.5	72.9±0.9
OF5B	8/23/96	0.2±12.3	3.7±1.2	BDL	BDL	0.4±3.4	84.5±0.8
OF7B	8/23/96	0.3±37.4	6.3±1.2	0.2±58.9	0.2±60.3	0.6±18.6	85.4±0.7
JZ1B	8/23/96	0.2±28.3	3.3±1.4	BDL	BDL	0.4±1.1	64.4±0.6
JZ2B	8/23/96	0.1±8.2	2.3±0.3	BDL	BDL	0.4±4.8	55.9±0.4
DS2B	8/23/96	0.1±66.1	5.5±1.5	BDL	BDL	0.4±2.0	62.8±1.0
Avg. OFB		**0.2±29.2**	**4.6±1.2**	**BDL**	**BDL**	**0.4±4.4**	**73.5±0.8**
Churchville							
CV1B	8/25/96	0.3±13.2	2.1±0.4	BDL	BDL	0.7±2.8	121.0±1.0
CV2B	8/25/96	0.3±17.6	1.3±0.5	BDL	BDL	0.7±3.4	114.0±0.8
CV3B	8/25/96	0.3±4.1	1.4±0.4	BDL	BDL	0.7±3.2	127.0±0.9
CV4B	8/25/96	0.3±29.0	1.3±0.8	BDL	BDL	0.6±2.5	93.8±0.5
CV5B	8/25/96	0.3±22.8	1.6±0.4	BDL	BDL	0.7±3.6	108.0±0.2
CV6B	8/25/96	0.2±15.6	1.4±0.6	BDL	BDL	0.7±5.4	103.0±0.4
CV7B	8/25/96	0.2±22.6	1.6±0.5	BDL	BDL	0.5±6.6	121.0±0.5
CV8B(CV1d)	8/25/96	0.2±24.5	1.6±0.4	BDL	BDL	0.4±2.1	135.0±0.4
Avg. CVB		**0.2±18.6**	**1.5±0.5**	**BDL**	**BDL**	**0.6±3.7**	**115±0.5**
Canal Cr							
CC1B	8/25/96	0.3±23.6	1.9±0.5	BDL	BDL	0.7±1.1	73.1±0.5
CC2B	8/25/96	0.2±43.1	2.4±2.1	BDL	BDL	0.6±4.4	73.7±0.6
CC3B	8/25/96	0.3±3.4	1.6±0.9	BDL	BDL	0.5±2.1	74.7±1.6
CC4B	8/25/96	0.3±36.1	2.7±0.6	BDL	BDL	0.6±4.2	110.0±1.1
CC5B	8/25/96	BDL	1.9±0.2	BDL	BDL	0.4±2.9	87.1±0.8
CC6B	8/25/96	BDL	2.5±0.8	BDL	BDL	0.4±11.5	98.1±1.7
CC7B	8/25/96	BDL	2.6±0.7	BDL	BDL	0.3±3.1	103.0±0.4
CC8B(CC3d)	8/25/96	BDL	1.5±1.7	BDL	BDL	0.3±4.4	74.8±1.0
Avg. CCB		**0.3±52.5**	**2.1±0.9**	**BDL**	**BDL**	**0.4±4.2**	**86.8±1.0**
CC2P	8/23/96	0.3±54.6	3.2±3.3	BDL	BDL	1.0±11.1	28.7±1.7
CC5P	8/23/96	0.1±47.9	2.5±2.0	BDL	BDL	0.6±3.9	34.2±2.6
CC2P(d)	8/23/96	BDL	3.5±0.5	BDL	BDL	0.8±6.5	29.7±0.6
CC5P(d)	8/23/96	0.3±43.3	2.9±0.6	BDL	BDL	0.7±1.3	31.2±0.9
Avg. CCP		**0.2±43.0**	**3.0±1.6**	**BDL**	**BDL**	**0.7±5.7**	**31.0±1.4**
APG BLANK	8/25/96	BDL	BDL	BDL	BDL	BDL	BDL

Table 4.7 (Cont.) Metal Analysis of Bees and Pollen by ICP/MS

		As	Ba	Be	Bi	Cd	Co	Cs
Location	Date	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.
O-Field		± % RSD	± % RSD	± % RSD	± % RSD	± % RSD	± % RSD	± % RSD
OF1D	9/25/96	0.4±6.0	2.4±0.2	BDL	BDL	0.5±2.0	0.2	BDL
OF2D	9/25/96	0.3±5.2	5.0±1.9	BDL	BDL	0.1±4.7	0.2±0.3	BDL
OF3D	9/25/96	0.7±6.5	3.7±1.8	BDL	BDL	0.2±4.1	0.2±7.4	BDL
OF4D	9/25/96	0.6±11.0	6.0±0.9	BDL	BDL	0.3±2.7	0.2±2.0	BDL
OF5D	9/25/96	0.4±10.8	4.5±1.2	BDL	BDL	0.1±3.4	0.2±5.7	BDL
OF6D	9/25/96	0.4±10.9	2.6±1.4	BDL	BDL	BDL	0.3±2.2	BDL
OF7D	9/25/96	0.5±11.6	3.4±2.7	BDL	BDL	0.1±4.8	0.2±0.6	BDL
OF 1P	9/28/96	0.3±4.0	4.2±1.9	BDL	BDL	0.1±4.8	BDL	BDL
OF7 P	9/28/96	0.3±9.4	3.0±0.6	BDL	BDL	BDL	1.5±6.9	BDL
Avg. OF	9/28/96	**0.4±8.4**	**3.9±1.4**	**BDL**	**BDL**	**0.2±2.9**	**0.3±2.8**	**BDL**
Churchville								
CV1D	9/24/96	0.4±11.6	4.1±2.9	0.1±89.2	BDL	0.1±56.2	0.3±34.8	BDL
CV2D	9/24/96	0.4±5.3	2.6±0.5	BDL	BDL	0.1±1.3	0.5±1.3	BDL
CV3D	9/24/96	0.3±16.9	1.7±1.9	BDL	BDL	BDL	0.3±1.8	BDL
CV4D	9/24/96	0.3±17.9	1.5±7.0	BDL	BDL	BDL	0.6±4.4	BDL
CV5D	9/24/96	0.4±11.6	1.7±0.7	BDL	BDL	BDL	0.2±1.3	BDL
CV6D	9/24/96	0.3±56.9	1.7±7.2	BDL	BDL	BDL	0.2±4.4	BDL
CV7D	9/24/96	0.3±10.4	3.0±1.4	BDL	BDL	BDL	0.2±2.5	BDL
CV2P	9/29/96	0.2±16.8	3.7±0.7	BDL	BDL	BDL	BDL	BDL
CV5P	9/29/96	0.2±25.5	3.1±1.8	BDL	BDL	BDL	BDL	BDL
CV2B	9/29/96	0.2±15.8	5.2±1.7	BDL	BDL	0.2±5.5	0.3±3.6	BDL
CV6B	9/29/96	0.2±6.6	4.5±2.0	BDL	BDL	0.3±4.8	0.2±2.1	BDL
Avg. CV		**0.3±17.8**	**3.0±2.5**	**0.1±89.2**	**BDL**	**0.1±6.2**	**0.3±5.1**	**BDL**
Canal Cr								
CC1D	9/23/96	0.5±5.0	2.1±1.2	BDL	BDL	0.1±3.7	0.3±1.2	BDL
CC2D	9/23/96	0.6±8.8	2.6±0.3	BDL	BDL	0.2±5.4	0.4±1.3	BDL
CC3D	9/23/96	0.7±10.6	3.7±0.8	BDL	BDL	0.2±0.2	0.4±2.0	BDL
CC4D	9/23/96	0.6±9.6	4.9±0.4	BDL	BDL	0.2±4.7	0.4±0.3	BDL
CC5D	9/23/96	0.9±5.0	8.7±1.1	BDL	BDL	0.3±0.5	0.7±2.0	BDL
CC7D	9/23/96	0.7±16.7	3.5±2.8	0.1±88.5	BDL	0.3±25.0	1.1±4.9	BDL
CC3P	9/28/96	0.4±10.0	2.6±1.3	BDL	BDL	0.2±8.2	0.2±1.9	BDL
CC6P	9/28/96	0.3±12.8	2.4±2.1	BDL	BDL	BDL	BDL	BDL
CC3B	9/29/96	0.7±6.9	3.6±1.3	BDL	BDL	0.6±0.5	0.4±3.0	BDL
CC5B	9/29/96	0.6±3.6	5.7±2.3	BDL	BDL	0.7±3.4	0.4±6.5	0.2±38.4
CC6B	9/29/96	0.7±7.9	3.5±2.5	BDL	BDL	0.5±4.3	0.3±5.6	BDL
CC7B	9/29/96	0.7±2.0	5.5±1.4	BDL	BDL	0.5±1.8	0.4±2.9	BDL
Avg. CCD/P/B		**0.6±7.2**	**3.9±1.8**	**0.1±88.5**	**BDL**	**0.4±3.0**	**0.3±3.3**	**0.2±38.4**
Edgewood								
EBB	9/27/96	0.8±5.0	5.7±1.2	BDL	BDL	0.6±7.2	0.6±5.3	BDL
YCB	9/26/96	0.7±0.6	5.8±2.1	BDL	BDL	0.7±1.6	0.4±2.0	BDL
BPB	9/27/96	0.7±5.8	4.3±2.2	BDL	BDL	0.2±8.7	0.3±6.4	BDL
LCB	9/26/96	0.6±8.1	6.3±2.2	BDL	BDL	0.6±2.5	0.4±5.2	BDL
GSB	9/26/96	0.6±4.7	5.5±1.3	BDL	BDL	0.5±3.8	0.3±1.6	BDL
NSB	9/27/96	0.6±3.1	3.9±1.7	BDL	BDL	0.8±2.3	0.1±3.7	BDL
Avg. Edgwd		**0.7±4.6**	**5.3±1.8**	**BDL**	**BDL**	**0.6±4.4**	**0.4±4.0**	**BDL**

Table 4.7 (Cont.) Metal Analysis of Bees and Pollen by ICP/MS

		Cr	Cu	Ga	Mn	Ni	Pb	Rb
Location	Date	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.
O-Field		± % RSD	± % RSD	± % RSD	± % RSD	± % RSD	± % RSD	± % RSD
OF1D	9/25/96	0.8±2.8	23.6±0.6	BDL	51.2±1.0	BDL	0.3±1.1	11.9±0.2
OF2D	9/25/96	0.7±2.0	16.9±0.5	0.2±3.5	109.0±1.1	0.2±6.3	1.1±2.1	10.8±1.3
OF3D	9/25/96	0.6±2.6	20.6±0.8	0.2±0.1	101.0±3.9	0.2±7.4	0.3±1.2	15.1±2.1
OF4D	9/25/96	0.6±1.2	21.5±2.2	0.3±1.1	138.0±1.7	0.1±2.1	0.4±1.3	12.4±1.4
OF5D	9/25/96	0.7±2.7	19.6±1.3	0.2±3.3	92.2±5.4	0.2±2.6	0.6±1.7	7.6±0.7
OF6D	9/25/96	0.8±2.0	18.7±0.3	0.1±4.4	49.5±0.7	0.2±8.0	0.4±1.0	9.2±1.4
OF7D	9/25/96	0.7±5.0	22.6±2.8	0.2±2.2	176.0±2.7	BDL	0.2±2.7	9.2±3.3
OF 1P	9/28/96	0.4±11.5	11.9±3.5	0.2±1.5	22.8±3.3	0.8±0.9	BDL	13.3±1.7
OF7 P	9/28/96	0.6±4.0	8.5±1.5	0.2±4.6	23.6±3.7	2.4±3.0	BDL	14.3±0.5
Avg. OF	9/28/96	**0.7±3.8**	**18.2±1.5**	**0.2±2.3**	**84.8±2.6**	**0.5±3.4**	**0.4±1.2**	**11.5±1.4**
Churchville								
CV1D	9/24/96	0.8±14.4	24.3±1.1	0.3±43.6	67.6±1.2	0.2±64.1	0.4±20.4	19.1±2.0
CV2D	9/24/96	0.7±4.3	27.8±0.9	0.1±3.1	95.5±1.5	0.3±1.3	0.2±0.8	16.0±1.0
CV3D	9/24/96	0.6±5.3	25.5±4.9	BDL	48.8±3.0	0.2±7.9	0.4±1.4	14.5±2.6
CV4D	9/24/96	0.9±7.9	21.7±5.8	BDL	39.1±5.2	0.5±8.3	0.3±4.6	14.2±5.8
CV5D	9/24/96	0.6±6.8	22.4±2.0	BDL	63.9±2.2	BDL	0.3±1.7	13.0±1.2
CV6D	9/24/96	0.6±5.4	21.3±8.4	BDL	36.3±6.5	BDL	0.3±6.3	12.6±9.8
CV7D	9/24/96	0.6±1.7	16.7±1.2	0.1±2.5	34.4±3.5	BDL	0.6±0.8	10.8±0.1
CV2P	9/29/96	0.5±2.7	11.0±3.4	0.2±6.8	32.9±4.6	0.4±1.4	BDL	14.1±0.5
CV5P	9/29/96	0.4±2.5	13.4±3.4	0.2±4.7	24.4±2.6	0.5±4.0	0.2±2.2	19.5±4.3
CV2B	9/29/96	0.6±3.3	24.1±0.1	0.2±0.5	117.0±3.1	BDL	0.2±1.3	8.2±0.7
CV6B	9/29/96	0.6±4.8	27.3±1.2	0.2±3.3	125.0±1.4	0.2±6.1	0.2±2.5	6.5±2.9
Avg. CV		**0.6±5.4**	**21.4±2.9**	**0.1±5.9**	**62.3±3.2**	**0.2±8.5**	**0.3±3.8**	**13.5±2.8**
Canal Cr								
CC1D	9/23/96	0.5±3.5	31.2±1.8	BDL	75.0±3.7	BDL	0.3±4.2	17.0±3.1
CC2D	9/23/96	0.7±2.3	27.6±2.0	0.1±6.3	113.0±2.5	0.2±8.5	0.2±1.5	10.7±3.6
CC3D	9/23/96	0.6±5.7	25.1±0.3	0.2±3.1	164.0±3.5	BDL	0.5±1.0	10.6±1.3
CC4D	9/23/96	0.6±3.6	27.0±1.6	0.2±4.1	175.0±1.9	BDL	0.5±1.5	9.8±1.4
CC5D	9/23/96	0.7±4.0	30.5±1.7	0.4±1.9	276.0±2.7	0.3±7.2	0.8±1.6	10.9±1.3
CC7D	9/23/96	0.7±10.3	31.4±0.8	0.2±46.6	131.0±6.0	0.8±11.7	0.3±30.5	14.4±0.7
CC3P	9/28/96	0.3±3.4	10.1±1.1	0.1±5.2	22.3±2.2	2.2±1.6	BDL	14.4±0.8
CC6P	9/28/96	0.3±7.3	8.2±2.4	0.1±6.8	22.1±4.1	0.4±10.0	0.1±2.1	10.2±2.0
CC3B	9/29/96	0.5±1.4	29.0±3.3	0.2±3.4	142.0±2.3	0.3±5.6	0.4±6.0	9.4±1.4
CC5B	9/29/96	0.6±4.8	25.2±2.4	0.4±8.5	277.0±3.9	0.2±20.1	0.8±2.1	6.3±1.8
CC6B	9/29/96	0.4±4.8	27.9±0.6	0.2±7.8	183.0±2.9	0.2±6.3	0.6±2.5	7.3±2.9
CC7B	9/29/96	0.4±5.2	29.3±1.7	0.3±4.8	151.0±2.3	BDL	0.4±1.7	6.4±2.4
Avg. CCD/P/B		**0.4±4.5**	**21.6±1.9**	**0.2±6.1**	**132.9±3.0**	**0.6±7.3**	**0.4±2.4**	**9.0±1.9**
Edgewood								
EBB	9/27/96	0.5±11.5	25.8±1.2	0.3±10.7	148.0±2.1	0.5±7.3	0.8±5.4	5.9±2.3
YCB	9/26/96	0.4±1.6	28.4±2.4	0.3±3.1	147.0±1.0	BDL	0.3±2.7	9.8±2.0
BPB	9/27/96	0.5±3.5	20.2±1.6	0.2±10.1	175.0±5.8	0.1±19.9	0.4±3.4	4.7±1.7
LCB	9/26/96	0.4±6.0	26.4±1.7	0.3±2.0	204.0±3.1	0.2±19.1	0.6±3.1	8.3±3.1
GSB	9/26/96	0.3±3.7	27.7±2.7	0.3±4.3	150.0±1.6	BDL	0.7±1.2	9.3±3.4
NSB	9/27/96	0.5±1.5	29.4±2.2	0.2±5.8	69.5±1.2	0.3±2.4	0.6±0.9	5.6±3.1
Avg. Edgwd		**0.4±4.6**	**26.3±2.0**	**0.3±6.0**	**148.9±2.51**	**0.2±8.1**	**0.6±2.8**	**7.3±2.6**

Table 4.7 (Cont.) Metal Analysis of Bees and Pollen by ICP/MS

		Se	Sr	Tl	U	V	Zn
Location	Date	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.	Ave. Conc.
O-Field		± % RSD	± % RSD	± % RSD	± % RSD	± % RSD	± % RSD
OF1D	9/25/96	0.3±1.2	2.0±0.4	BDL	BDL	1.2±3.7	70.4±0.5
OF2D	9/25/96	0.3±16.8	3.3±1.8	BDL	BDL	1.0±1.5	97.9±0.9
OF3D	9/25/96	0.3±14.3	3.1±1.2	BDL	BDL	0.8±2.0	72.9±1.9
OF4D	9/25/96	0.4±20.6	3.5±1.6	BDL	BDL	0.7±2.3	95.5±2.4
OF5D	9/25/96	0.3±8.7	3.0±0.7	BDL	BDL	0.8±6.7	96.1±0.7
OF6D	9/25/96	0.3±23.7	1.8±1.3	BDL	BDL	1.2±1.9	80.3±0.7
OF7D	9/25/96	0.4±16.4	4.3±1.4	BDL	BDL	1.0±2.4	75.1±1.9
OF 1P	9/28/96	BDL	4.5±0.9	BDL	BDL	0.7±8.1	36.3±1.8
OF7 P	9/28/96	BDL	5.0±0.4	BDL	BDL	0.5±6.1	27.3±1.2
Avg. OF	9/28/96	**0.3±11.3**	**3.4±1.1**	**BDL**	**BDL**	**0.9±3.9**	**72.4±1.3**
Churchville							
CV1D	9/24/96	0.5±26.6	1.1±13.1	BDL	BDL	1.0±8.7	88.6±1.8
CV2D	9/24/96	0.4±24.5	1.1±0.4	BDL	BDL	0.9±1.0	93.2±0.7
CV3D	9/24/96	0.3±15.8	0.8±2.9	BDL	BDL	0.6±2.9	87.0±3.0
CV4D	9/24/96	0.5±18.7	0.8±5.4	BDL	BDL	1.2±4.4	76.2±5.2
CV5D	9/24/96	0.3±29.6	0.7±0.7	BDL	BDL	0.7±3.3	76.0±1.3
CV6D	9/24/96	0.3±25.4	0.7±8.0	BDL	BDL	0.7±3.9	72.4±8.6
CV7D	9/24/96	0.3±16.1	1.1±0.4	BDL	BDL	0.9±0.4	77.2±0.4
CV2P	9/29/96	BDL	1.3±0.5	BDL	BDL	0.7±2.7	37.0±0.6
CV5P	9/29/96	BDL	1.4±0.9	BDL	BDL	0.7±2.4	40.3±2.4
CV2B	9/29/96	0.2±14.2	1.9±1.5	BDL	BDL	0.9±6.3	107.0±0.5
CV6B	9/29/96	0.3±20.5	1.6±1.6	BDL	BDL	0.9±4.9	105.0±1.9
Avg. CV		**0.3±17.4**	**1.1±3.2**	**BDL**	**BDL**	**0.8±3.7**	**78.2±2.4**
Canal Cr							
CC1D	9/23/96	0.4±32.8	1.1±1.5	BDL	BDL	0.7±2.1	89.7±2.3
CC2D	9/23/96	0.5±14.8	1.8±1.2	BDL	BDL	0.8±1.1	83.2±1.8
CC3D	9/23/96	0.4±9.0	2.5±0.8	BDL	BDL	0.8±4.9	92.2±0.5
CC4D	9/23/96	0.3±8.6	2.3±0.7	BDL	BDL	0.9±0.8	104.0±1.2
CC5D	9/23/96	0.4±27.3	4.7±1.0	BDL	BDL	0.8±2.1	138.0±0.4
CC7D	9/23/96	0.5±15.8	2.1±4.8	BDL	BDL	0.9±11.2	97.8±0.3
CC3P	9/28/96	BDL	2.3±2.1	BDL	BDL	0.5±1.9	26.3±0.9
CC6P	9/28/96	BDL	2.2±0.7	BDL	BDL	0.5±2.7	30.0±1.3
CC3B	9/29/96	0.3±20.0	2.2±0.5	BDL	BDL	0.7±3.8	114.0±0.3
CC5B	9/29/96	0.3±2.3	3.5±2.0	BDL	BDL	0.8±3.0	120.0±1.7
CC6B	9/29/96	0.2±18.4	2.2±1.4	BDL	BDL	0.6±3.0	116.0±1.2
CC7B	9/29/96	0.3±47.7	2.8±1.2	BDL	BDL	0.6±0.4	119.0±1.9
Avg. CCD/P/B		**0.2±14.7**	**2.5±1.3**	**BDL**	**BDL**	**0.6±2.5**	**87.6±1.2**
Edgewood							
EBB	9/27/96	0.2±14.1	3.2±1.1	BDL	BDL	0.6±9.9	124.0±1.4
YCB	9/26/96	0.2±8.7	2.9±1.9	BDL	BDL	0.7±2.7	138.0±1.7
BPB	9/27/96	BDL	3.3±0.3	BDL	BDL	0.7±4.3	99.1±1.7
LCB	9/26/96	0.2±41.4	3.7±2.1	BDL	BDL	0.7±6.1	175.0±2.2
GSB	9/26/96	0.2±7.5	2.6±1.9	BDL	BDL	0.6±1.5	118.0±0.9
NSB	9/27/96	0.2±37.3	2.6±2.6	BDL	BDL	0.6±3.8	105.0±2.6
Avg. Edgwd		**0.2±18.7**	**3.1±1.7**	**BDL**	**BDL**	**0.7±4.7**	**126.5±1.8**

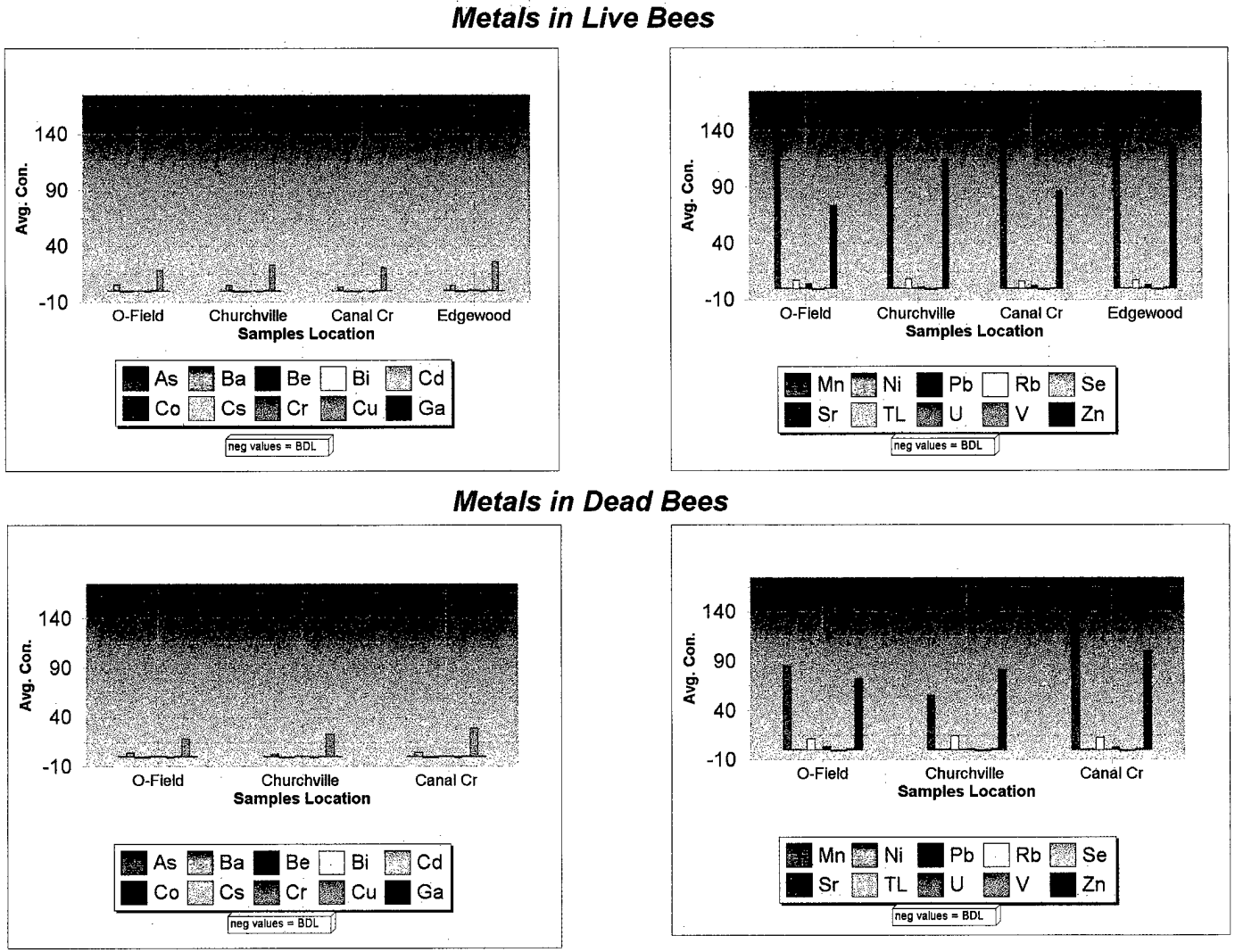


Figure 4.31. Metals in 1996 Live and Dead Honey Bee Samples

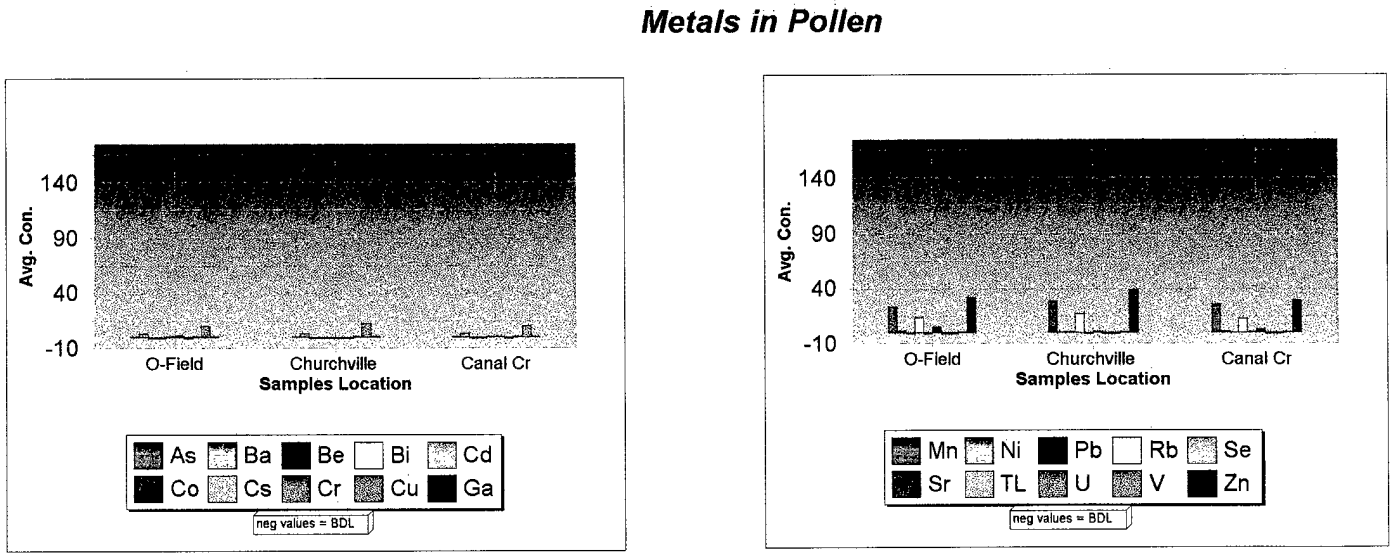


Figure 4.32. Metals in 1996 Pollen Samples

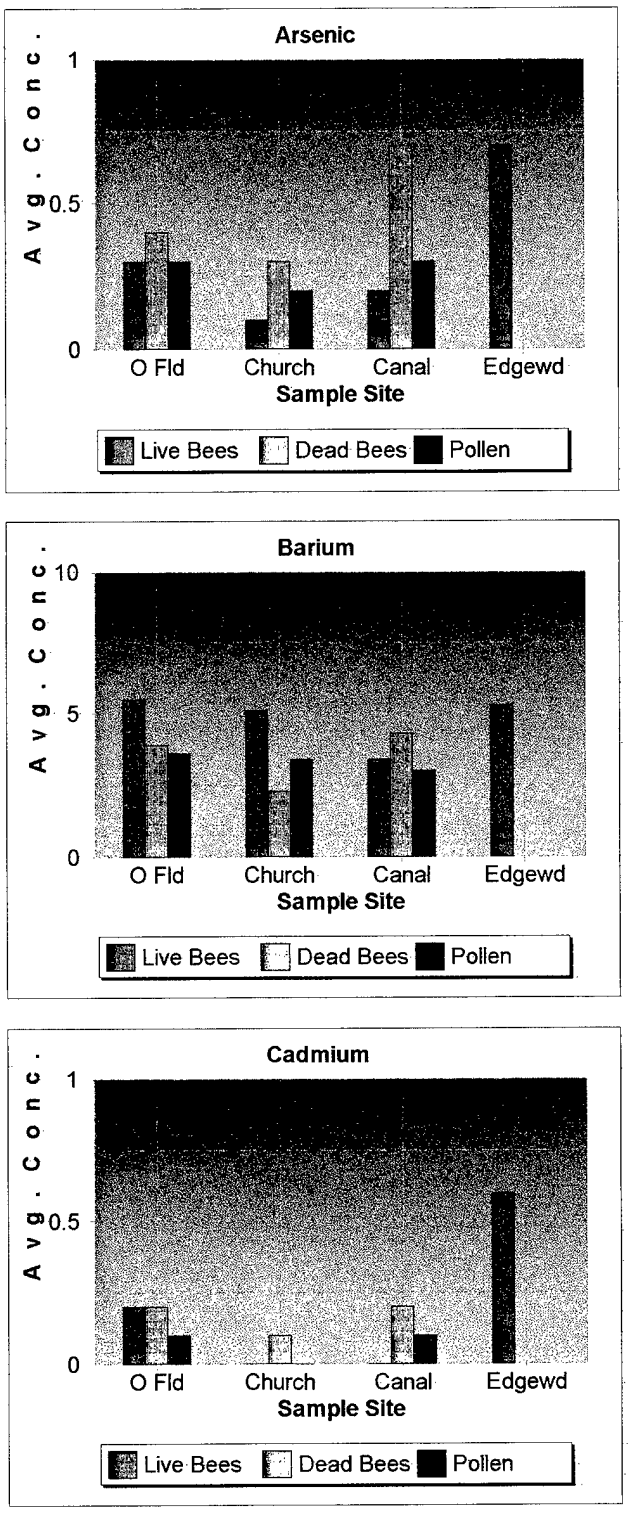
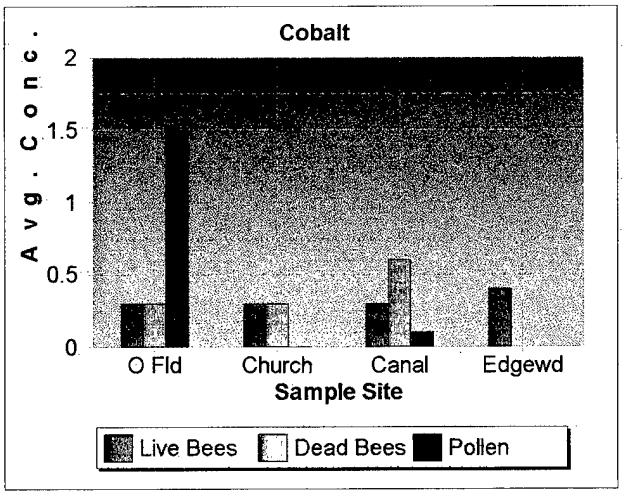
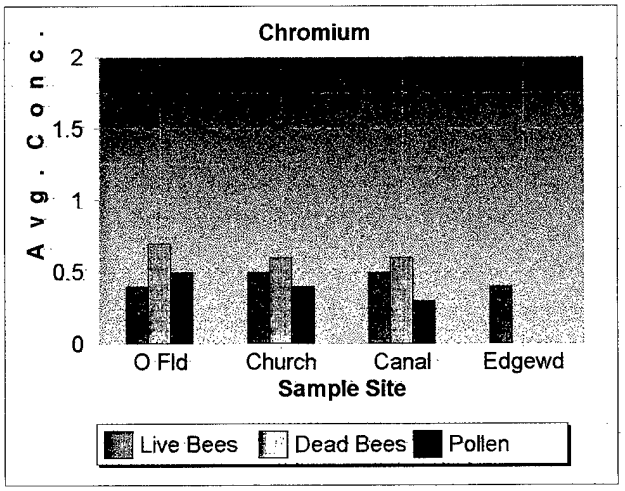


Figure 4.33. Element Levels from 1996 APG Field Applications
(ppm oven-dried weight)





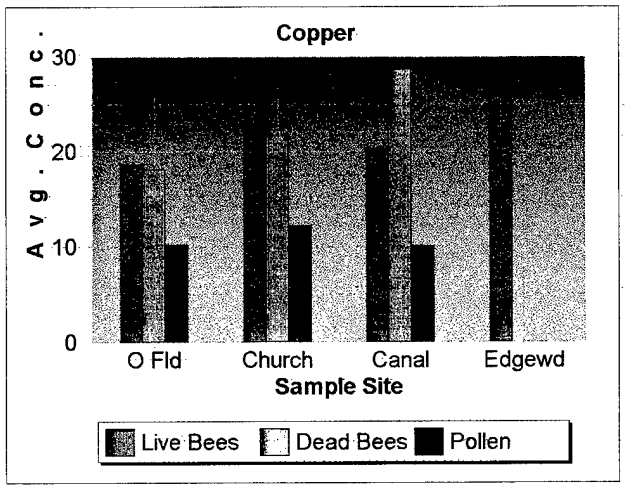
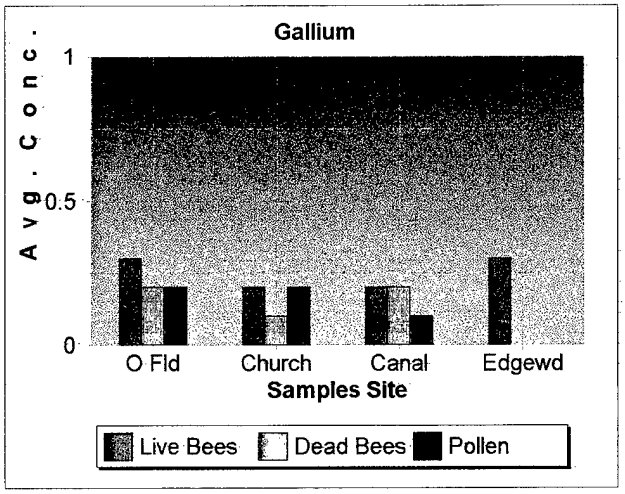
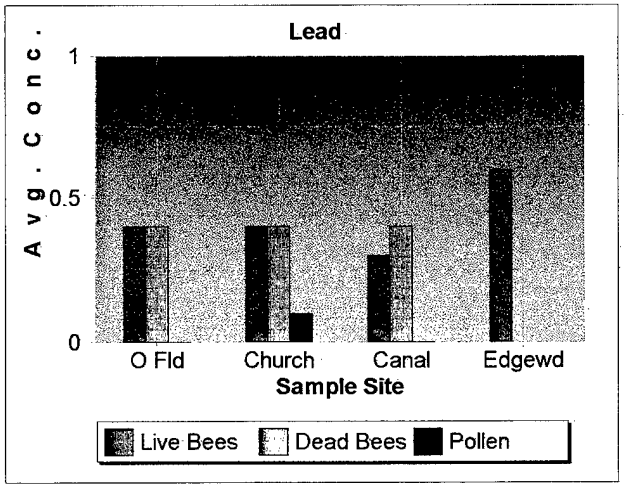


Figure 4.34. Element Levels from 1996 APG Field Applications
(ppm oven-dried weight)





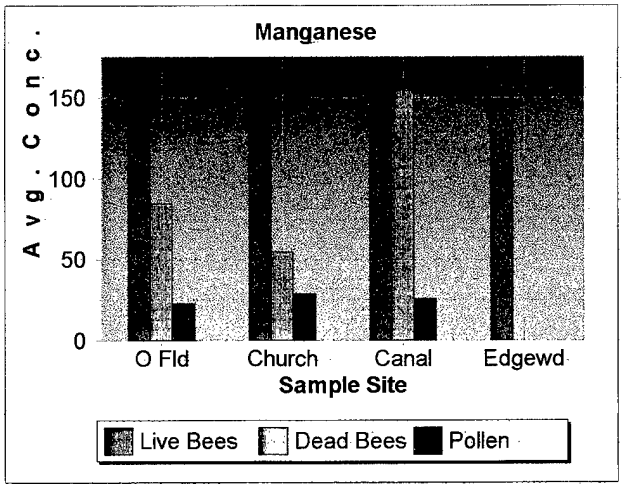


Figure 4.35. Element Levels from 1996 APG Field Applications
(ppm oven-dried weight)

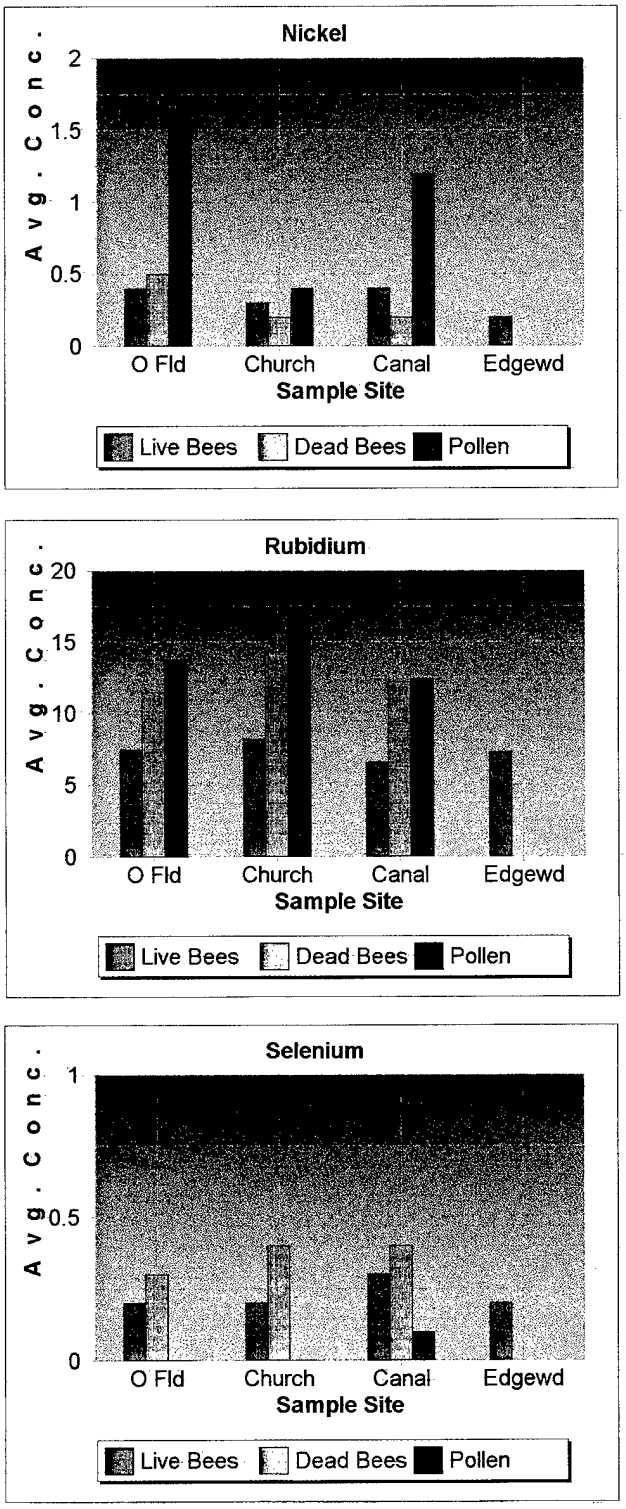
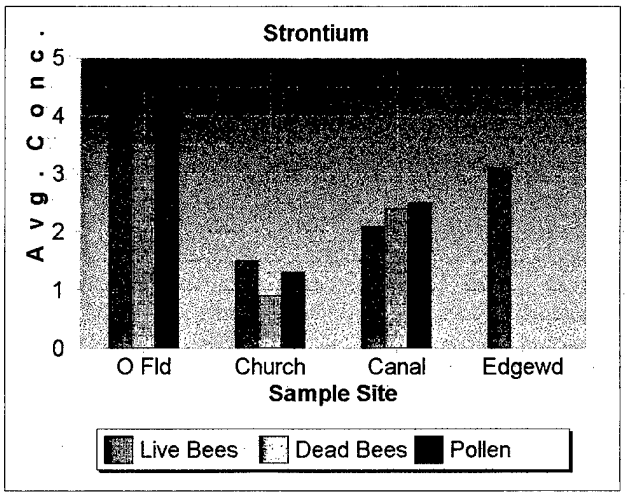
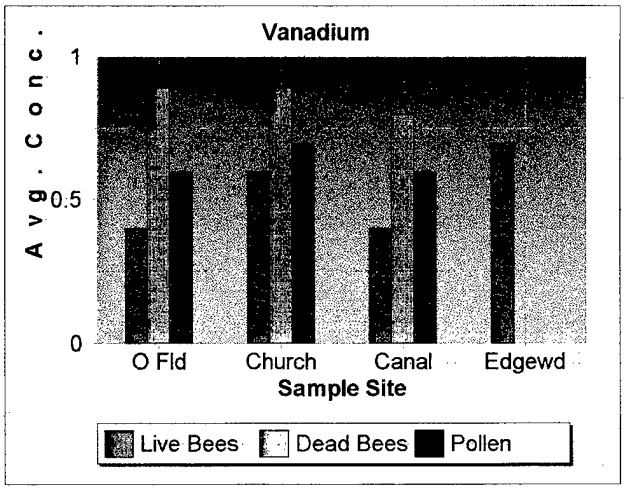


Figure 4.36. Element Levels from 1996 APG Field Applications
(ppm oven-dried weight)





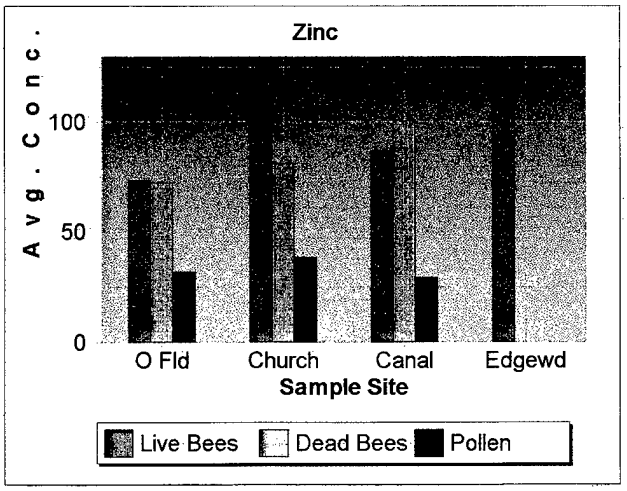


Figure 4.37. Element Levels from 1996 APG Field Applications
(ppm oven-dried weight)

foraged in the West Branch Canal Creek and Edgewood areas, registering up to 0.7 ppm of each. In uncontaminated bee populations, cadmium is generally in the 0.1 to 0.2 ppm range and arsenic in the 0.1 to 0.5 ppm range. APG levels of all three elements, though, are well below those observed in bees from smelter and mine waste sites where arsenic can approach 18 ppm, cadmium can approach 7 ppm and lead can approach 100 ppm (10).

Uptake of several elements by plants is suggested from the pollen analysis results. The cobalt concentration in pollen collected at Old O Field is five times the level measured in honey bees. Cobalt was not recorded in the off-site pollen at Churchville. There is no evidence of assimilation by the bees, however, since bee levels of cobalt are similar at all sites. Elevated nickel values are found in pollen from both Old O Field and West Branch Canal Creek. Again, within the time frame of the field season, no increase in bee burdens of nickel can be substantiated.

Strontium is the only element among the suite of 20 that were analyzed which shows evidence of uptake by bees from a pollen-borne source. The high strontium level found on average in pollen at Old O Field (about 4.5 ppm) is reflected in both live bee and dead bee tissue levels. To a lesser extent, the same pattern is seen at the West Branch Canal Creek site where the pollen level at 2.5 ppm is reflected in the bees. Churchville, at 1 ppm, has the lowest observed levels.

4.4 Chemical Applications of Artificial Neural Networks (ANNs)

4.4.1 Use of ANNs for Classification of Samples

Post-chemistry activities in the project have focused on processing GC/MS data from the field trials with artificial neural network software (11). The ultimate objectives of neural network applications are to develop techniques through which subtle changes in the chemical fingerprint of a honey bee colony can be detected and correlated with known exposures and bee colony metrics. This is needed: 1) to quickly discern, in an acute exposure event, the presence of a new compound against the normal background of compounds that are normally present in a hive; and 2) to recognize the accumulation of a contaminant in a beehive over time, as would be the case in a chronic exposure scenario.

Because the 1995 APG Pilot Study at West Branch Canal Creek did not place the bee colonies in a highly contaminated area, we chose to develop our initial neural network differentiation activities around exploring the suite of volatile and semi-volatile compounds in individual beehives. The research question was: Is the chemical fingerprint for each hive sufficiently unique to unambiguously distinguish it from the others? We felt that if this challenge could be overcome by the neural network software, then detection of new contaminant compounds arriving in a hive would be feasible.

4.4.2 Manipulation of GC/MS Data for Artificial Neural Network Analysis

The preliminary stage of network design demands that we condense the wealth of spectral information for a single sample into a concise numeric format. The number of data points retained for each air sample was dictated by standard recommendations in the literature on artificial neural networks. Accepted guidelines suggest that the number of points used as input for any classification scheme should be less than half the total number of samples that will be examined by the neural network (12). Since we collected a total of 55 samples during 1995 at APG, our input file was, therefore, restricted to 24 points.

The 24 input points for each air chemistry sample in these preliminary investigations was extracted from its Total Ion Chromatogram (TIC). The TIC simply charts the retention time and total ion abundance for each peak that emerges from the capillary column on the GC. We chose to divide our TIC into eight 5-minute intervals and recorded three items of information for each 5-minute interval: 1) the number of peaks observed in the 5-minute interval; 2) the retention time of the largest peak observed in the interval; and 3) the total ion abundance for the largest peak in the interval. A schematic diagram of this procedure is shown in Fig. 4.38. The finished product from this activity was a matrix of numbers whose rows corresponded to individual samples and whose columns held one of the three values for a given interval.

Concatenated onto the right side of the data matrix was a second grid that provides the network software with the correct identification of the sample that it should learn, i.e., the hive from which the sample was collected. We had seven possible identification categories -- one for each of the six hives in the condo cluster and a seventh category for non-hive samples (blanks or ambient air). A portion of the overall input file is shown in Table 4.8. In the table one can see the three values for intervals 1, 2 and 8, as well as the encoded identification of the hive.

4.4.3 A Back-Propagation Neural Network for Colony Identification

Artificial neural networks are a form of computer intelligence that learn by example. They differ from expert systems in that there are not built on rules. Expert systems reach a conclusion by applying a rule at each stage and selecting the next deductive step based on the results of the test. Neural networks, on the other hand, use a series of examples to extract the numerical linkages between a set of measured parameters (inputs) and the category or result associated with them (outputs). Each set of inputs and outputs constitutes a fact. Between the input and output of the neural net is one or more layers of hidden processing units (neurons). Each neuron in a hidden layer is connected to every input and every output neuron. And each connection has an adjustable weighting factor. An overall picture of our Hive Discrimination Network is show in Fig. 4.39.

The first step in using a neural network is called training. It consists of supplying the network with a set of examples from which to draw the mathematical linkages between the

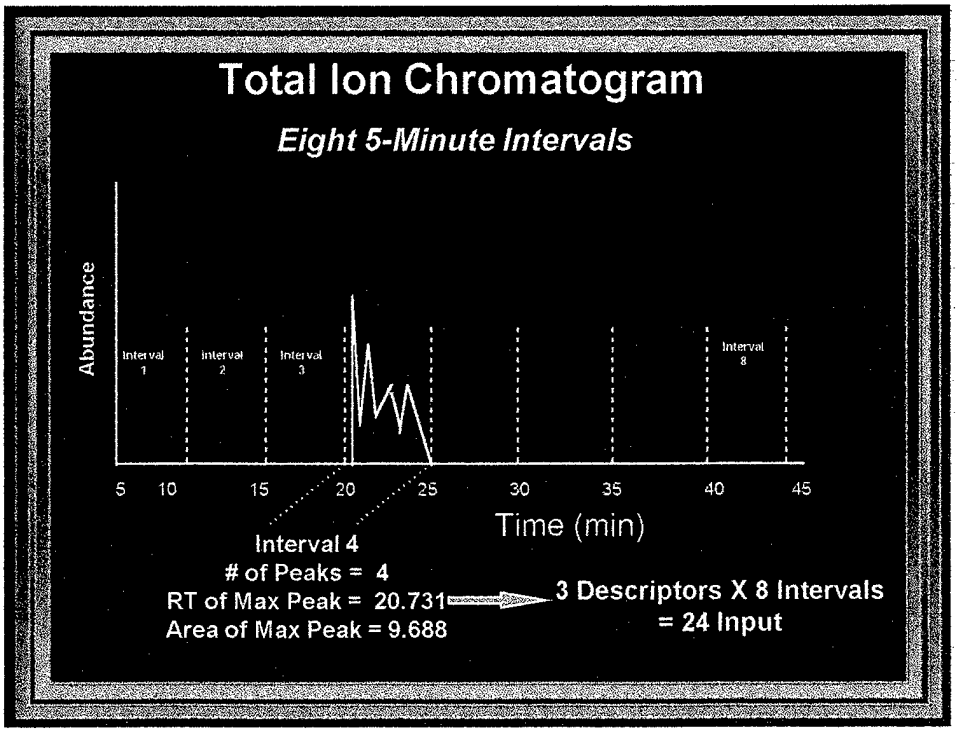


FIGURE 4.38. Digitizing a Total Ion Chromatogram

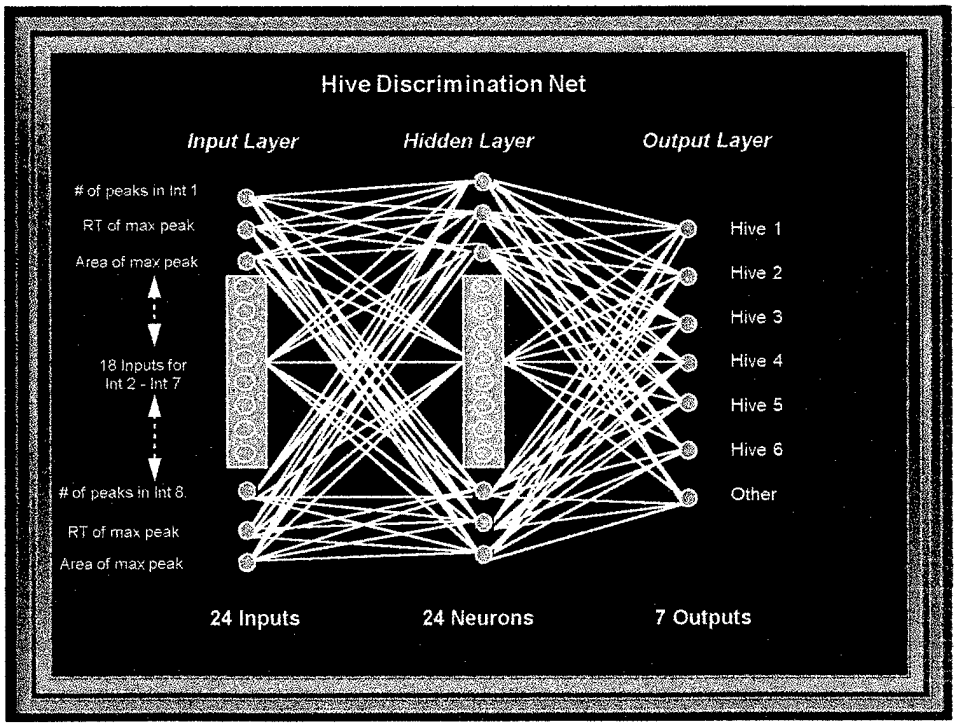


FIGURE 4.39. The Hive Discrimation Network

TABLE 4.8. Digitized TIC Input for ANN Training

Sample	Pk 1	RT 1	Abun 1	Pk 2	RT 2	Abun 2	...	...	Pk 8	RT 8	Abun 8	Hv1	HV2	HV3	HV4	Hv5	Hv6	Oth
1545.1	6	8.103	18.562	2	14.063	7.848			1	45.868	4.361	0	0	0	1	0	0	0
1546.1	3	9.478	100	3	14.176	14.763			1	41.332	1.754	0	0	0	1	0	0	0
1546.2	1	9.926	22.518	0	0	0			1	45.892	1.928	0	0	0	1	0	0	0
1546.3	5	9.934	100	3	14.064	15.595			5	45.878	4.55	0	0	0	1	0	0	0
1546.4	1	8.145	5.443	1	10.09	60.442			1	45.889	2.256	0	0	0	1	0	0	0
1546.5	1	9.942	25.598	4	10.453	16.225			1	43.999	100	0	0	0	1	0	0	0
1546.6	0	0	0	2	11.681	14.335			2	43.998	100	0	0	0	1	0	0	0
1546.7	0	0	0	1	11.689	30.689			2	44.014	100	0	0	0	0	1	0	0
...						...				...								...
...						...				...								...
...						...				...								...
1560.1	1	7.302	9.207	2	10.236	48.77			0	0	0	1	0	0	0	0	0	0
1560.2	1	6.324	100	1	10.796	1.687			0	0	0	0	1	0	0	0	0	0
1560.3	2	7.966	12.79	2	10.665	100			1	45.85	2.331	0	0	1	0	0	0	0
1560.4	1	9.687	1.419	1	10.665	100			1	45.855	1.774	0	0	0	1	0	0	0
1560.5	2	5.94	100	0	0	0			0	0	0	0	0	0	0	1	0	0
1560.6	1	6.499	100	1	11.029	2.158			0	0	0	0	0	0	0	0	1	0
1560.7	2	8.17	42.16	5	10.033	40.408			2	45.867	4.846	0	0	0	0	0	0	1
1562.1	8	8.129	100	3	11.616	14.162			0	0	0	0	0	0	0	0	0	1

inputs (chemical parameters from the TIC) and the outputs (the hive ID). We conducted a training run using 80% of our samples, i.e., 44 of the 55. The balance of samples, 11 in number and selected at random from the total set, were withheld from training so that they could be used to test the ability of the trained network to correctly identify the origin of known samples.

The neural network uses the training files to adjust the weighting factors on each connection so that application of a set of input data is routed to the correct output. This usually requires the network to iteratively run through the entire training set and readjust its weights many times. The results presented in this report were the response of the network after 500 runs through the training set. The number of runs required to successfully train a network is not readily predicted and must be evaluated through the testing procedure described in the following section.

4.4.4 Testing the Trained Hive Discrimination Networks

As the network trained, the cumulative error associated with a given refinement run was shown on the computer screen. In this manner, we could observe when the network seemed to have reached a stable configuration for identifying samples. Once this had occurred, the network was ready for testing.

In testing, one supplies a trained network with samples it has never seen. The network sends each sample through the weighted set of neuron connections and a value is reported at each output. Outputs for a classification scheme, such as we were performing here, yield values between 1.00 (a perfect match) and 0.00 (no similarity whatsoever).

The 20% withheld samples (11 out of 55) were submitted to our trained network. The network output is provided in both tabular form (Table 4.9) and graphical form (Figs. 4.40 through 4.42). The height of the bar above each hive ID registers how much similarity the network found between the test case and its learned hive fingerprint. Note that for each test fact, strong similarity is shown to only one hive location, and in each case the large bar corresponds to the correct hive number. All the others are practically zero.

Thus, we have successfully been able to correctly identify the hive from which an air sample is derived. From examination of the chemical fingerprints for the hives, we presume the hive signature is carried, at least in part, by the suite of terpenes characteristic of the pine boards used in hive construction. Essentially, we can tell one pine board from another based on its terpene mix! Given this sensitive discrimination ability, we feel confident in our capacity to detect the presence of contaminant compounds brought back to the hive by the forager bees.

Development of a network capable of recognizing the appearance of contaminants in hive atmospheres is underway. Instead of using the entire TIC for entry into the training base, we

TABLE 4.9. Hive ID Test Results

Test #	1	2	3	4	5	6
Hive 1	0.0027	0.0005	0.0003	0.9900	0.0003	0.0008
Hive 2	0.9725	0.9734	0.0144	0.0069	0.0066	0.9673
Hive3	0.0284	0.0144	0.0791	0.0013	0.9429	0.0347
Hive 4	0.0452	0.0435	0.9009	0.0003	0.0403	0.0967
Hive 5	0.0152	0.0025	0.0103	0.0721	0.0169	0.0018
Hive 6	0.0003	0.0262	0.0245	0.0044	0.0081	0.0008
Other	0.0037	0.0005	0.0008	0.0096	0.0342	0.0584

Test #	7	8	9	10	11
Hive 1	0.0003	0.0005	0.0003	0.0003	0.0013
Hive 2	0.0279	0.0054	0.0061	0.0003	0.0262
Hive3	0.9751	0.0008	0.9522	0.0201	0.0003
Hive 4	0.0555	0.9656	0.0787	0.9913	0.0552
Hive 5	0.0061	0.0088	0.0284	0.0044	0.9102
Hive 6	0.0042	0.0013	0.0005	0.0203	0.0003
Other	0.0066	0.0008	0.0071	0.0657	0.0093

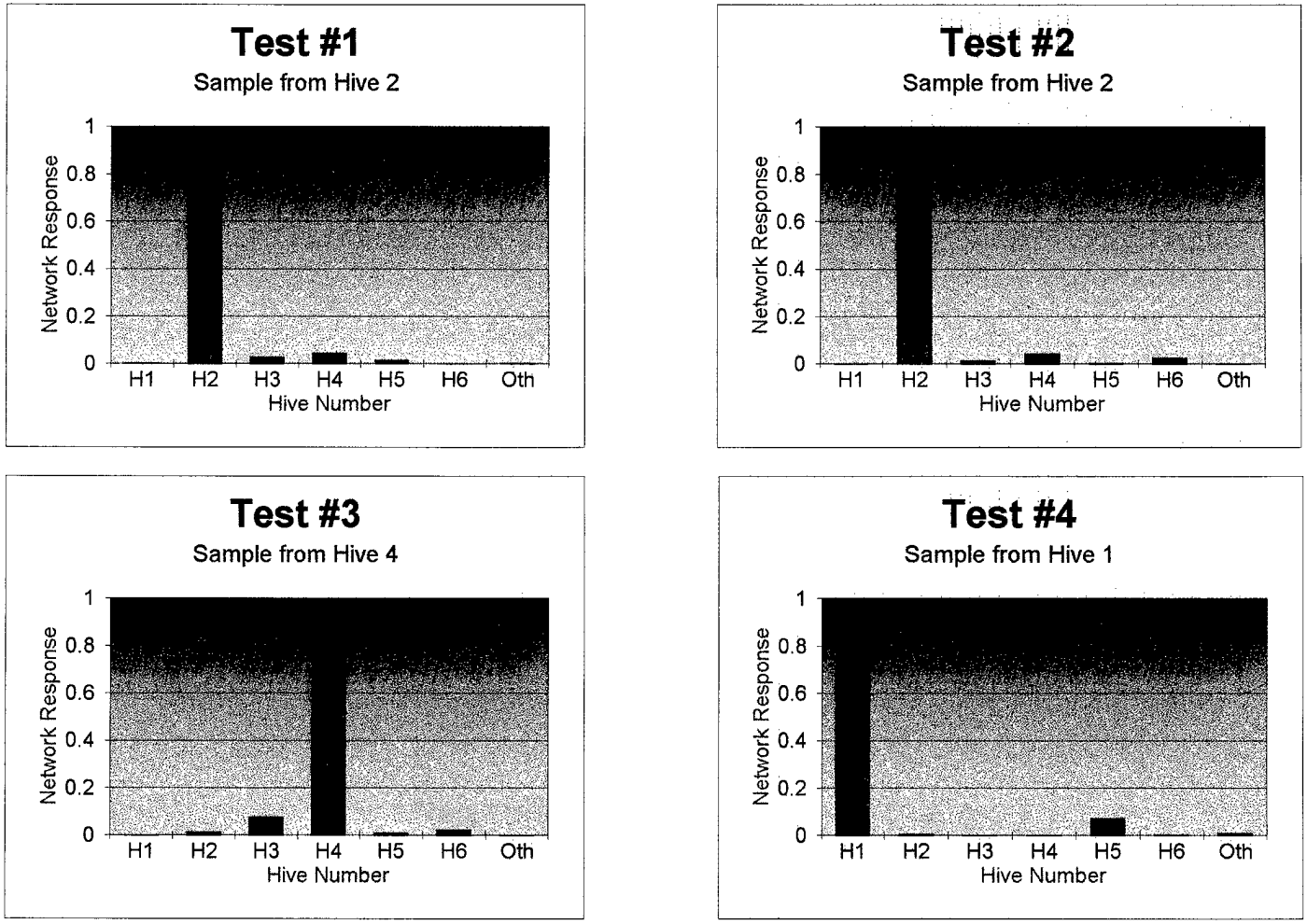


Figure 4.40. Network Response to Previously Unseen Samples

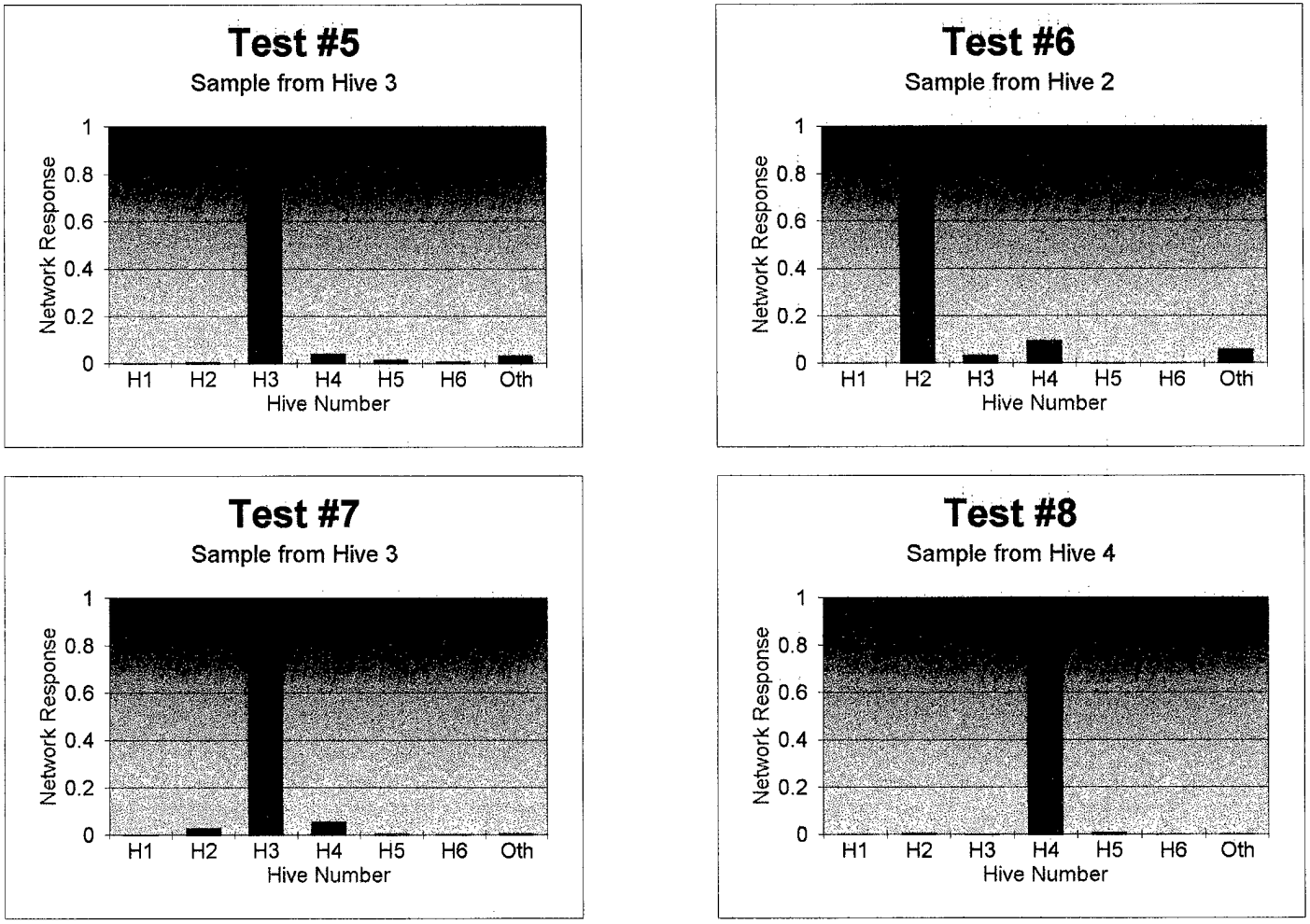
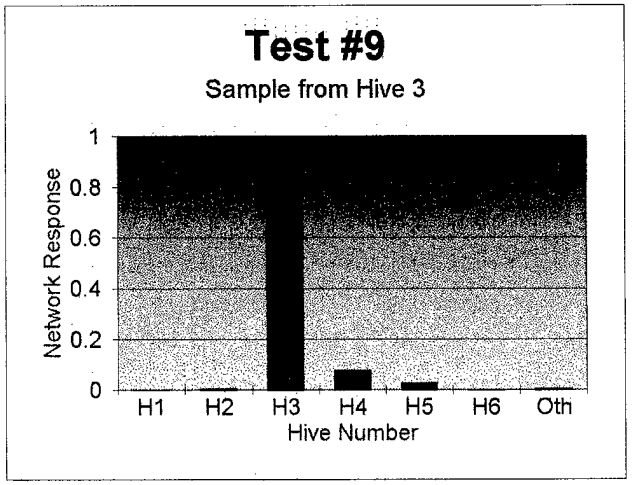
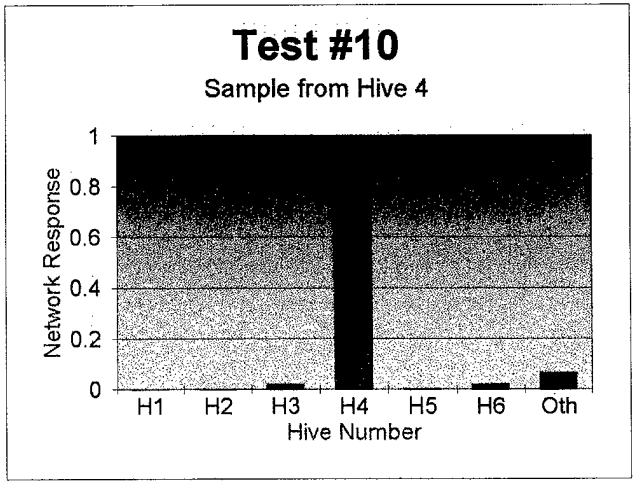


Figure 4.41. Network Response to Previously Unseen Samples





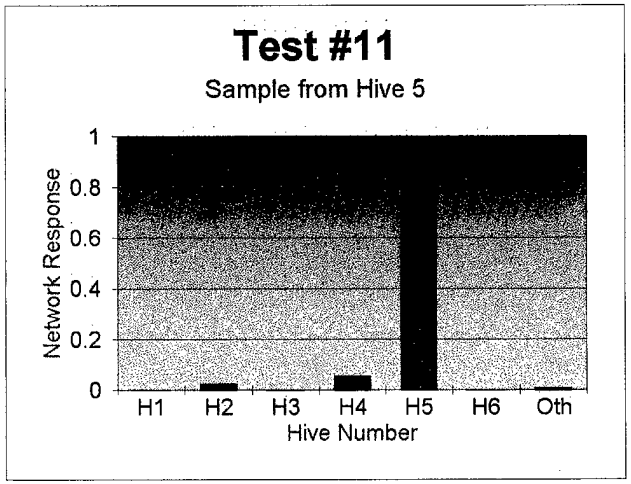


Figure 4.42. Network Response to Previously Unseen Samples

are codifying a specific retention times, TIC abundances and the strengths of characteristic mass spectral peaks for our training basis. This should minimize the amount of data to be processed and speed up response time of the trained network. An added feature of a trained network is that it can be used to generate a visual display of the "general" TIC fingerprint for a given colony. Graphic visualization of a generalized TIC should allow rapid identification of previously undocumented components in hive atmospheres.

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Section 5

HAZARD ASSESSMENT AND CONCLUSIONS

5.1 Hazard Assessment Framework and Guideline Development

The 1996 deployment of honey bee colonies across the APG post provided both an opportunity to further refine the use of honey bees as continuous monitors in the context of military unique chemicals and to conduct a preliminary survey of environmental conditions at APG. The 1996 tests spanned the period of late June through November. Monitoring will be resumed at APG in mid-April of 1997 and continued through June. Combined with the 1996 biomonitoring at APG, this investigation will provide an extensive database for a full year, excluding the winter months of December through March when bees do little foraging. Because contaminant bioavailability may vary depending on time of year, weather conditions, plants in blossom, and other factors, any biomonitoring program using bees should span an entire forage season.

The APG field applications are part of an ongoing five-year program aimed at refining methods for accomplishing real-time monitoring of colony behavior to detect the presence of a bioavailable contaminant or multiple contaminants. An additional goal is to identify the data needed to establish a specific cause and effect link. Much of this activity will be focused on controlled, dose-response trials to be conducted in Montana beginning in the spring of 1997.

Because real-time measuring of exposures to bioavailable chemicals with honey bees is a new technology, a discussion of some of the advantages and limitations of the approach in the context of hazard assessments is warranted.

5.1.1 Advantages and Limitations of Honey Bees as Real-Time Monitors

Probably the greatest strength of honey bees as biomonitors relies upon their multi-media, spatial and temporal averaging. Based on the 1996 data, nucleus colonies of bees made thousands of foraging trips per day (e.g., the actual numbers ranged as high as 70,000 trips per day in Montana, with 10,000-30,00 trips typical of a day in Maryland). Although events such as hurricane Fran reduced flight activity at APG sites to zero, on all but the stormiest days, each colony made a few hundred to thousands of forays from the hive. However, the advantages of honey bees as biomonitors of a wide array of bioavailable contaminants across large areas must be balanced against the limitations of the technique.

Because each colony may vary its foraging patterns from day to day, the best sampling coverage is accomplished by deploying more than one colony at each sample location. Thus, seven colonies were deployed at each of the three primary sampling sites (i.e., West Branch

Canal Creek, Old O Field, and the Churchville reference site), two colonies were set out at each of the APG residential and work areas, and seven colonies were used for additional sampling at Old O Field. Similarly, because floral resources change with seasons, as may contaminant bioavailability, continuous monitoring of colony response across an entire foraging season rather than intermittent monitoring is desirable. These strategies increase the likelihood that at least one of the colonies will contact contaminants of interest.

In order to link colony responses to specific stressors, chemical sampling is needed to determine exposure baselines. Additional chemical sampling should be conducted when a pre-determined colony response parameter exceeds an action threshold. However, during the initial development stages of the present studies, it was necessary to conduct periodic chemical sampling because the real-time, critical colony response functions were still being identified and software programs were still being written to provide the action alert flags.

Another limitation of the technique is that bees do not fly at night, during inclement weather, or during the winter in northern climates when temperatures are low and plants are not in blossom. In warmer, southern climates, honey bees actively forage all year. Although the inability to fly at these times precludes sampling of a large area, the colonies can still provide single point monitoring data. Bees in cages have been shown to accumulate heavy metals near a lead smelter (1), despite their inability to forage. In addition, Dr. Bromenshenk reviewed a European study that examined metals in bees in hives during the winter, when the colony was clustered and no forage was available. The authors of the study believed that the confined bees would provide a point source monitor for metals, and their data supported that conclusion. Whether this paper has been published is unknown.

Because bees fan to move air through the hive, it is reasonable to view the hive itself as a sampler of an air column, somewhat like a high-volume air sampler. It seems likely that a bee colony acts like a point monitor at night, but becomes an area monitor during the day when weather conditions permit and flowers are available. The advantage over more traditional air monitoring instrumentation is that bee colonies provide area sampling capability, reducing the probability that the monitor will not detect the substances of interest because of where it is placed. It should be pointed out that detection of one or more contaminants of interest by a multi-media, area monitor may require additional investigation to identify the contaminant sources.

Unlike instruments, honey bee colonies specifically address issues of bioavailability, including transport into the colony, fate, depuration, and any associated adverse effects. On the other hand, these same processes introduce lags in response time and can lead to delayed effects.

5.1.2 The Individual versus the Population as the Characterization Unit

Both the individual organism and the population have been proposed as the fundamental unit of ecological risk analysis (2). The members of EPA's Ecological Risk Assessment Guidelines Strategic Workshop disagreed about whether the fundamental unit for risk analysis is the individual or the population. All agreed that population-level effects really reflect the aggregate response of individual organisms; whereas, exposure to stressors occurs at the level of the individual and individuals respond to stress. Assessments focused on the individual can take advantage of toxicity assays, behavioral information, and other well-established methods. Population-level effects reflect the combined response of individual organisms. By summarizing individual response at the population level, one can develop risk methods in terms of classic demographic models, including estimates of stress on natality, mortality, net reproductive potential, age/size class structure, and yield and other traditional demographic descriptors.

When considering community and ecosystem level endpoints the degree of complexity of interconnections is high. The community cannot be separated from either populations or ecosystems. Also, the overall assessment endpoint of interest is sustainability. However, real-time monitoring of community endpoints such as diversity and productivity or of ecosystem endpoints such as physical structure and carbon flows is a challenging task. By comparison, population-level assessments can provide: 1) responses that are better-defined and more predictable with available data and methods, 2) more tractable scope and scale, 3) economic, recreational, aesthetic, and biological significance that is appreciated by the general public, and 4) protection of groups of organisms that may be perceived as having greater biological or social significance than individuals, organisms or suborganisms (3).

5.1.3 Honey Bee Colony Populations

Honey bee populations offer a dual approach to an ecological characterization or hazard assessment—the individual and the colony population. A colony is a social structure composed of individuals from subfamilies that are structurally and functionally more like a superorganism than a diverse assemblage of individuals. Each hive contains a colony population, and the individuals are more closely related genetically than in typical animal populations.

Exposures occur to individuals, and the responses of individuals can be determined. Thus, a puff of smoke calms individuals, but the effect may alter the population's foraging rate. Similarly, insecticides kill individual bees, and rarely kill entire colonies. Small losses of field bees may have little affect on the colony population. More severe exposures may cause the population to shift duties (e.g., recruitment of hive bees to replace lost field bees), change age structure (e.g., loss of brood), fail to thermoregulate, or even collapse.

Our equipment and methods allow simultaneous and continuous measurements of the responses of individuals (e.g., numbers of bees entering and leaving the hive) and of the colony population (e.g., temperature regulation, total mortality, hive biomass).

Individual-based population models have been developed to describe population dynamics. Models integrate demographics, behavior, bioenergetics, and life-history information. For many species of interest, the kinds of detailed biological information needed for individual-based modeling is seldom available. This is not the case for honey bees. Bees occur globally and are economically and ecologically important as producers of honey and wax and as pollinators. Because of their value, extensive databases exist for bee population biology and for honey bee responses to toxic substances (as a result of hazard testing required for pesticide label registration). Also, based on over 20 years of research and applications, we have developed dynamic, interactive ecotoxicological models for honey bee colony populations exposed to natural and anthropogenic stress (4).

Between the aggregate demographic models and the detailed individual-based models lies an intermediate level of population description. These intermediate models (5) permit the simultaneous consideration of disparately scaled biological phenomena (e.g. foraging over seconds or minutes, field force strength and colony growth over days). The properties of these models are distinct from those of typical demographic models. The meaning and use of these differences for hazard assessments is one of our research objectives. Real-time, multiple endpoint, behavioral response data combined with state-of-the-art data processing, including advanced statistics and pattern recognition by artificial neural networks, are some of the tools we are developing under this five-year project.

5.1.4 Principles of Characterization of Stress and Ecological Effects

The ecological risk assessment framework is composed of characterization of exposure and of effects. Exposure generally refers to chemical stressors, but can be extended to nonchemical stressors that can affect a variety of ecological components. In the broadest sense, exposure applied to a wide range of stressors conveys the important concept of the co-occurrence and interaction of the stressor with the biological component (5).

For the purpose of this report, we often employ the term hazard assessment rather than characterization of ecological effects. Characterization uses the results of measuring effects and exposures to evaluate the likelihood that adverse ecological effects are associated with exposure to a stressor. An ecological risk characterization is aimed at providing a complete analysis and results. Historically, hazard assessment has been defined as either the intrinsic effects of a stressor (6) or as a comparison of an estimate of an exposure concentration with a toxicological endpoint (7). Although our overall goal is to eventually conduct ecological risk assessments, our pilot trials of 1995 and the field applications at APG in 1996 were focused at the level of hazard assessments. Colony behavioral responses were continuously measured and periodic measures of contaminants in or on the bees and pollen as well as in hive atmospheres

were made to assess exposures to bioavailable chemical contaminants as part of an initial hazard assessment.

5.2 Exposure Characterization at APG

With respect to chemical exposures, this project puts in place the capability of looking at volatile and semi-volatile chemicals in the air inside a beehive. Semi-quantitative chemical exposure data collected to date in combination with the use of artificial neural networks provided us with: 1) an inventory of a complex array of over 200 of these chemicals inside a beehive, 2) fingerprints for the chemicals associated with various components of the bees and the hives (i.e., metabolic products from the bees, terpenes from the wooden hive), and 3) the ability to reliably identify the particular hive from which any sample had been taken via ANN pattern matching.

Residues of five chlorinated hydrocarbons, a polycyclic aromatic hydrocarbon, and a ketone were found in colonies at all of the sites sampled in Maryland, including the off-post reference location (summarized in Fig. 5.1). The highest relative abundance levels of chemicals of particular interest to the Army (detailed in Section 4) occurred near the landfill being capped at Old O Field. Low to intermediate levels of many of these organics occurred at several locations across the residential area of APG—Edgewood. The lowest levels of most volatile and semi-volatile organics generally were found near the office complexes northwest of the National Guard Armory and at West Branch Canal Creek. Usually, the levels of organic solvents in the hives at these APG locations were as low or lower than those at the Churchville reference site. For all sites, levels of solvents inside beehives usually were equal to or higher than those of the ambient air. At some sites, levels inside the beehives exceeded those of air by several orders of magnitude, especially at Old O Field. Detection of solvents in both air and bees at residential sites such as the Youth Center may warrant additional investigation to determine whether an unidentified source exists.

We speculate that the bees at Old O Field were obtaining the organic contaminants from a more condensed media, such as water. As previously discussed in Section 4, bees utilize water for drinking, liquefying crystallized honey, making food for larvae, and evaporative cooling of the hive. Bees prefer to gather water from standing pools, rather than flowing water, probably because of the risk of drowning in moving water. If bees utilize contaminated surface water, such as a puddle covered with an oily film, a concentrated or condensed sample could be brought back to the hive either in the water carried by the bee or as chemicals clinging to the outside of the bee's body. In addition, the contaminant may be concentrated in the puddle itself by evaporation, a situation that has been documented for fluoride (8).

In addition, bioaccumulation from other sources, including air, may occur inside the hive. Semi-volatile and volatile chemicals brought back to the hive in or on the bees themselves, or in or on bee-collected materials including water, nectar, pollen, or resin, should be present in the hive atmosphere. On a typical day, the air inside a hive should reflect substances collected

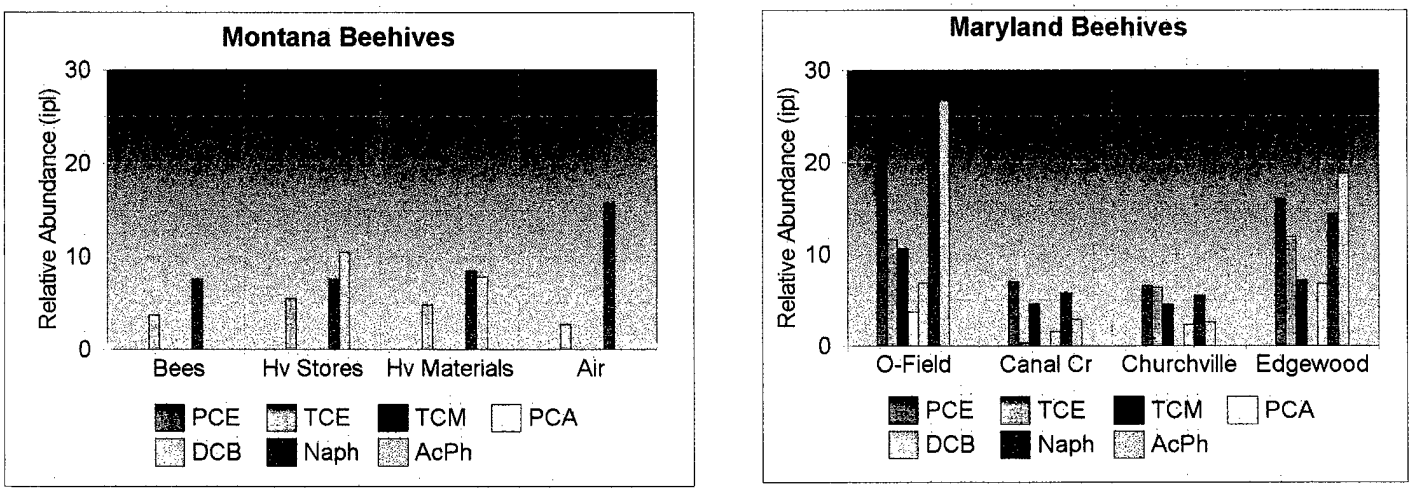

Figure 5.1. Volatile and Semi-Volatile Organics Inside Beehives from Montana and Maryland

by bees via hundreds to thousands of forager flights. Also, the hive box itself confines these materials. Because the colony usually keeps temperatures inside the hive box at 34-35 °C, the more volatile substances should thermally disturb. On cool days, the rate of volatization of these chemicals inside the hive could exceed that of the same materials in the ambient air.

Because we did not know what organic contaminants would occur in beehives placed at APG, our initial approach focused on a semi-quantitative survey of the relative abundance of the semi-volatile and volatile chemicals found inside a hive. Having identified specific organic chemicals that can be found in hive atmospheres at APG, we are now developing appropriate calibration standards to provide actual concentrations for these chemicals. However, the semi-quantitative results should be within $\pm 10\%$ of the true value. Thus, small differences observed among the sampling locations across the residential areas of the post and at the Churchville reference site may not be statistically different, but the increased relative abundance by many orders of magnitude at Old O Field undoubtedly differ from the other locations.

The quantitative inorganic analyses of bees and pollen did not reveal any gross contamination by inorganic elements, including heavy metals, but some chemicals known to be present at APG, such as arsenic and strontium, were slightly elevated in bees and pollen at Old O Field and at some of the upper Edgewood post locations, compared to the Churchville off-post reference site (e.g., **means of 0.4, 0.6, and 0.7 ppm of As for Old O Field, Canal Creek, and Edgewood versus 0.3 ppm for Churchville in August; and means of 3.4, 2.6, and 3.1 of Sr for the APG sites compared to 1.1 at Churchville**). Also, cobalt was not recorded in pollen at Churchville, but was detected in one Old O Field pollen sample at five times the amount measured in honey bees from this same site.

Because slightly elevated levels of metals in bees and pollen occurred at some of the APG sites compared to Churchville, the data suggest that the foragers did detect some potential hot spots of these contaminants at APG. Although the differences detected were slight, it is possible to map spatial distributions of metals at these low levels, provided that the sampling includes sufficient sampling frequency to investigate seasonal changes in bioavailability and that enough sites are sampled to permit construction of geostatistical plots for the area. In a previous study of the Department of Energy's Hanford site and the surrounding agricultural locations, bees were shown to be capable of differentiating spatial patterns of arsenic distribution at levels ranging from 0.12-0.4 ppm (9). However, it should be kept in mind that exposure monitoring simply demonstrates where chemicals are bioavailable and does not necessarily imply that the bees will be adversely affected.

5.3 Seasonal Variations in Exposures and Responses

During both years, the bees were deployed at APG beginning in mid-summer. The 1996 field season extended from late June through mid-November, when cold temperatures and a

light snow flurry ended the study. To complete a biomonitoring cycle, bees will be deployed at the same APG sites in April and monitored through the end of June. The first part of the growing season may be a critical time for measuring colony performance. During the spring and early summer, deep-rooted trees and shrubs flower which produces nectar, pollen, and resin that the bees gather. In addition, carpets of dandelions and other earlier season lawn and meadow flowers will force the bees to more intensively forage grassy areas. By late summer, these floral resources are more or less absent, nectar sources are limited, and much of the foraging occurs in marshy areas and estuaries where moisture sustains late season blossoms. Spring is usually associated with the highest elevation of the water table. Liphophilic contaminants floating atop ground water sources may find surface expressions in springtime seeps and become accessible to bees. Completing an annual biomonitoring cycle should allow us to determine the influence, if any, of seasonal changes on contaminant exposures and effects at APG.

As discussed in 5.1.1, a recognized limitation of using bees as area samplers is that the bees, in northern climates, do not forage in the winter. However, the colonies continue to function as point monitors. Also, clustering to generate heat means that the colony will continue to thermally disturb semi-volatile and volatile chemicals (compared to outside air temperatures). By monitoring the temperature inside the cluster, a feedback system is still available for warning of the need to take a chemical sample. In other words, if the colony begins to fail to maintain a constant core temperature, further investigation may be warranted.

5.4 Exposure Characterization at Montana versus APG

All of the organic chemicals found at APG—Edgewood were also detectable at Churchville. In addition, some contaminants (e.g. a derivative of furfural and para-dichlorobenzene) were either only seen or were first seen at Churchville or occurred at levels equal to or higher than at APG. Given the distance of the Churchville site from APG, the upwind location, and the surface and subsurface origin of many contaminants found at APG, we believe that it is unlikely that the solvents found in bees at Churchville came from APG. This issue could be inexpensively addressed by conducting a landscape scale grid sampling using existing colonies of bees kept by local beekeepers (10). The resultant spatial patterns of chemicals in hive atmospheres should be helpful in discerning potential sources of the industrial solvents detected in the Churchville reference hives.

Air inside hives in Montana did not contain detectable levels of four of the seven solvents found in all of the Maryland hives (Fig. 5.1). Of the seven organics commonly found in Maryland samples, only trichloroethylene (TCE) and naphthalene (Naph) were found in Montana bees and air. TCE is among the most ubiquitous degreasing compounds and Naph is found in petroleum products, so detection of these substances in Montana bees was not unexpected. Some acetophenone (AcPh) was detected in the polyethylene components of the bee condos and the plastic comb foundation of the honey supers, probably as a result of AcPh

being used as an olefin catalyst in the manufacture of these plastics. On the other hand, there were no detectable amounts of perchloroethylene, tetrachoromethane, hexachloroethane, or dichlorobenzene in the Montana bees, air, or hive components.

Most of the inorganic chemicals observed at APG approximated or were only slightly elevated compared to levels in bees and pollen collected from the Missoula valley of Montana. Concentrations of potentially toxic inorganics at APG were far below those found in bees from metals' contaminated areas of the Pacific Northwest, such as industrial and smelter areas. For example, based on our in-house databases, arsenic concentrations in honey bees from most parts of the United States generally fall below 1.0 ppm (dry bee tissue weight) and usually average about 0.1-0.2 ppm. In heavily contaminated areas, As levels in or on the tissues of forager bees may exceed 18 ppm.

5.5 Effects Characterization at APG

5.5.1 Classical Toxicity Measurement Endpoints

The computerized data acquisition systems utilized at APG provided continuous measures of colony behavioral performance. In addition, a more traditional assessment of adult bee mortality was provided by the traps located under each of the condo hives. These traps collected dead and dying bees that were removed from the colony by housekeeper bees. Adult bee mortality as measured by trap contents provided a commonly accepted measure of acute toxicity. However, the weakness of this method is that it only measures toxicity in terms of the bees that return to the hive. Bees dying in the field can not be sampled. This is a serious limitation in addressing exposures to toxic substances. Many of the most severely affected bees may die in the field, especially with exposure to an acute poison such as might occur from a pollutant emission or drift from application of an insecticide.

Based on the trap data for dead bees, no acute toxicity was detected at any of the test locations in Maryland, nor was there any statistically significant difference in total bee loss at any of the sites (discussed in Section 3.2.2 and data presented in Tables 3.2 and 3.3 and Figure 3.2). Statistically significant date by site interaction for adult bee death indicated that there were site-specific differences, but the bee loss was small. During both years, bee mortality at West Branch Canal Creek was minimal. The somewhat elevated bee mortality at the Churchville reference site appears to have been a reflection of the drone-laying condition of one colony and the unintentional trapping of bees that by-passed the counters in another hive and couldn't find their way into the hive. Compared to bee colonies in Montana and other western states inspected over the past 20 years, we judged these losses to be well within the normal range for expected attrition due to old age and to loss while foraging.

As with any population, some individuals in the colony are lost daily due to factors other than contaminant exposure. Excess bee mortality by itself may warn of a potential exposure,

but chemical sampling and other testing, such as checking for disease or lack of a queen, is needed to identify the cause. Also, one of the strengths of using bees is that a loss of individuals may adversely affect colony structure and performance, but only in the most severe cases is the entire colony lost. Thus, although the individual may be susceptible to exposure to a toxic material, the colony itself is relatively robust and often continues to serve its monitoring function, including providing the opportunity to monitor its own recovery.

5.5.2 New Measurement Technologies

The 1995 pilot study and the 1996 field applications produced the most advanced technologies ever applied to continuously measuring honey bee colony behaviors. Whereas occasional studies have equipped one or a few colonies with a single sensor such as a temperature probe, placed a hive on a balance, or attached a counter to the entrance, no other investigation has ever simultaneously and continuously monitored multiple measurement endpoints for a number of colonies at more than one site. Having accomplished this capability, we added real-time data collection for a full complement of meteorological conditions and also used samplers that provided estimates of the amount of pollen collected by time of day. Inclusion of hive-mounted traps to catch dead bees provided another measurement endpoint, as did continuously measuring hive weights using pressure transducers (1995) or strain gauges (1996). In 1995, we tested six prototype electronic hives. In 1996 we fielded 21 of these electronic hives in replicates of seven at three sites.

Our data clearly demonstrated the reliability and usefulness of real-time measuring methods to assess colony condition. The flight activity counters proved to be capable of quickly distinguishing subtle differences between hives and among sites. Some colony responses were expressed in less than five minutes, indicating rapid response times that offer considerable potential as an early warning system of exposure. Easily identifiable changes in flight activity in response to a variety of internal and external factors provided unique data sets. These can be used to assess the ability of various data filtering and smoothing procedures as well as advanced statistics and neural networks to relate colony responses to these stressors. Preliminary data analysis has demonstrated that these approaches can delineate effects in ways that a computerized decision-making tree could be used to provide a feedback alert of a change warranting sampling for exposure or further inspection of the hive to determine its condition (e.g., healthy, diseased, queen-right).

Some of the hive sensors are still in the process of development. Strain gauges proved to be more reliable and stable than pressure transducers for observing colony weight, but further refinement is needed to maximize sensitivity. The hot wire anemometers used to assess air flow are being evaluated for accuracy and resolution. Relative humidity sensors were prone to calibration drift, and the bees did not appear to exercise much control over humidity inside the hive. As such, we concluded that humidity was unlikely to be useful for evaluating the colony homeostasis.

Systems and performance audits of accuracy and resolution showed the temperature probes and flight activity counters to be the most reliable of the tested electronic devices. Flight activity and colony core temperature also provided the best estimates of colony condition. Based on these two parameters, we could determine net loss of bees returning to the hive, within site variability related to total flight activity by each colony, relative colony vigor and strength, presence of an egg-laying queen, and we could predict the structure of the brood nest (e.g., presence of uncapped or capped brood) and assess the ability of the colony to maintain homeostasis (i.e., thermoregulation) within the hive.

5.5.3 Colony Performance as Evidenced by Real-Time Measuring of Behavioral Endpoints

Based on the flight activity and temperature regulation measures, the bee colonies at West Branch Canal Creek performed well during both years. None of the queens were lost, and all of the colonies actively foraged. Two of the colonies had moderately sized bee populations, but these same colonies were smaller at the onset of the field trials. Although no acute bee mortality occurred at Old O Field, 50% (6 of 12) of the queens disappeared from both the condo hives and the additional nucleus hives used to supplement the chemical sampling grid. The presence of emergency queen cells suggested sudden queen loss (i.e., death or escape from the hive) rather than queen absconding or swarming with most or all of the bee population. In addition, one marked queen was found walking about on the ground outside of a nucleus hive near the Old O Field bee condos. Two queens (2 of 7) absconded from hives at Churchville.

Queen disappearance from colonies was clearly evident by depressed flight activity and reduced core temperatures. In addition, population size and activity were more variable at Old O Field and Churchville than at West Branch Canal Creek. Queen disappearance at Old O Field occurred from condo colonies brought from Montana in July and from nucleus colonies obtained in Maryland in June. Four of these Old O Field colonies exhibited the highest levels recorded for solvents found in beehives in Maryland (data presented in Section 4, page 4-26). Although colonies occasionally sustain the loss of a queen, a 50% (6 of 12) disappearance suggests severe stress. Although no toxicity or bioconcentration factors for solvents such as PCEs are available with respect to honey bees, the lack of any statistically significant adult bee mortality in the Old O Field colonies combined with the observation of a queen outside the hive suggests that the queen disappearance was not the result of toxicity. However, because most of the workers only live for a few weeks, whereas the queen can live for several years and also is fed the richest diet, there is a potential for selective toxicity to the queen. It also should be noted that chemicals such as formic acid which is used to control mites in colonies (legal only in Canada) are known to occasionally drive queens from the hive. Whether industrial solvents could cause the queen to leave the hive will be the subject of dose-response tests planned for the summer of 1997 in Montana.

It seems reasonable to assume that at least one of the queens at Churchville departed due to transportation stress, considering that the queen vacated the hive as soon as it was deployed in Maryland. The cause of absconding by a second queen is unknown. Overall, levels of exposures to volatile and semi-volatile organic chemicals at this site were intermediate to those for bees from residential areas of APG. However, occasionally individual chemicals appeared at high levels in one or two of the Churchville hives, so exposures were variable.

5.6 Stress Characterization

Historically, hazard assessments have focused on chemical stresses. Our approach incorporates nonchemical stresses, the interaction of multiple stresses, and the abiotic modulation of stresses. The population itself, in this case the colony, is continuously monitored, not exposures.

Consideration of exposure is needed to define the temporal (i.e., acute, episodic, or continuous) and spatial (i.e., local, regional, or global) scope of a problem. Following this initial scoping stage, exposure assessment seeks to quantify the magnitude, frequency, and duration of contact of the receptor (bee colony) with the stressor. The metric produced through the stress characterization must be keyed to the requirements of the stress-response measurement. For example, a metric keyed to net loss of foragers as defined by the number of bees leaving and returning to the hive serves both as an indication of bee mortality (which kills individuals and may adversely affect the colony population) and provides a rate function relatable to pollination efficacy (which may potentially affect the community). Simply stated, fewer forager flights mean fewer visits to blossoms and less pollination. Although the loss of one or a few colonies of bees would probably have little adverse effect on most agricultural and natural plant communities, harm to honey bee colonies may signal the potential for more widespread impacts, including loss of other insect pollinators. It also provides a warning that additional investigation, such as sampling for the presence of toxic chemicals, is warranted.

5.6.1 Bee Population Monitoring Endpoints

Because pesticides can be hazardous to the honey bee, the U.S. Environmental Protection Agency requires that a hazard assessment be conducted to assess the potential for harm to honey bees. Guidelines are provided that outline methods for determining the degree of toxicity, both to groups of adult bees in cages and to colonies under field conditions. A commonly used device is the hive mounted trap to assess adult bee mortality. However, all of these classical methods rely upon periodic measures, such as the percent bee death at 48, 96, and 120 hours. In addition, assessments of acute or chronic toxicity to individuals (in cages) often have to be extrapolated to potential impacts to colonies. Comparison studies indicate that the responses of caged bees may not adequately predict the responses of colonies (11).

This project adds the ability to continuously monitor an array of colony responses, some of which reflect traditional endpoints, and some add unique capabilities. Over the last year, real-time measuring of colony behavioral responses has been achieved. A form of real-time monitoring has also been accomplished. Real-time measurement data is displayed in real-time via graphical displays to computer screens. In theory, if one were to constantly watch the computer displays, real-time monitoring of effects has been accomplished. However, interpretation of the raw display data is not straightforward.

To date, three measurement endpoints have yielded data that can be used by a computer software program to provide a real-time feedback or an alert of the presence of a stressor. These include:

- Net loss of forager bees.
- Coefficient of variation of total flight activity at a site.
- Core temperature in the brood nest.

Net loss of forager bees provides a measure of acute bee loss on a daily basis. The numbers of bees returning to the hive by the end of each day should nearly equal the number leaving the hive. Some bees will fail to return due to old age, predation, entry into the wrong hive, and other factors. Estimates of the mean number of returning bees can be extracted from the database generated at APG in 1996. From this information, the expected rates of return for a hive can be generated and adjusted for weather and food resource conditions. Following a control chart format, the feedback software can be written to provide a warning of a net loss exceeding a pre-determined warning limit or a control limit. In addition, the software can flag a systemic bias such as a slowly increasing daily loss of bees.

Although acute toxicity is one cause of a net loss of foragers, other factors could affect the numbers of bees returning. For example, bees caught by a storm might not return or may be delayed. The digital weather stations at each of the three honey bee colony performance monitoring sites provide the information needed by decision-making software to adjust for this potential source of error. In addition, the hive mounted dead bee traps provide another measure of mortality and bees for body-burden residue analysis to ascertain exposure to toxic chemicals.

Whereas net loss of returning foragers provides a measure of impacts to individual bees, total flight activity (number of trips per day per hive) provides a measure of overall colony activity and condition. Populous colonies should have more foragers than smaller colonies. Stormy days, cold weather, or a lack of floral resources will curtail flight. On warm, calm days when blossoms are abundant, flight activity will be high.

Based on the Maryland and Montana data from the last two years, flight activity at a given site is mainly a function of weather and floral resources. The colonies at each site display similar diurnal patterns of activity. The coefficient of variability (C.V.) for daily flight

activity adjusts for colony size. C.V.s for the flights of West Branch Canal Creek, Old O Field, and Churchville hives tended to be relatively low during the summer, and increased at the end of the season. The highest C.V. values were observed for the colonies at Old O Field (discussed in Section 2). By comparing residuals from one site to another, it became apparent that the C.V.s for Old O Field departed dramatically from the other sites, particularly after the passage of hurricane Fran. Interestingly, this effect was not seen the day immediately following the storm, suggesting the change was not a direct impact on the forager force. In other words, the colonies did not lose foragers to the storm. As with net loss of forager bees, software can be written to provide a feedback system that provides a warning of increasing C.V. values. Unlike net loss of foragers (i.e., individuals), the variability in flight activity provides a measure of the condition of the colonies (i.e., populations) at each site.

Because honey bee colonies tightly control temperatures in the brood nest and in the winter cluster, core temperatures can provide a measure of the colony's ability to interact socially to control environmental conditions inside the hive. As apparent from the Figures and discussion contained in Section 3.3.5, core temperature provided a reliable indication of the queen and brood status of each colony. During the growing season, departures from the 34.5± 1°C normal temperature can be readily flagged. Also, the software can be programmed to adjust for late fall, winter, and early spring, in northern climates, when brood is not present and cluster temperatures may drop as low as 18 °C. Measuring colony core temperature provides a measure of the physiological status of the colony.

Ongoing activities include development of computer feedback software that will provide real-time monitoring capability for the three aforementioned measurement endpoints by the summer of 1997. Advanced statistics and data processing systems such as Artificial Neural Networks may provide other potentially useful measurement and assessment endpoints for honey bee colonies. In addition, these tools may help in the interpretation of complex colony behaviors and population dynamics.

5.6.2 Evaluating Causal Associations

Data used for hazard assessments or characterization of ecological effects must be analyzed to quantify the stressor-response relationship and to evaluate the evidence for causality. Controlled laboratory and field tests can provide strong causal evidence linking a stressor with an observed response and helping to discriminate between multiple stressors and other confounding factors such as food resource availability. Field designs using comparable reference sites or evaluating changes along a stressor gradient produce a weight of evidence that improves confidence in assessing a causal relationship. In addition, a variety of techniques including statistical methods, mathematical modeling, and applications of pattern discriminating forms of computer intelligence may be used to quantify the stressor-response relationship and to evaluate the evidence for causality (5).

Another important aspect of a characterization is to evaluate the strength of the causal association between the stressor, the measurement endpoint, and the assessment endpoint. Ideally, the assessment endpoint can be directly measured rather than extrapolated from indirect or qualitative information. This project focuses on identifying colony endpoints that can be directly measured.

Activities planned for the 1997 field season include testing in Montana to assess the effects of exposure to the solvents identified in APG hives, completion of seasonal testing, and additional field proofing.

5.6.3 Evaluation Criteria for Causal Associations

An evaluation of causality augments the risk assessment. Many of the concepts from applied human epidemiology can be applied to observational field studies. Hill (12) recommended nine evaluation criteria for evaluating causal associations. These criteria provide a conceptual framework for developing honey bees as real-time monitors. Some of these criteria were examined during the initial field applications at APG. The data needs for meeting these criteria are described below.

- **Strength**: A high magnitude of effect associated with the stressor—50% queen disappearance from colonies at Old O Field indicates a high level of stress. Additional (dose-response) investigation is needed to determine whether this response was associated with exposure to the industrial solvents found in these hives.

- **Consistency:** The association is repeatedly observed under different circumstances— nucleus colonies will be placed on the same sites during the spring and summer of 1997. Time series statistical analysis of the data from both years should provide additional assessments of spatial and temporal patterns of colony response.

- **Specificity:** The effect is diagnostic of the stressor—we propose use of a biomarker such as acetylcholinesterase inhibition to examine potential exposure to organophosphate-based nerve gas agents at Old O Field. Under the former federal indemnity program that reimbursed beekeepers for pesticide kills, acetylcholine-esterase assays were used as legal evidence of exposure to toxic concentrations of organophosphate pesticides. In addition, honey bees have been shown to produce metallothioneins in response to exposure to cadmium (13).

- **Temporality:** Temporally, the stressor precedes the effect or conversely the effect provides a warning of immediate exposure. With implementation of real-time monitoring of net loss of forager bees, increased variability of within site flight activity, and colony thermoregulation combined with chemical sampling when an effect is observed, this capability should be realized in 1997.

- **Presence of a biological gradient:** Colonies are continuously compared (for exposures and responses) to each other (within site) and to replicate colonies at other sites (i.e., Old O Field versus West Branch Canal Creek, Churchville, and additional sampling units deployed throughout the residential areas of APG—Edgewood)

- **A plausible mechanism of action:** Information available from existing databases for some chemicals and categories of chemicals (e.g., arsenic, many pesticides) or can be obtained by controlled laboratory or field bioassays.

- **Coherence:** The hypothesis does not conflict with known biology or history. With respect to a site like APG, both bee biology and the history of the site need to be considered, including other site surveys and characterizations.

- **Experimental evidence:** Measurement endpoints can be easily and accurately measured using the electronic hives equipped with multiple sensors and chemical sampling probes. Controlled dose-response experiments are planned for the summer of 1997 using chemicals injected into electronically monitored colonies.

- **Analogy:** Similar stressors cause similar responses. For example, tests of formic acid for mite control indicate that high dosages harm the queens. The proposed 1997 studies will examine whether the disappearance of queens from the Old O Field colonies was a response of high levels of industrial solvents in hive atmospheres.

EPA's Risk Assessment Forum concluded that "not all of these criteria must be satisfied, but each incrementally reinforces the argument for causality. Negative evidence does not rule out causation, although it may indicate incomplete knowledge about the relationship (13).

5.7 Summation

Based on the 1995 pilot study and the 1996 field application, it is premature to attempt an ecological characterization of APG. However, by referring to Hill's criteria for evaluating causal associations, it should be apparent that we already have a considerable amount of information about colony responses at APG sites and some of the tools needed to evaluate the strength of suspected associations.

Minimally, we can rank areas according to overall colony condition, detectable chemicals in hive atmospheres, and the relative abundance of semi-volatile and volatile chemicals of interest to APG managers. Areas such as the Youth Center and Beach Point, where both bees and air demonstrate elevated levels of several organic solvents warrant additional investigation of possible exposures to people working in these areas.

When levels of contaminants in hives approximated those of air, by sampling at least two colonies, we greatly increased the chance of detection. In theory, placement for sampling is less critical when employing widely foraging colonies of bees rather than a stationary instrument. On the other hand, colonies do vary in their foraging habits. As such, uisng one or more of the colonies increases the likelihood of detecting an exposure to contaminants in hot spots. We recommend the use of at least two colonies at any given sample location.

The high exposures to solvents observed inside hives combined with the disappearance of queens at Old O Field warrants additional investigation. By comparison, the bee colonies at West Branch Canal Creek thrived and received minimal exposures to many of the volatile and semi-volatile chemicals observed at APG sites and at the Churchville reference location. Previously, bees have been shown to be useful, multi-media monitors of inorganic chemicals over large geographical areas. The present study has shown that bees also can detect biologically available volatile and semi-volatile chemicals in their surroundings. Because the relative abundance of several solvents inside the hives at Old Field exceeded that of air or of other Maryland sites by many orders of magnitude, we suspect that the results may reflect a multi-media sampling capability of bees for these substances.

5.7 References

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SECTION 6

GLOSSARY

This list contains terms that have special meaning in beekeeping, computer electronics, chemistry, and risk assessment. It is provided to facilitate reading this report without having to refer to other references. The risk assessment terms follows definitions developed by EPA's Risk Assessment Forum, a committee of EPA scientists.

A/D Interface. An analog to digital signal conversion device placed between a computer and the electronic sensors.

Age polythethism. The changing of activities of members of the colony as they age.

Apiary. The place where a group of beehives is kept. Also called a beeyard.

Artificial Neural Networks. Software programs that learn real-valued functions from examples.

Assessment Endpoint. The environmental value that is to be protected.

Brood. The immature members of the colony, including eggs, larvae, and pupae.

Broodnest. The region of a colony's nest where brood is reared, usually a central, roughly spherical area below the stored honey.

Burr comb. Pieces of comb that bridge the main combs in a hive.

Carbotrap. A form of thermal desorption sampling tube used to collect samples of volatile and semi-volatile organic chemicals.

Cell. The hexagonal tubes that make up the beeswax comb and in which the brood develops and food is stored.

Colony. The honey bee social unit, each consisting of a queen and workers. During the growing season the colony also will contain drones and brood. Normally, a hive contains a single colony.

Cluster. The behavior by which bees group together for warmth and then coalesce into a single group to conserve heat and to minimize surface area relative to volume. Clustered bees generate metabolic heat.

C.V. Coefficient of variation, a statistical function used to compare the relative amounts of variation in populations having different means. Also termed relative standard deviation.

Dead bee trap. A trap used to collect dead and dying honey bees.

Digital Interface. A digital signal device placed between a computer and the electronic sensors.

Direct effect. The consequence of a stressor acting on the ecological component of interest.

Drone. A male honey bee.

Forager. A worker bee that gathers and brings back to the hive water, resin, pollen, or nectar.

Flight counters. The hive mounted infra-red detector units used to track the numbers of incoming and outgoing bees.

Frame. The rectangular wooden structure that surrounds and supports each beeswax comb in a man-made hive.

Functional Organization. The organization of the colony that contributes to the survival and reproduction of the social unit.

Ecological Component. Any part of any ecosystem, including individuals, populations, communities, and the ecosystem itself.

Ecological Risk Assessment. An evaluation of the likelihood that adverse ecological risks may occur or are occurring as a result of exposure to one or more stressors.

Ecological Significance. The interpretation of risk estimates in the context of the types and extent of anticipated or observed effects. The interpretation step usually relies on professional judgement and considers the nature and magnitude of the effects, the spatial and temporal patterns of the effects, and the potential for recovery if the stressor is removed.

Exposure. Co-occurrence of or contact between a stressor and an ecological component.

Exposure characterization. The evaluation of the interaction of a stressor with one or more ecological components.

Exposure profile. A summary of the magnitude and spatial and temporal patterns of exposure used in the analysis phase of an ecological risk assessment.

Hive. The structure that houses a bee colony. Man-made hives are usually made of wood. Natural hives usually are cavities in trees.

Honey stomach. The expandable portion of the alimentary canal used to store and carry nectar and water.

Hydraulic pressure transducers. Electronic devices for measuring changes in pressure, used to weigh hives.

Hymenoptera. The order of insects that includes wasps, bees, and ants.

Hypopharyngeal glands. Glands in the head of the bee that produce proteinaceous secretions which are fed to the larvae and various enzymes that serve in the conversion of nectar to honey.

ICP/MS. Inductively coupled plasma mass spectrometry.

Indirect effect. The consequence of a stressor acting on supporting components of an ecosystem, which in turn influence the ecological component of interest.

Instar. Any stage between molts (casting off of outgrown skin) during the course of development of insects.

Larvae. The stage between the egg and the pupae in insects. In honey bees a crescent-shaped grub that floats on a pool of liquid food, intensively feeding, and rapidly growing.

Measurement endpoint. A measurable characteristic that is related to the valued characteristic chosen as the assessment endpoint. Measurement endpoints are often presented as statistical or arithmetic summaries of the observations that comprise the measurement.

Mini-hive. A small, mailbox-sized beehive used to house nucleus colonies for mating and for research.

Nasanov's gland. A gland on the apical end of a bee's abdomen that secretes a pheromone that attracts other bees.

Nectar flow. A period of intense nectar secretion by plants during which a honey bee colony collect large amounts of nectar and produce surplus stores of honey. Also called a honeyflow.

NOEL. No observed effect level, the highest level of a stressor that does not cause a statistically significant difference from the control.

Nucleus colony. A small colony of honey bees.

Nurse bee. An age-specific member of the colony that is specialized for the care of brood and other tasks in the brood nest.

Parallel port. A standard device usually used to connect a printer to a computer, usually the LPT port on an IBM-compatible computer.

Pollen basket. A specialized structure on each hind leg for the collection and transport of pollen back to the hive. A smooth area, bordered by a fringe of long curved hairs, on the outer surface of the leg.

Proboscis. The extensible, tubular mouthparts of a bee.

Propolis. The material used by bees to seal cracks in the hive, reinforce comb walls, and create a smooth coating over interior surfaces. Propolis is made up of plant resins collected by bees.

Pupae. The nonfeeding developmental stage between the larvae and adult form.

Queen. The reproductive female member of the colony. Generally, a colony only contains a single queen.

Queen cell. A special beeswax cell built to house a developing queen.

Queen excluder. A screen placed between the boxes of a hive to confines the queen to a particular region of the hive.

Queen loss. Disappearance of the queen from a colony without a concurrent loss of worker bees (see swarm). Queen loss may be a result of queen death or of the queen vacating the hive.

Queenright. A bee colony that contains a fully functioning queen.

Recruit. A forager bee that looks for new food sources after following the waggle dances of a returning forager in the hive.

RH probe. An electronic relative humidity sensor.

R.S.D. Relative standard deviation which is the same as the coefficient of deviation.

Scout. A forager bee that looks for new food sources by independent searching.

Serial port. A standard port for connecting devices to a computer, usually the COM port of an IBM-compatible computer.

Social physiology. The highly organized functioning of a colony comprised of the integrated activities of the individuals and the group as a whole. An example is thermoregulation inside the colony.

Strain gauge. A bi-metal foil resistor that measures flexion of a beam. Strain gauges are used to measure hive weight changes.

Stressor. Any physical, chemical, or biological entity that can induce an adverse response.

Stressor-response profile. A summary of the data on the effects of a stressor and the relationship of the data to the assessment endpoint.

Supersedure. The replacement of the queen by her daughter.

Swarming. The method of colony reproduction in which the queen and a large portion of the worker bees leave the hive and find a new nest cavity.

Temperature probe. An electronic probe consisting of a thermal transistor.

TD/GC/MS. Gas chromatography/mass spectrometry for analysis of organic chemicals.

Waggle dance. The dance that causes foragers to leave the hive in search of specific food sources.

Weight of Evidence. A process that provides insights into the confidence of the conclusions reached in a risk assessment. Weight of evidence considerations often include the sufficiency and quality of the data, corroborative information, and the degree of correlation between the presence of one or more stressors and some adverse effect.

Worker. One of the non-reproductive females in the colony. The bulk of the population consists of workers.