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13. ABSTRACT Honey bees (*Apis mellifera* L.) have been shown to be multi-media monitors of chemical exposures and resultant effects. This five-year project has developed an automated system to assess in real-time colony behavioral responses to stressors, both anthropogenic and natural, including inclement weather. Field trials at the Aberdeen Proving Ground— Edgewood included the Old O Field and J Field landfills, the Canal Creek and Bush River areas, and a Churchville, MD reference site. Preliminary results show varying concentrations of bioavailable inorganic elements and chlorinated hydrocarbons in bee colonies from all Maryland sites. Industrial solvents in the air inside beehives exhibited the greatest between site differences, with the highest levels occurring in hives near landfills at Old O Field, J Field, and at some sites in the Bush River and Canal Creek areas. Compared to 1996, the 1997 levels of solvents in Old O Field hives decreased by an order of magnitude, and colony performance significantly improved, probably as a consequence of capping the landfill. Recent chemical monitoring accomplishments include development of a new apparatus to quantitatively calibrate TD/GC/MS analysis, a QA/QC assessment of factors that limit the precision of these analyses, and confirmation of transport of aqueous contaminants into the hive. Real-time effects monitoring advances include development of an extensive array of software tools for automated data display, inspection, and numerical analysis and the ability to deliver data from remote locations in real time through Internet or Intranet connections.

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, SITEVIEW, artificial neural networks.

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FOREWORD

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LIST OF ACRONYMS

AIA	Absolute Ion Abundance
ANL	Adjusted Net Loss
ANN	Artificial Neural Network
APG	Aberdeen Proving Grounds
As	Arsenic
Be	Beryllium
Ba	Barium
Benz	Benzene
BP	Beach Point Site
BR	Bush River Site
BTEX	Benzene, Toluene, Ethylbenzene, Xylene
BRSA	Bush River Study Area
CD-ROM	Compact Disk-Read Only Memory
CGI	Common Gateway Interface
CC	Canal Creek Site (West Branch Site)
Cu	Copper
CV	Churchville Reference Site
C.V.	Coefficient of Variation
DCB	Dichlorobenzene
DER*DIF	Derivative*Differential Analysis

EB	East Branch Canal Creek Site
Ethbenz	Ethylbenzene
FM	Fort Missoula Site
GS	G Street Site
Hz	Hertz
ipl	ions per liter
JF	J Field Site
JFN	J Field North
JFS	J Field South
LC	Lauderick Creek Site
mg	milligram
Mg	Magnesium
Mn	Manganese
Napth	Naphthalene
ng/m^3	nanogram per cubic meter
Ni	Nickel
NL	Net Loss
OF	Old O Field Site
PCR	Percentage of Bees Returning
ppt	parts per trillion
QA	Quality Assurance
QC	Quality Control

Pb	Lead
PCE	Perchloroethylene
Rb	Rubidium
r^2	Regression coefficient
SSE	Summed Square Error
SOPs	Standard Operating Procedures
SSYYMMDD	File naming format: Site, Year, Month, Day
Sr	Strontium
TCE	Trichloroethylene
TCM	Tetrachloromethane
TD/GC/MS	Thermal Desorption/Gas Chromatograph/Mass Spectrometry
TFA	Total Flight Activity
Tolu	Toluene
USACEHR	U.S. Army Center for Environmental Health Research
VOCs	Volatile Organic Chemicals
SVOCs	Semivolatile Organic Chemicals
WP	Work Plan
WWW	World Wide Web
YC	Youth Center Site
Zn	Zinc

EXECUTIVE SUMMARY

The objective of this five-year study is to develop and apply real-time biomonitoring using honey bee colonies to assess toxic chemical contaminants in military-unique, terrestrial ecosystems. The Edgewood Area of Aberdeen Proving Ground (APG) provided appropriate test locations for conducting top down (field to laboratory, colony to individual, effects to exposures) testing.

This report covers the 1997 field season project activities and goals, including:1) monitoring of bee colony behavioral responses to contaminants, weather and other environmental factors; 2) electronic hive improvements and site descriptions for the 1997 field season; 3) monitoring of exposures to bioavailable contaminants; and 4) planned activities for the 1998 field season.

Monitoring of Bee Colony Responses to Contaminants, Weather and Other Factors

The 1997 project year saw an extensive array of software tools (SITEVIEW©)developed for the display, inspection, and automated numerical analysis of colony behavioral data. This was especially true for the bi-directional flight activity counters. Custom data acquisition software was enhanced with automatic updates and aggressive error correction routines to improve the reliability of data collection and transmission as well as to reduce the number of computers required at each site. The complete set of software tools plus the entire colony response and meteorological data sets for 1996 and 1997 have been archived on a CD-ROM included with this report.

A four-tier approach to the analysis of counts from flight activity has been developed. Tier 1 includes analysis of total flight activity (TFA), the percentage of bees returning (PRC) and adjusted net loss (ANL). In Tier 2, individual colonies at a site are compared via methods such as inter-colony coefficient of variation (C.V.). Tier 3 analysis invokes a menu of smoothing and derivative algorithms to enhance features in counter traces and extract the signal of interest from the noise. Finally, Tier 4 examines the counts in individual passages in the 14-tunnel counter assembly, used mainly for systems audits. Flight activity analysis can easily distinguish events that induce departures from normal bee behavior - storms, hive manipulations, colony robbing and swarming, exposure to toxic chemicals.

Following the APG field season, further software developed in Montana allowed direct delivery of data from field sites in real time via Inter- or Intranet connections. Updated data can be ported every 15 minutes by Java™ and Common Gateway Interface (CGI) scripts to provide graphical displays of colony responses. An example of the data that can be delivered at a distance can be seen at the Bee Alert! Web site -- http://www.umt.edu/biology/bees. Real-time data transmission from Maryland sites should be accomplished during the 1998 field season.

An artificial neural network (ANN), trained on flight activity and weather data collected during the 1997 field season, identified days when colony behavioral metrics deviated from expected. A hive swarm event, hive experimental manipulations (including chemical exposures), and a staged "robbing" episode (where the bees were allowed to forage from an artificial external field source) were readily spotted by the ANN. A Sum Squared Error approach improved the ability to assess modeling accuracy.

Design Improvements in the Electronic Hives and 1997 Field Sites

The physical enclosures of the electronic hive condos were altered during 1997 to permit easier access to the hives for inspection as well as hive atmosphere sampling. A flip-top outer enclosure design permitted all condo electronics to be inspected without disturbing the colony.

Since hive core temperature proved to be a critical metric regarding queen and brood condition, a second temperature probe was added inside each hive box. The second probe replaced relative humidity probes that were not yielding significant information about hive conditions. Electronic strain gauges for following hive weight were improved. They are undergoing additional development to increase robustness and sensitivity.

Field applications for the 1997 field season were expanded from the previous year with respect to both number of colonies and length of the field season. In 1997, we fielded 70 nucleus colonies from May through October - 28 in four clusters of electronic condos (Old O Field, West Branch Canal Creek/J Field, Churchville and Missoula, MT), 33 as stand-alone nucleus survey hives (Canal Creek Study Area, Bush River Study Area, Old O Field, and J Field), and 9 spare test hives. By contrast, only 34 colonies were at APG and Churchville from July to October in 1996.

Monitoring of Bioavailable Contaminant Concentrations in Bees, Pollen and Hive Air

While a variety of organic and heavy metal contaminants were detected in samples from the 1997 field season, no acute toxic exposure scenarios were evident. Preliminary results for Bush River colonies indicated no presence of gamma-emitting radionuclides at levels that were statistically distinct from background levels, although a couple of samples exhibited slightly elevated Cesium at concentrations that were questionably above background. There also did not appear to be a significant health risk to either honey bees or humans from metals or radionuclides.

Results from 1997 indicate a marked reduction in bioavailable contaminants at Old O Field. The term "bioavailable" refers to any transport of contaminants from the ecosystem into the hive box - ingested, assimilated into tissue or adhering to the bees' exoskeletons. Although this site continued to exhibit the highest hive and ambient air loads of chlorinated hydrocarbons among the APG sites, the maximum levels of percholorethylene (PCE) decreased by an order of magnitude and petroleum-derived residues (BTEX) fell to one-third the 1996 means.

Unlike 1996, when half of the Old O Field hives became queenless, no queen loss was noted in 1997-- suggestive of less chemically induced colony stress. Analysis of 1996 field data revealed that impacted colonies could be picked up by four different metrics: (1) levels of organic solvents in hive air, (2) loss of queen, (3) failure of the colony to thermoregulate, and (4) a dramatic increase in the coefficient of variability in flight activity among hives.

The initial assessment of J Field by bee colonies revealed kinds and levels of bioavailable chemicals that resembled those found at Old O Field (e.g, TCE, TCM, PCE, DCB, BTEX, Napthalene). Trichlorethylene was most prominent. Among the Edgewood sites, the Youth Center had consistently high (but not alarming) levels of four (of the eight commonly observed contaminants. Survey hives in the Cluster 3 section of the Bush River study area produced the highest observed levels for five (i.e., TCM, PCE, DCB, toluene, and ethylbenzene) of the eight reported contaminants for the Bush River area. The PCE and DCB concentrations were also the highs across all APG sites for the entire 1997 sample season. As in previous years, the actual concentrations of these organics in hive atmospheres and in ambient air were in the parts per trillion range, or even lower. The resultant levels of organic chemicals in the hive atmospheres tended to equal or exceed those of ambient air, sometimes by as much as five orders of magnitude, especially if the contaminant had a water origin. Also, depending on the site and sample date, specific organics could be detected in both the ambient air and in the air inside a hive or just in the hive air. Whereas specific organic chemicals sometimes appeared in the air inside the hive but not in the ambient air, the converse was rarely true.

Three new aspects of chemical sampling are presented in this report:

1) Quantification of the thermal desorption/gas chromatography/mass spectrometry (TD/GC/MS) results are reported in parts per trillion (ppt) and ng/m^3.

2) Propagation of error analysis on the measurements and calculations used to produce contaminant concetrations revealed that results have a relative uncertainty of ±10% when 1000 ng of contaminant has been trapped during a pumping period. Uncertainties on concentrations from samples with 100 ng of contaminant are larger -- in the range of 17% to 141%. Thus, trace level observations of contaminants are restricted to one significant digit.

3) Two initial dosing experiments were performed in Montana to demonstrate a possible mechanism for transport of contaminants by forager into the hives. Bees were trained to utilized a watering station that had been scented with anise as an attractant. Subsequently, the water supply was contaminated with liquid PCE, the organic contaminant noted prominently in APG hive air samples. It was immediately observed in the hive at levels above ambient air, suggesting that the bees contacted or ingested it at the watering station. Another contaminant observed at APG, naphthalene, was used in a second watering station trial. Disbursed as solid flakes, it was not as efficiently transported as the liquid compound. At APG naphthalene was probably transported as a dissolved component of petroleum-based mixtures.

Project Activities Proposed for the 1998 Field Season

Proposed activities for 1998 include additional APG applications at J Field (to monitor a removal activity and to investigate the potential for interactions between bees and the trees used for phytoremediation), and original site assessments at D Field. Three transects of survey hives will be used in a boundary area study to assess the extent of off-site, non-military sources of contaminants. The electronic hive systems will be outfitted with either telephone or wireless modems for remote delivery of real time honey bee behavioral and weather data.

Chemistry goals for 1998 include increased use of external and internal standards to improve data QA/QC, modification of the air sampling train to remove moisture and varnish-forming terpenes and sugars, and an extensive set of dose-response trials with solid, liquid and gaseous contaminants.

SUBJECT TERMS:

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

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SECTION 1

MONITORING OF COLONY BEHAVIORAL RESPONSES TO CONTAMINANTS, WEATHER, AND OTHER ENVIRONMENTAL FACTORS

1.1 Colony Dynamics and Behavioral Responses

For this project, colony response metrics are guided by two overall objectives:

1) Developing real-time monitoring of colony population dynamics to establish the relationship between acute exposures to specific chemical agents and measured behavioral endpoints; and

2) Assessing the responses of honey bee colony populations for site-to-site comparisons with respect to the effects of chronic as well as acute ecosystem exposures to bioavailable chemical agents.

We have made considerable progress toward accomplishing automated, real-time monitoring of honey bee colony performance. During the summer of 1996, 21 electronically-equipped, mini-hives containing nucleus (small) colonies of bees were deployed at two Aberdeen Proving Ground (APG) and a rural Maryland reference site. Another set of seven electronic hives were established in 1997 at a Montana reference site. All of 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. The field trials produced an extensive data set needed to: (1) determine the sensitivity, variability, and usefulness of several population-level, toxicity assessment endpoints, (2) further develop and refine models of honey bee population dynamics, and (3) conduct ongoing hazard assessments at APG.

These automated systems provided real-time monitoring capability for a select set of colony responses, as well as a wealth of data and experience from applications at the APG field sites. Colony response data was continuously displayed on computer screens in real-time at the field locations. However, the data could not be easily accessed from a distance, nor could it be simply retrieved from the resultant databases that increased daily in size by several Mbs. Also, bee flight data, collected at a rate of 200 counts per second, produced enormous, data-rich files. The data then needed to be reduced and simplified via averaging, smoothing, and other numerical processing procedures. With appropriate data processing, it was possible to isolate response signals from a background of rapidly changing bee activity and to interpret bee movements as reactions to a variety of internal and external stressors.

Two new aspects of behavioral monitoring are presented in this year's report. First, we produced an extensive array of software tools for the display, inspection, interpretation, and automated numerical analysis of colony behavioral data, with an emphasis on the bi-directional flight data. These software routines have been bundled as a set and can be distributed on a CD-ROM along with the colony response and meteorological data from 1996 and 1997. Second, we developed the software required to deliver colony response data in real time via Internet or Intranet connections.

All of the custom and commercial interface software that had been used until the fall of 1997 has been replaced with custom software that we wrote. The new software offers several improvements. Automatic updates and aggressive error correction routines are more robust than those in the "commercial software" and have virtually eliminated occasional data losses by the interface hardware used for the hives and for the meteorological stations. The new software ports the data to Linux (a Unix operating clone) for a more stable multi-tasking environment. JAVA and CGI scripts can post data summaries in text format every 15 minutes and provide interactive graphical displays of the data. An on-line weather station updates every 15 seconds. The on-line weather station and two weeks of archived data from August, 1997, at the Missoula reference locations can be accessed and interactively displayed via our web site ***(http://www.umt.edu/biology/bees)***.

1.2 Materials and Methods

Design and construction of the electronic bee-counters and other hive sensors was discussed in detail in the previous report submitted to the Army (Bromenshenk *et al.*, 1997). Because all of the real-time monitoring devices, software, and data acquisition software were developed during the first two years of this project, materials and methods development continues to be an important aspect of this project. The 1996 annual report discussed our prototype off-line data analysis tools used to characterize honey bee forager flight activity as applied to the three Maryland study sites. In 1997, four field sites were monitored for *Apis mellifera* flight activity, three in Maryland in a biomonitoring application mode, and one in Montana used as a research and development station for further identification and characterization of flight activity disturbances. Because the 1995 and 1996 trials indicated that flight activity and core temperature of the brood nest were critical assessment and measurement endpoints, we focused the 1997 investigations on real-time monitoring of these two metrics and upgrading of the software from prototypical to robust, finalized versions. Real-time monitoring was continued from the previous year with stations set up at West Branch Canal Creek, Old O Field, and a rural Maryland reference site at Churchville (see Section 2 for site descriptions). J Field was added September 1997, using electronic hives that had been at West Branch Canal Creek earlier in the season.

Software bugs encountered during the 1996 field season were addressed prior to field deployment in 1997. A duplicate channel problem described in the 1996 annual report (Bromenshenk *et. al.*, 1997) was solved by correcting errors in the data acquisition sub-program. In this case, the program assigned incorrect channel inputs from the interface to arrays designated

to other channels. Another problem was addressed by reformatting the daily compressed file and directory naming system. The new system labels files and directories with a two letter code for each site followed by six numbers representing the date (i.e., YY/MM/DD). Thus the files and directories are labeled as SSYYMMDD. Table 1.1 provides examples of the new filenaming scheme, which anticipates the year 2000 changeover.

Table 1.1
Explanation of 1997 File Naming System.

Name of Site	Site-Code	Date and Year	File Name
West Branch Canal Creek	CC	August 7, 1996	CC960807
J Field	JF	September 5, 1997	JF970905
Old O Field	OF	June 22, 1997	OF970622
Churchville Reference	CV	September 1, 1997	CV970901
Fort Missoula	FM	July 21, 1997	FM970721

The basic structure of the flight activity data files has remained unchanged. Originally, stopping data acquisition to service the counter system posed a problem to the new file-naming format. This was overcome by automatically appending previously opened files.

A final software fix implemented prior to the start of 1997 field sampling addressed problems encountered with temperature dependence of the oscillation frequency of the square-wave generator used to trigger sampling. The square wave generator was based on a 555 oscillator circuit that included a temperature sensitive capacitor. The thirty second sampling interval used in data acquisition is based on the number of samples collected and the sampling rate (6000 samples for a 30 second period at 200 Hz). Typical operating frequencies to trigger sampling at 200 Hz were in the range of 2000 to 3000 Hz. The thirty second period was found to range from 28 to 39 seconds, because temperature variations affected the oscillation frequency of the 555 based square-wave generator circuit. During the 1996 field season, the 555 square-wave generator was recalibrated every 15 to 30 minutes to account for these weather induced temperature changes. In addition to the temperature variations, transients were discovered when the raw output of the square-wave generator was saved to a disk file. The transients sometimes caused premature triggering of sampling.

A more rigorous sampling algorithm was tested that eliminated problems caused by the transients but was unable to remedy the temperature dependence of the square wave generator. The problem was finally resolved by abandoning the 555 square-wave generator circuit and instead using the 8254 timer circuit used for sound generation in computers. The revised sampling algorithm operating at 200 Hz was able to generate sampling intervals to within ±0.07

seconds of the desired thirty second period on computers ranging from 120 MHZ Pentiums to 80286's. The increase in triggering accuracy guaranteed consistent thirty-second sampling periods throughout the year regardless of temperature or the computer used to acquire data.

Several software enhancements were implemented after field placement of bee-counter systems during the 1997 flight season. First, the data analysis code was optimized to decrease the length of time required for processing raw data collected during each thirty second sampling interval. To aid the code-optimization process, a test version of the field data acquisition program was written that accessed a simulated data file instead of the IOTech interface. The simulated data file was created from raw data collected during the Spring of 1996 at the U of M Prescott House apiary. This program allowed improvements to be tested and benchmarked as the code was written. Next, disk caching via the DOS utility SMARTDRV.EXE was used to improve the hard drive access speed. For new features and code optimization described below, this program proved a reliable testing ground prior to field deployment of software.

1920 thirty second intervals exist during a 16 hour sampling period (6:00 to 22:00). The computer processes raw data for approximately 1 to 5 seconds at the end of each thirty second interval, during which time data acquisition does not occur. Using the Fort Missoula site as an example, the typical number of thirty second sampling intervals collected during a day was 1680 before the code was optimized and 1830 after the code was optimized. This increased the sampling coverage from 85.5% to 95.3% of the total flight population. This 9% increase in number of samples collected over the course of a day did not affect the daily percent return rates in any way, indicating that the unoptimized code had adequately characterized flight activity.

Real-time delivery of data over the Internet, via a World Wide Web (WWW) home page, was a principal focus of the 1997 Methods Development (see Section 1.10). The bee-counter data acquisition software was modified to communicate via the RS-232 (serial) port to a computer equipped to receive data on fifteen minute intervals. The fifteen minute interval was used to minimize the impact of data transfer on the bandwidth required for data collection and transmission. The time interval can be further reduced to fit specific real-time application demands. The field data acquisition and display software was modified to transfer data to a computer running the Linux operating system. For field-based delivery of real-time data, the bee-counter computer will connect to the WWW via a Linux based computer (described later in this section). Flight counts for bees entering and leaving each hive are updated throughout the day. A compressed data file that includes count information for each of the 14 individual bee passageways in each hive is transferred across the network at the end of the sampling day.

Finally, the field data acquisition and display program was updated to address problems encountered during brief power outages in which the computer interface would shut-down and fail to restart. This was fixed by adding a command to restart the interface on fifteen minute intervals throughout the day. In other words, within 15 minutes of restoration of power, the system will be back on-line.

The Fort Missoula Research and Development Apiary also was the home to three colonies of *Bombus occidentalis* (bumble bees) that were placed in a man-made burrow with specially designed bee-counting devices attached. *B. occidentalis* was chosen as a model native species for use in environmental biomonitoring. The counters were housed in ABS plumbing material and constructed with 1 large bee-passageway to accommodate the larger sized bumble bees. These colonies housed less than 100 bees each. A single passageway proved to be sufficient for counting bumble bees. There were never more than 4 bees passing through the counter in 1 thirty-second interval. By comparison, honey bee counters averaged 34 bees passing through each channel per thirty second interval during a swarm event. Comparisons were made between bumble bee and honey bee flight activity. Both species responded to changes in weather in a similar manner. The bumble bee prototype systems and tests were funded by the National Science Foundation (NSF), The University of Montana, and the Montana Beekeepers Association. There was no cost to the army. Costs to these other sponsors were relatively low because we were able to apply lessons learned from designing the equipment and software already developed under this DOD project.

Correlation of forager flight activity with weather events was aided by the introduction of a solar radiation data-logger at the Fort Missoula apiary (loaned to this project by Dr. V. Watson, UM Environmental Studies Program). Data containing mean, high and low solar radiation values were collected every 10 minutes. The amount of sunlight present each day was correlated with flight activity data to further characterize daily flight activity patterns.

1.3 Off-line Data Tier Analysis Methods

As mentioned, electronic bee-counters were deployed at five locations during the 1997 field monitoring season. Analysis of flight activity data by site followed the Tier-based approach established during the 1996 monitoring season. The Tier system for flight activity is based on a biological organization (i.e., among populations, between populations, among and between individual bees by site and year) and is summarized in Table 1.2.

Table 1.2
Overview of the Flight Activity Data Tier System.

Tier	Capabilities and applications
1	Site-wide daily flight activity indicators compared between sites Site-wide daily flight activity indicators compared from year to year for each site
2	Flight activity indicators compared among hives at a site Can be used to compare seasonal changes at a site
3	Monitor short-term response of single counter or all counters at a site Site-wide short-term flight activity response compared between sites
4	Site-wide counter diagnostics using data from 14 bee passageways on each counter

1.3.1 Tier 1

Tier 1 flight activity analysis includes total flight activity (TFA), inter-colony coefficient of variation (C.V.) of total activity, the percentage of bees returning to the colonies at each site at the end of each day (PRC), the net loss (NL) of bees at the end of each day, and the adjusted net loss (ANL) of bees at the end of each day. The PRC for each colony at each site at the end of the day was calculated as the total number of bees that entered the counters divided by the total number of bees that exited the counters at each site for a given day. Net loss (NL) was calculated by subtracting the total number of bees that entered the counters from the total number of bees that exited the counters at each site for a given day. Adjusted net loss (ANL) was calculated by dividing the net loss of bees for the site by the total activity for the site.

Although similar, the flight activity analysis methods have individual strengths that determine their function. The percentage of bees returning (PRC) to the hive at the end of each day usually remained in the range of 92% to 98%. Deviations outside this range were due to events such as rainshowers at the beginning or end of the day, natural colony dynamics (swarms), exposures to acutely toxic chemical events, or equipment malfunction (power outages). The daily net loss (NL) of bees was affected by the level of total flight activity for each day. As such, it was used as a secondary indicator in conjunction with adjusted net loss (ANL). Net loss must be corrected for the total flight activity to be a reliable indicator. For example, on a warm sunny day total flight activity may exhibit a net loss of bees (by count) that exceeds the total flight activity on a low activity day. Recruitment (or drift) of bees from other nearby colonies could result in a net gain of bees for an individual colony, but no change when averaged across all of the hives at a site.

Adjusted net loss normally ranged from -4 to +6, depending on whether there was a net loss (positive numbers) or net gain (negative numbers) of bees for each hive/day. If a large net gain or loss occurred, the net loss and total flight activity data were then reviewed to investigate the nature of the event. Based on the results, appropriate action could be taken, such as initiating chemical sampling or inspecting the colonies. All Tier 1 results were represented using 2-dimensional X-Y plots.

1.3.2 Tier 2

Tier 2 analysis applied Tier 1 analysis methods to individually compare the colonies at a site. The PRC, NL and ANL methods were all expected to demonstrate greater fluctuations for individual colonies than observed in the Tier 1 results. Normalized Total Activity (NTA) was calculated as the total activity for each colony divided by the total activity for the seven colonies at the site. This indicator provided an easy method of determining the strength of individual colonies in relation to one another. With seven colonies at a site, the optimal NTA value for colonies of equal strength was about 14.3%, larger values indicated a stronger than average colony and lower values indicated a weaker colony. All Tier 2 results were represented using color maps for formal analysis and 2-dimensional X-Y plots using Option 10 of the SITEVIEW program on the included CD-ROM. A SITEVIEW Manual is included in Appendix A.

1.3.3 Tier 3

Tier 3 flight activity analyses included all data analysis methods that operate on short-term flight activity data. SITEVIEW offers many analysis options for Tier 3 data. Each option is a custom software utility program written at UM. These utilities offer data processing and visualization capabilities ranging from: (1) data file formatting and storage, (2) simultaneous display of raw or smoothed data from seven colonies at each site, (3) site by site comparisons, (4) honey bee and bumblebee species comparisons, and (5) complex numerical analysis methods that are highly sensitive to unusual flight activity behavior. The following text is taken from the main menu of the SITEVIEW program.

WELCOME TO THE BEE-COUNT DATA MANIPULATOR AND VISUALIZATION

THERE ARE SEVERAL SELECTIONS AVAILABLE FOR PROCESSING AND VIEWING DATA OBTAINED FROM SITES CONTAINING SEVEN BEE COUNTERS.

(1) PLOT TOTAL ACTIVITY FOR 7 HIVES (NOISY DATA)
(2) PLOT SMOOTHED TOTAL ACTIVITY DATA FOR 7 HIVES
(3) PLOT DIFFERENCE BETWEEN IN AND OUT ACTIVITY FOR 7 HIVES
(4) PLOT IN AND OUT DATA SIMULTANEOUSLY TO SEE OVERLAP
(5) PLOT SMOOTHED PERCENT RETURNED FOR 7 HIVES
(6) CALCULATE AND PLOT SMOOTHED DERIVATIVES OF FLIGHT ACTIVITY
(7) PLOT COMPONENTS OF THE DER*DIFF ALGORITHM USED IN OPTION 7
(8) PLOT DERIV*DIFF RATIO TO SEE PHASE SHIFT SENSITIVE ANALYSIS
(9) DISPLAY AN HOURLY OR USER SELECTED INTEGRATION PERIOD
(10) PLOT SITEWIDE DAILY ACTIVITY FOR ENTIRE SEASON
(11) SIMULATE THE FIELD DISPLAY
(12) PLOT FOUR DAYS/SITES SIMULTANEOUSLY
(15) REVERSE BACKGROUND / FOREGROUND COLOR
(19) DELETE OPENED DATA FILES FROM DISK, LEAVES COMPRESSED FILES ALONE
(20) END SITE/STUDY VIEWER MENU AND RETURN TO MAIN MENU
PLEASE ENTER A NUMBER FROM THE ABOVE LIST:

Each of these options are described in detail in the SITEVIEW Program Description and Trouble Shooting Manual found in Appendix A and included on the CD-ROM disk. The SITEVIEW Manual includes instructions and suggested parameters for use of each option. The CD-ROM contains a complete archive of the 1996 and 1997 field data for this project. Significant aspects of the flight activity data are described to aid the user in interpretation of the results from each option. Specific data sets are used to illustrate visualization and interpretation of the output of many of the SITEVIEW routines. Equations are described to clarify the numerical methods used to process data from the bee-counters.

The options available in the SITEVIEW program provide rapid viewing of the flight activity data using a PC based computer. Some of these options illustrate the daily flight activity patterns for the colonies at a single site, while others allow comparisons of the site-wide total flight activity pattern for several sites simultaneously. For example, tracking events such as a storm moving from one site to another can be accomplished by concurrently displaying the total flight activity throughout a given day for two or more sites (Option 12 of the SITEVIEW program). Established metrics of flight activity such as TFA, C.V., PRC, ANL, and NL can be plotted to rapidly provide information about changes in colony behavior.

1.3.4 Tier 4

Tier 4 analyses used count data from each of the 14 bee-passageways of each bee-counter as a diagnostic tool. Tier 4 diagnostics used the coefficient of variation among passageways on a single bee-counter, as well as among the similar passageways for each of the seven bee-counters at a site. Large values for the C.V. among passageways on a single bee-counter alerts the operator to the presence of defective channel-counters but does not provide information about which passageways were blocked. Large values of the C.V. among similar passageways for bee-counters at a site helped pinpoint which passageways were blocked or defective. When an unusually large C.V. was observed, direct observation of the data from each bee passageway identified the defective or blocked units that required replacement. Several options of the SITEVIEW program can be used to perform these Tier 4 analyses. Application of Tier 4 diagnostics allowed quick evaluation for detection and assignment of hardware problems.

1.4 Results of Tier 1 Evaluations of Flight Activity Data

Flight activity data collected during the Summer and Fall of 1997 were summarized and compared with data from the 1996 field monitoring season. Figure 1.1 presents Tier 1 summaries comparing the corrected (i.e., adjusted for the number of sampling periods and number of bee-counters being monitored) total daily flight activity throughout the season for each of the test sites. A detailed summary of events that occurred during the 1996 field monitoring season was presented in the previous annual report (Bromenshenk *et al.*, 1997). In general, flight activity at all of the APG sites displayed similar day to day seasonal trends, probably as a consequence of colony responses to local weather conditions. All sites showed a similar seasonal trend characterized by high activity during the summer, a slow decline in activity as summer progressed into fall, and then very little activity after the middle of October.

Weather events had a similar effect on all APG sites during both the 1997 and 1996 monitoring programs. In 1997, large drops in activity were visible at all APG sites on July 23-24, August 20 and September 10, indicating the presence of rain. Unlike the 1996 season, in 1997 both the West Branch Canal Creek site and the Old O Field site displayed a general drop in activity from July 29 to August 14. This decrease was not mirrored by the bees at the Churchville reference site. During the same period bees at the Fort Missoula site were busy foraging nearby knapweed and were increasing activity. The drop in activity at Old O Field was similar to that

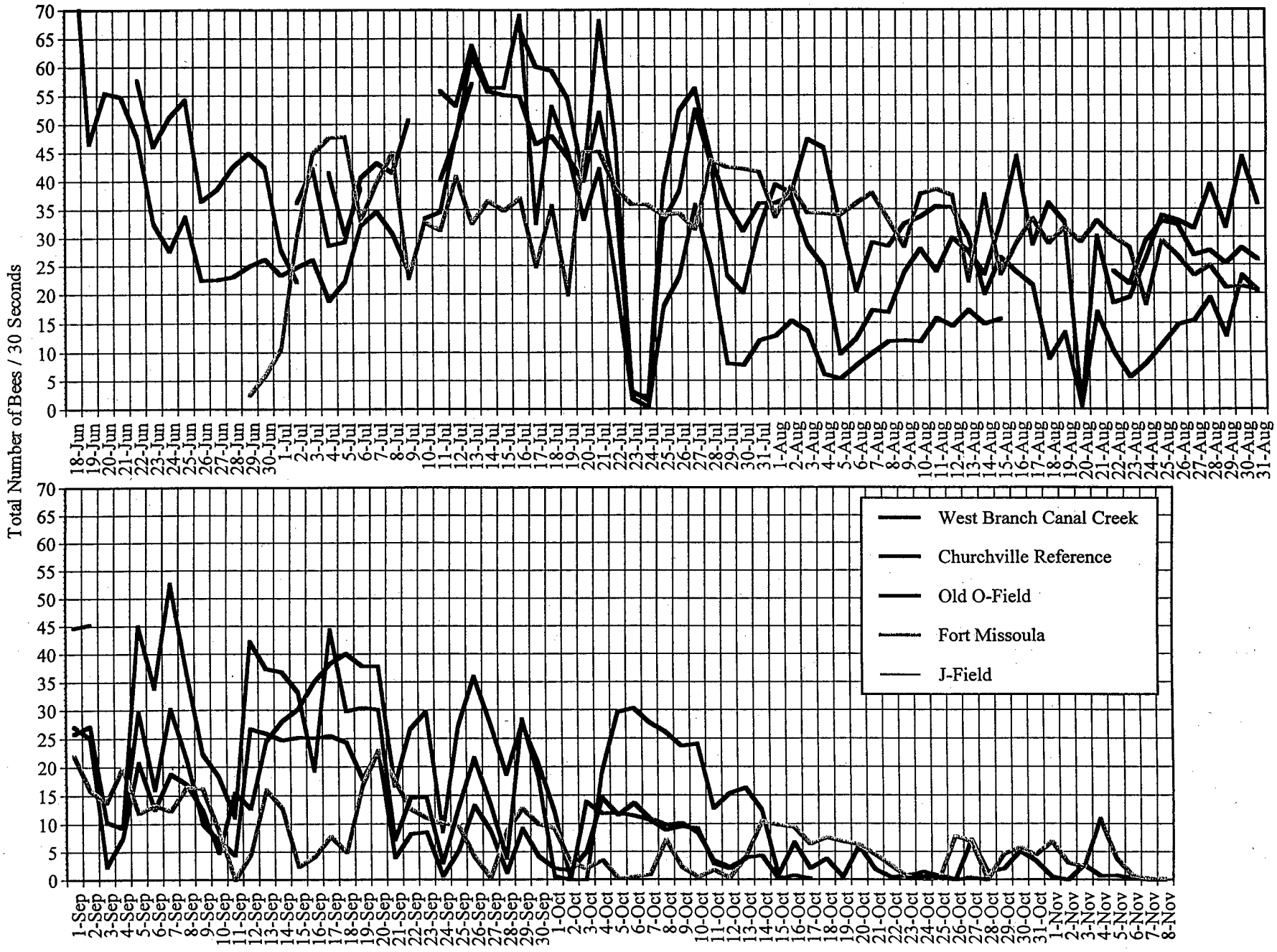


Figure 1.1. Cumulative daily flight activity by site, 1997. Total number of bees entering and leaving the hives every 30 seconds. Each site had seven colonies of bees.

seen in the previous year after a hurricane passed through the area. The drop in activity for West Branch Canal Creek was not as severe as that seen for Old O Field. This response could be due to a lack of available foraging materials on the APG peninsula. Because the Old O Field change in activity again corresponded with an increase in the levels of PCE and other organics in the air inside the hives (see Section 3), it is possible that greater change at Old O Field might have been due to exposure to these chemicals. In 1996, a severe drop in activity and loss of the queen from 50% of the hives at Old O Field corresponded with a period of high exposures to organic solvents (relative to other APG sites).

Coefficients of variation of flight activity among the colonies at a site for the 1997 monitoring season also were plotted and compared. As seen in Figure 1.2, the C.V. for all sites remained near or below 50% throughout the summer and into October, with a few notable exceptions. A two day long rain-event during July 23 and 24 produced the large peak found at West Branch Canal Creek and the Churchville reference site. Oddly enough, this peak is barely discernable for only the first day at Old O Field. The swarm at one of the Churchville colonies raised the C.V. from around 30% to nearly 50% until the colony was reconstituted.

Power failures, usually following thunderstorms, continued to plague all of the Maryland sites as evidenced by gaps in data, resulting in high C.V. peaks for these days. The Fort Missoula site was brought on-line in stages, with the last four colonies installed on July 13, explaining the unusually high C.V. at the beginning of its season. The increase in C.V. at Fort Missoula from the beginning of October until the end of the monitoring season was attributed to a combination of low flight activity, and experiments testing the effects of a misaligned counter.

Extremely low flight activity or incomplete data sets have a detrimental effect on the calculation of C.V. Low flight activity increased the C.V., if even one colony had modest activity, while other colonies had little or none. As expected, missing data from the daytime period increased the C.V., although missing data during the night had no effect since bees don't fly at night. If total daily flight activity was lower than 3000 bees entering and exiting the counter, or an incomplete data set was collected, the C.V. was not analyzed.

Sister queens were used during the 1997 season to test whether reducing genetic variability would improve the correlation of responses between colonies and across sites. All of the Maryland colonies were established with sister queens. At Fort Missoula in Montana, colonies 1, 2, and 3 received sister queens; colonies 4-7 had queens from different genetic stocks. The presence of sister queens appeared to lower the C.V.s for 1997 compared to 1996 at Old O Field and the Churchville reference site (Table 1.3). At West Branch Canal Creek, the C.V. remained around 50% until the end of July when it dropped to levels similar to the previous year. The higher C.V. at the beginning of the season was probably a result of a strong colony swarming. Although the C.V. at the Old O Field site improved dramatically over the previous year, it remained higher than both West Branch Canal Creek and the Churchville reference site.

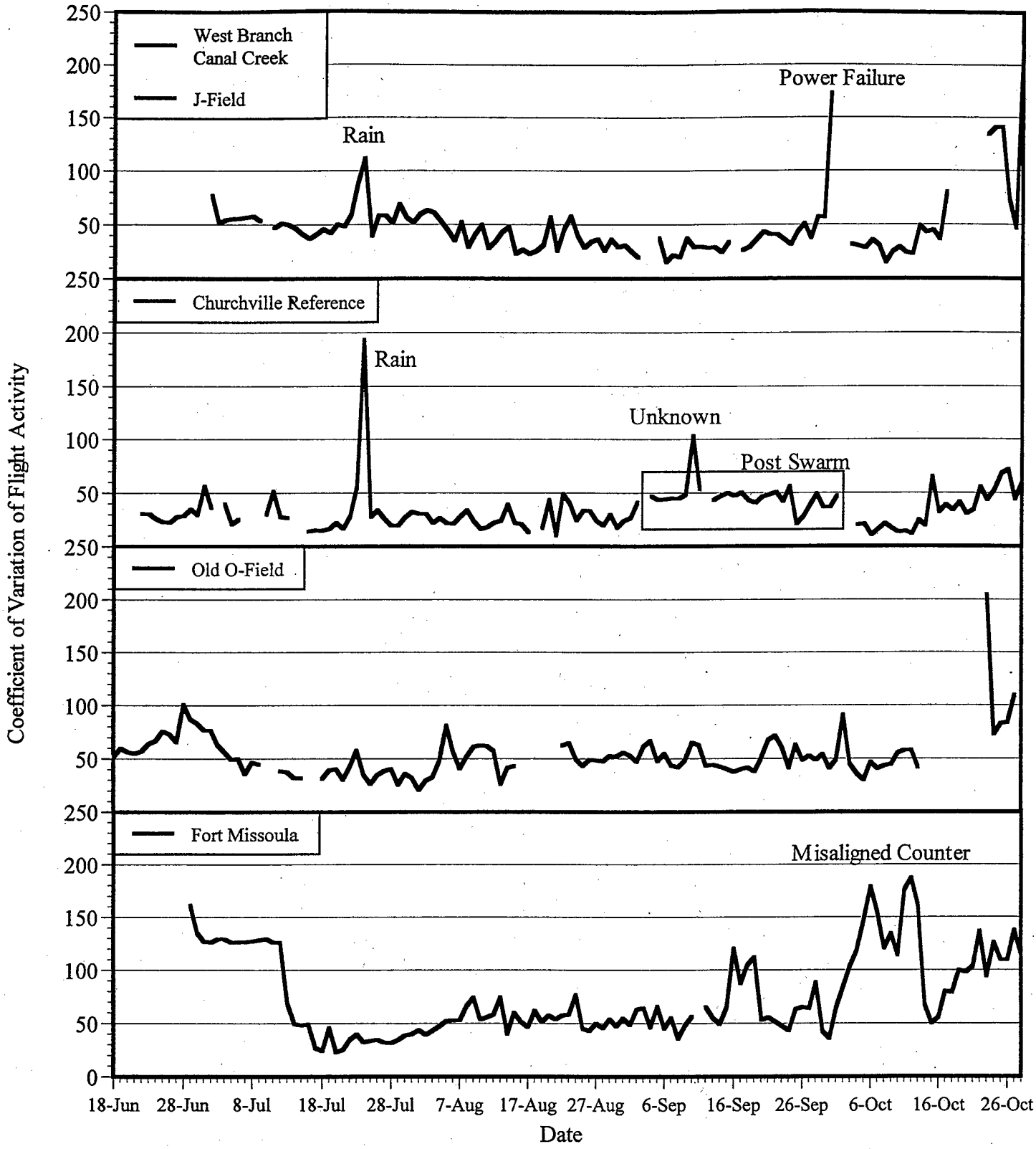


Figure 1.2. Coefficient of variation of flight activity by site, 1997.

The percentage of bees returning to all of the hives at a site at the end of the day was calculated by the site-wide PRC analysis (Table 1.3). This metric provided an indication of the general health of the colonies at each site. Figure 1.3 illustrates the percent returned to the hive at the end of the day for sites monitored in 1997. Generally, for West Branch Canal Creek and the Churchville reference site, the sitewide PRC results stayed within the range of 94% to 98% during the foraging season. A rain event on July 23 and 24 produced PRC peaks for West Branch Canal Creek, Old O Field and the Churchville reference site. A swarm that occurred on September 1 at the Churchville reference site yielded a PRC of less than 90% for the entire site, due to a 43% PRC for the colony that swarmed. More information on the swarm is included in Tier 2 and 3 analysis. The site-wide PRC at Old O Field and Fort Missoula varied from 94% to 104% and 94% to 108%, respectively, during the foraging season. Both sites experienced occasional days with PRCs greater than 100%, especially from late July to mid-August. At the Fort Missoula site this correlated with the knapweed bloom occurring in adjacent fields. The increase in PRC at the Old O Field site is still unexplained. We suspect that during heavy nectar flows, some of the bees may stay in the field overnight, returning the next day. Tests of the flight counters indicated that the PRC in excess of 100% were real and not an artifact of some equipment malfunction.

Table 1.3

Comparison of Tier 1 (Site-Wide) Indicators.

Percent values in this table represent "normal" colony variability and do not include maximums induced by events such as severe rainstorms, swarming, or power outages.

Site	C.V.		PRC		ANL	
	1996	1997	1996	1997	1996	1997
CC	15%-60%	20%-50%	90%-108%	94%-98%	-4 to +4	0 to +5
OF	50%-130%	25%-75%	90%-102%	94%-104%	-2 to +6	-2 to +3
CV	50%-90%	15%-50%	92%-100%	94%-98%	0 to +4	0 to +3
JF	-	15%-50%	-	92%-98%	-	+1 to +5
FM	-	30%-140%	-	94%-108%	-	-4 to +3

Adjusted net loss and corrected PRC results for total flight activity were calculated so that a quality control window could be established for daily forager flight behavior. Values of ANL between 0 and 4 are considered to be acceptable, while values outside of that range require closer inspection. As seen in Table 1.3, variation of ANL improved dramatically from to 1996 to 1997.

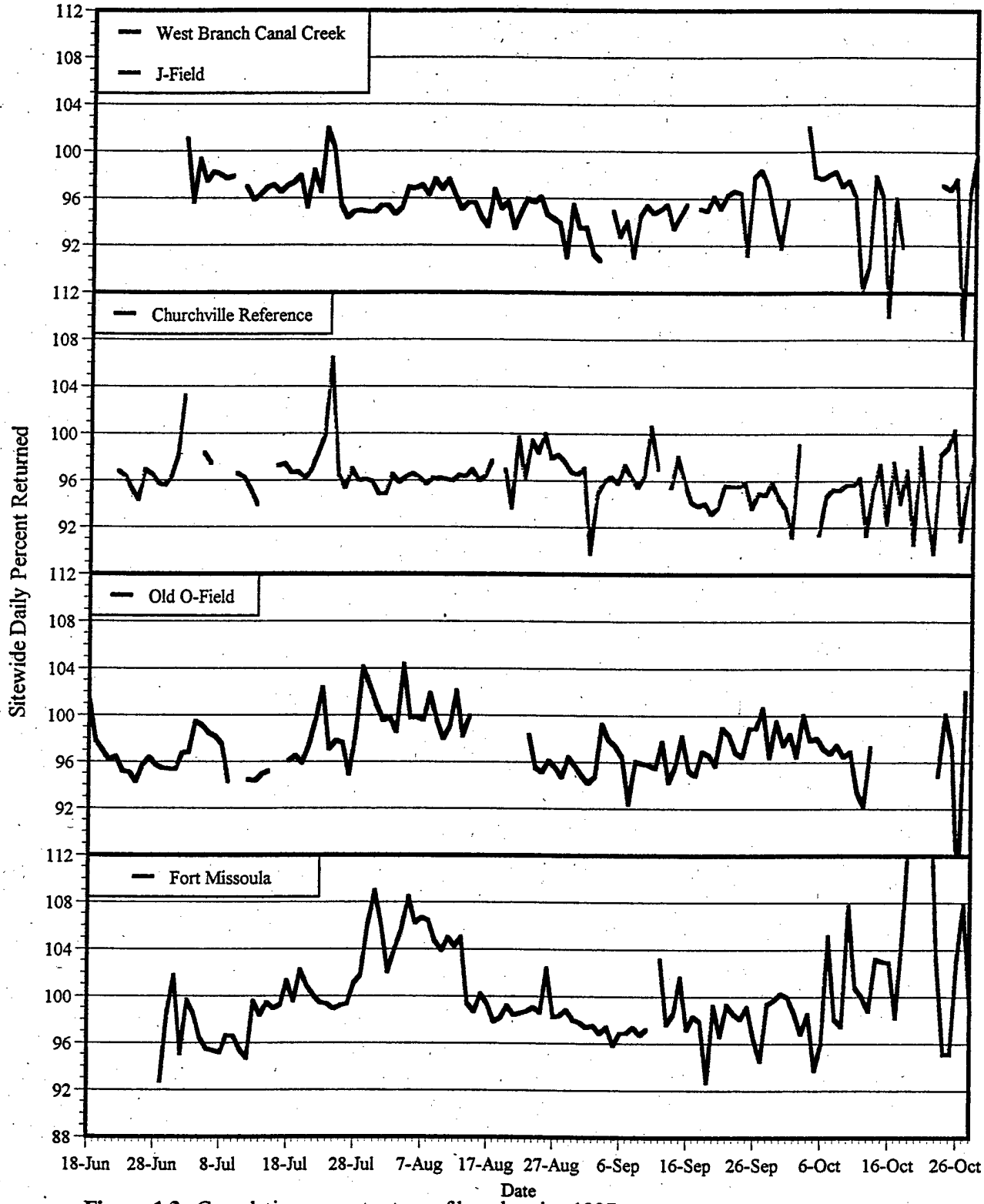


Figure 1.3. Cumulative percent return of bees by site, 1997.

As seen in Table 1.3, ANL variation improved dramatically in 1997 compared to 1996. PRC results from the 1997 monitoring season also showed lower day to day variations and a smaller range for the sites under study, compared to 1996. In 1996, West Branch Canal Creek colonies had a PRC that ranged from 90% to 108%. In 1997, the West Branch Canal Creek/J Field colony mean return rates varied from 94% to 98%. The Churchville reference site saw a similar change, with the range being from 92% to 100% in 1996 and then from 94% to 98% (with the exception of the day of the swarm) in 1997. Old O Field varied from less than 90% to 102% in 1996 compared to 94% to 104% in 1997. During both years, we observed drift from weak to strong colonies at Old O Field, contributing to PRCs in excess of 100%. Table 1.3 summarizes the C.V., PRC and ANL results for all of the sites under study.

Large day to day variations in C.V., PRC and ANL at the end of each year indicated the close of the productive forager flight season. The queens stop laying eggs and foraging drops off with as nectar and pollen floral resources disappear due to frost and plant senescence. The onset of increased variability correlated with the time when NL consistently remained below 2000 for each of the sites. In 1997 the end of the productive forager flight season for J Field colonies occurred on October 3. At the Churchville reference site the season ended on October 1, while the forager flight season ended on September 21 at Old O Field. Assignment of the exact end of the productive forager flight season for Fort Missoula colonies was hindered by the tests documenting the effects of a misaligned bee-counter. September 24 appeared to be the end of the forage period for Fort Missoula.

In 1996, a high degree of variability evidenced in the late season data made determination of the exact end of the productive foraging season difficult. Because naturally colonies suspend foraging in the fall, variability for all of the flight metrics is expected increase. The time when this occurs effectively determines how the end of the period when these metrics can be reliably used as bioassessment indicators. Obviously, the non-foraging period will be longest in northern states with cold winters, and short or non-existent in warmer southern states.

1.5 Results of Tier 2 Evaluations of Flight Activity Data

Daily flight activity data from individual colonies were compared with data from other colonies at each site. Sites that were part of a continuing study were compared to results from the previous year using Tier 2 analysis methods. Figures 1.4 - 1.8 present total daily flight activity for the sites under study. Colonies at West Branch Canal Creek were moved to J Field on September 4. Therefore, the initial behaviors of the colonies when placed at J Field should reflect patterns while still residing at West Branch Canal Creek.

With the exception of the Churchville reference locations, one or more colonies at each site exhibited unusually high flight activity when compared to the other colonies at the same site. Colony (#1) at West Branch Canal Creek was found to have 2 queens in early-August. One of

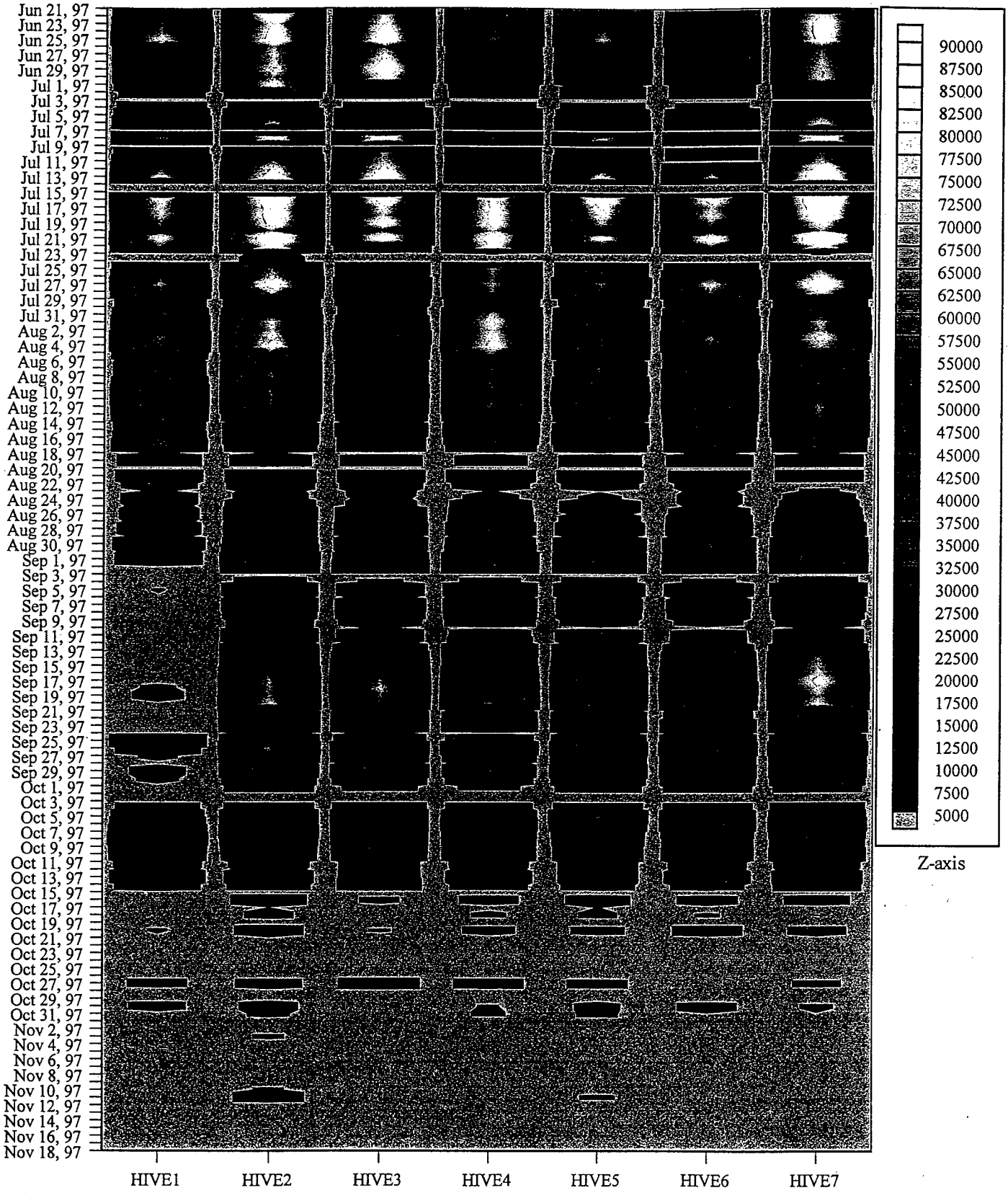


Figure 1.4. Cumulative daily flight activity, Churchville, 1997. Hotter colors denote higher activity.

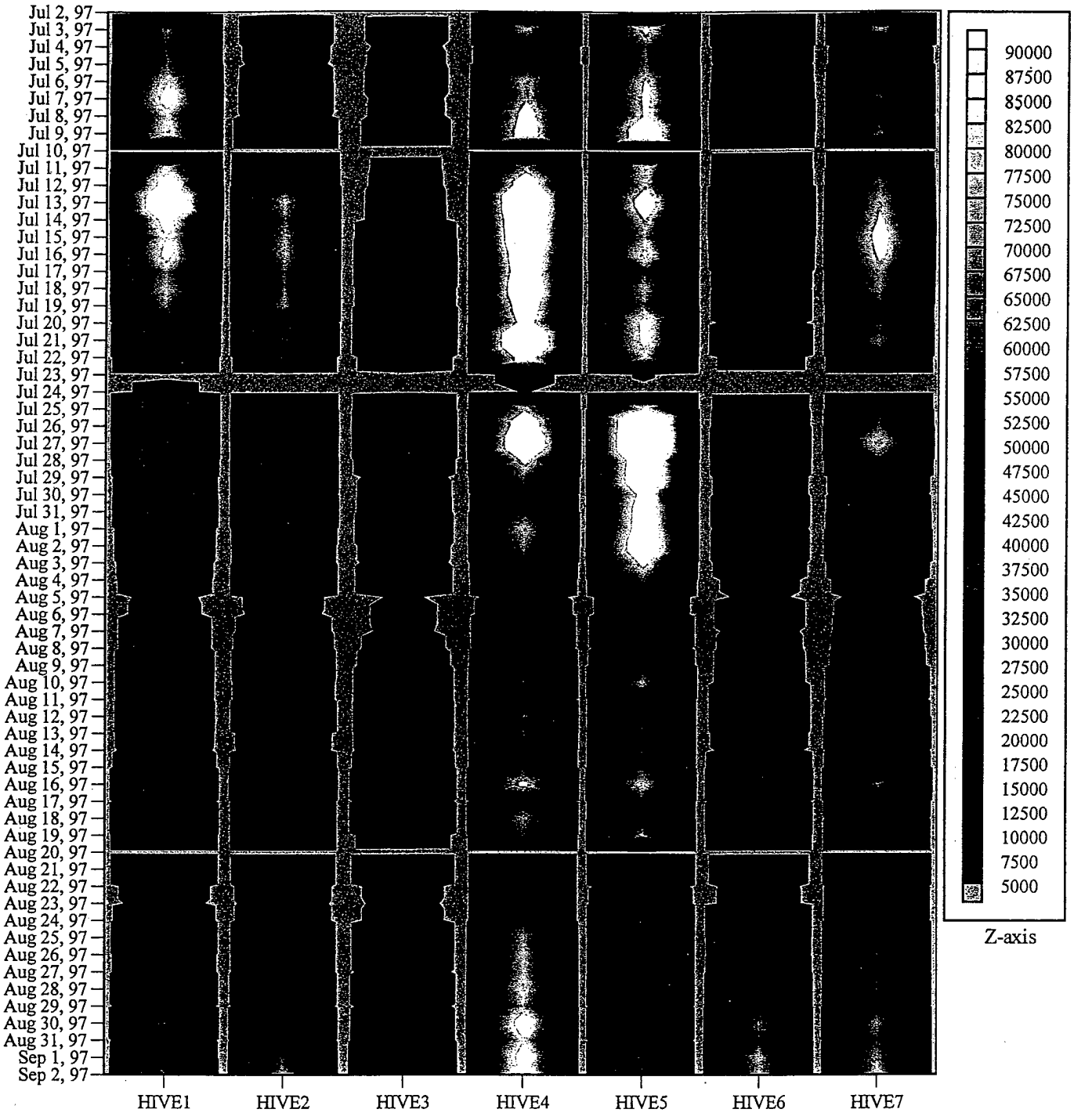


Figure 1.5. Cumulative daily flight activity, West Branch Canal Creek, 1997. Hotter colors denote higher activity.

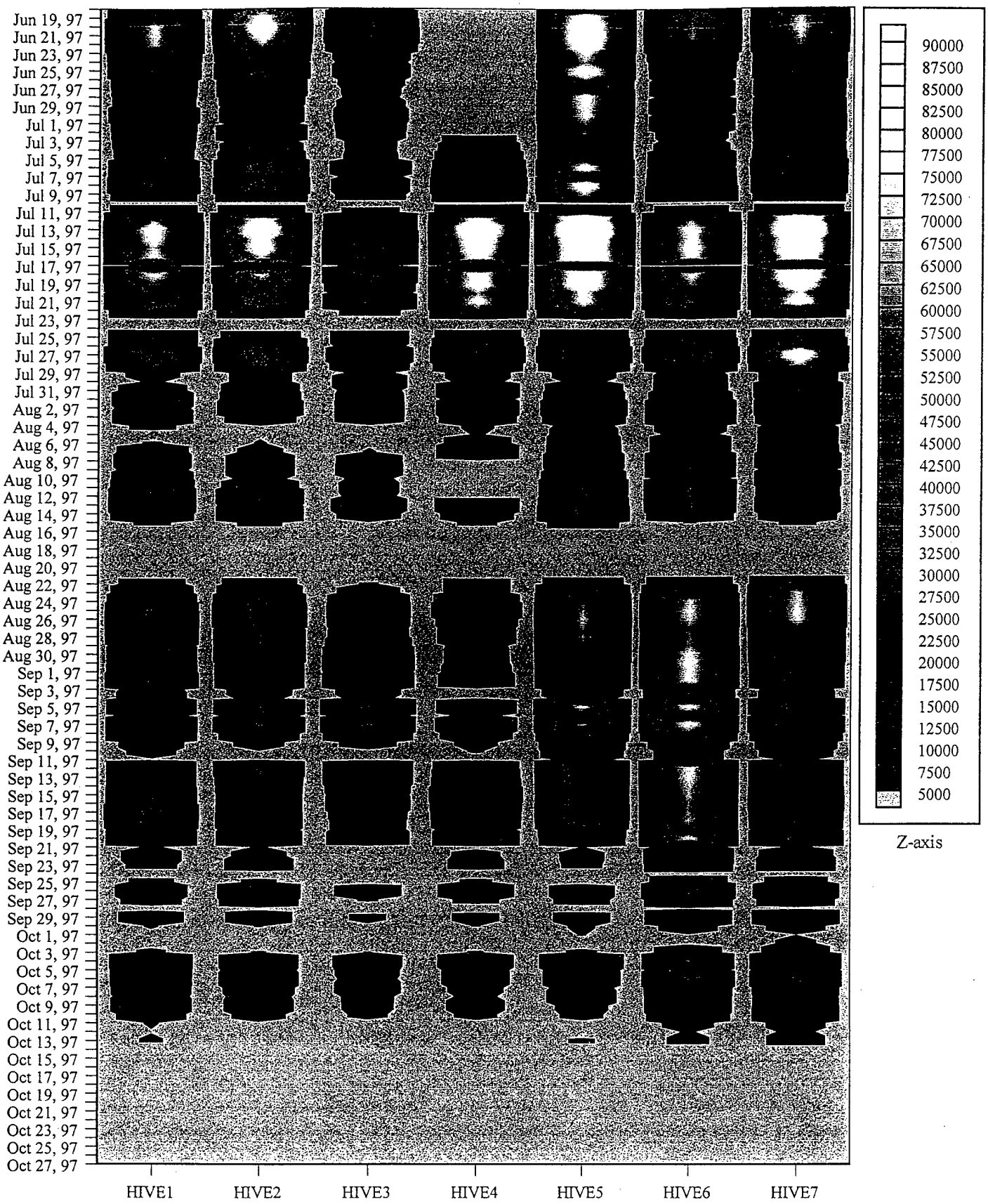


Figure 1.6. Cumulative daily flight activity, Old O-Field, 1997. Hotter colors denote higher activity.

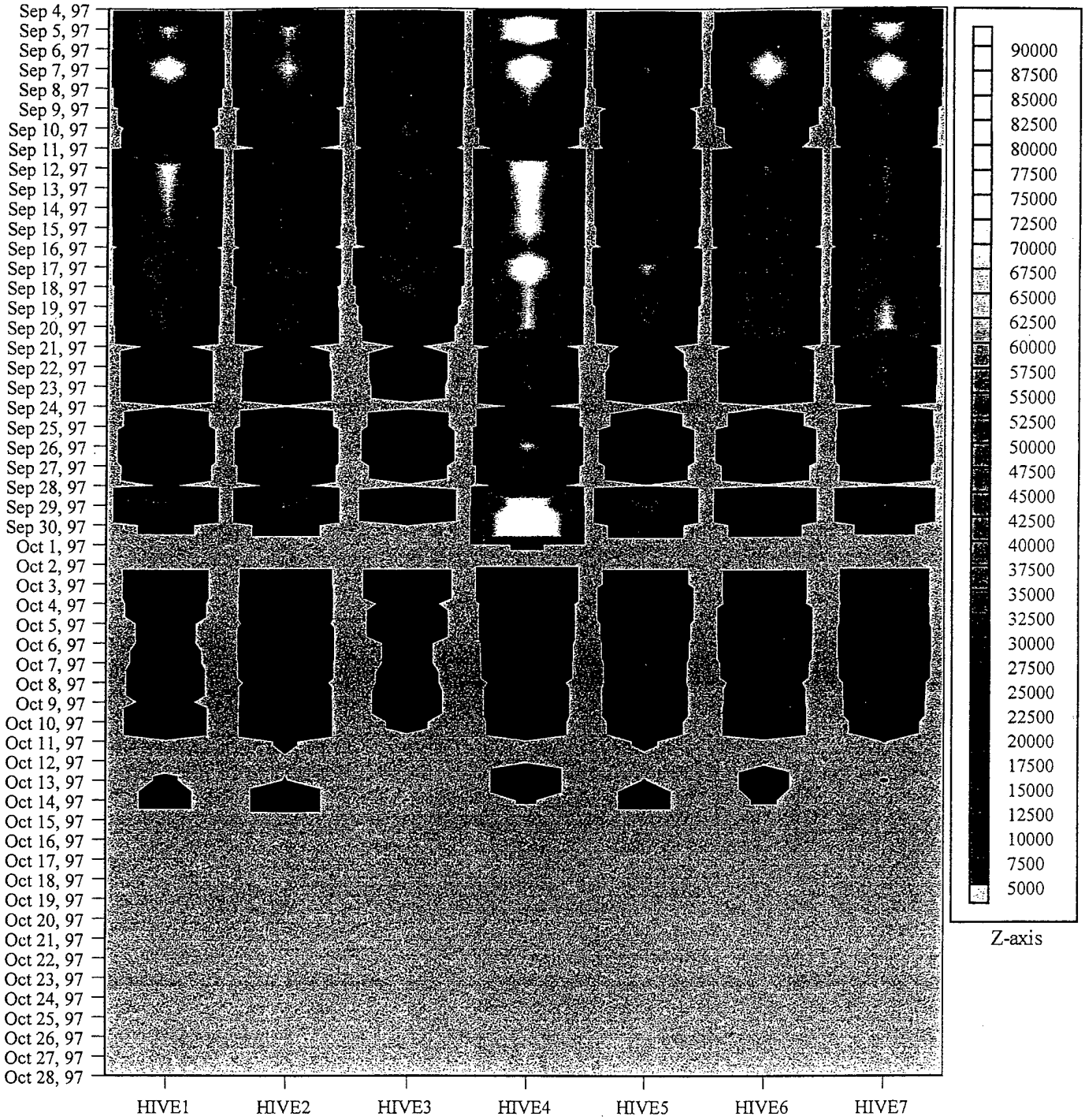


Figure 1.7. Cumulative daily flight activity, J-Field, 1997. Hotter colors denote higher activity.

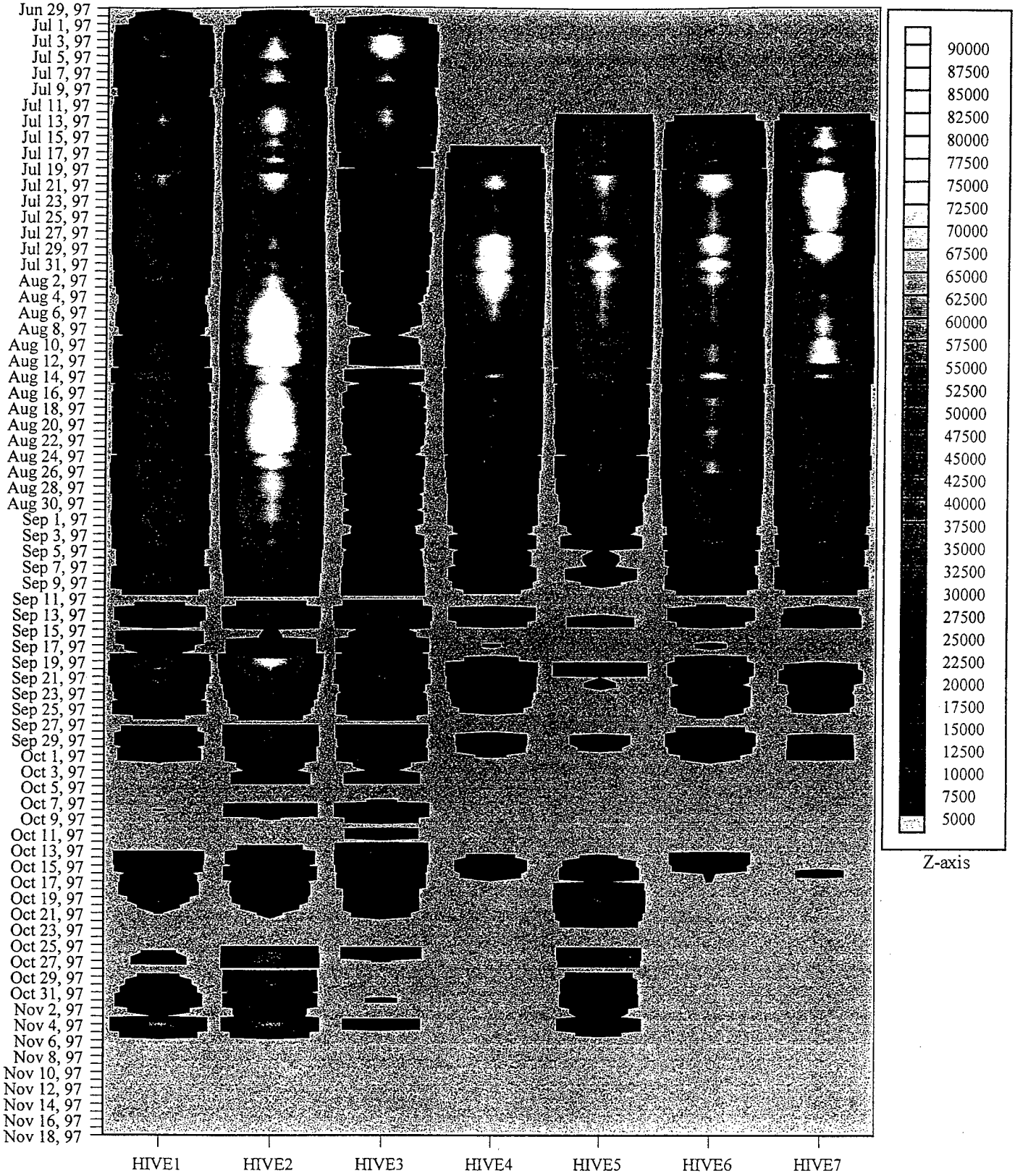


Figure 1.8. Cumulative daily flight activity, Fort Missoula, 1997. Hotter colors denote higher activity.

these queens was removed on August 10. Colonies #4 and #5 exhibited flight activity levels of 75,000-80,000 round trips per day. Figures 1.5 and 1.7 show that the transfer of colonies from West Branch Canal Creek to J Field did not affect the relative flight activity levels in any way.

Figure 1.4 shows the flight activity levels for the Churchville reference site during 1997. In comparison to the combined West Branch Canal Creek and J Field plots, flight activity trends were similar for all colonies except for #1 which swarmed on September 9. This colony was re-queened on September 27, and returned to normal flight activity in less than 10 days.

Flight activity maps for Old O Field are plotted in Figure 1.6. On August 24, the queen in colony #4 was discovered to be laying drones, indicating that she had exhausted her supply of spermatozoa. This colony was re-queened with a fertilized queen and supplied with frames of brood on September 2. It returned to normal flight activity levels by mid-September.

Figure 1.8 is a flight activity map for the Fort Missoula site. Colonies 1-3 were brought on-line early in the summer. Colonies 4-7 were brought on-line as more equipment became available for use. Colonies 1-3 were re-queened with sister queens, while 4-7 had queens of mixed genetic origin. Initially, colony #2 exhibited extremely high flight activity levels. At the same time, colony #3 exhibited unusually low flight activity. A colony check revealed that a brood break had occurred, but that a healthy, laying queen was present, so no corrective action was taken. The brood break probably resulted because this queen took longer than the other sisters to start laying. This colony eventually exhibited high forager flight activity levels, but peaked later in the summer than the other colonies.

Variations in flight activity can be quantified as C.V.s among hives (Tier 1) or graphically visualized for individual hives (Tier 2) by using normalized flight activity color maps for each site as displayed in Figures 1.9-1.13. This analysis provides a visual benchmark on which to compare colonies based on the assumption that all colonies contribute an equal amount to site-wide flight activity if conditions are optimal.

In general, colonies at all sites displayed higher levels of activity during the 1997 flight season than the 1996 flight season. Decreased variability in total flight activity among the hives can be seen at most sites during the 1997 flight season, compared to 1996. Colonies at West Branch Canal Creek displayed similar colony to colony variability, but higher levels of flight activity than at the same time during the previous year. Colonies at the Churchville reference site showed a much higher level of flight activity, but lower degree of variability (with the exception of the colony that swarmed) than the previous year. In 1996, the Churchville site was the last to be established. In 1997, it was set up earlier in the summer and had more time to become established. At Old O Field, the flight activity was much higher and the degree of variability among hives was much lower than the previous year.

In Figure 1.10, two colonies (#4 and #5) at West Branch Canal Creek displayed a higher than average contribution to the site-wide total flight activity, while two other colonies exhibited

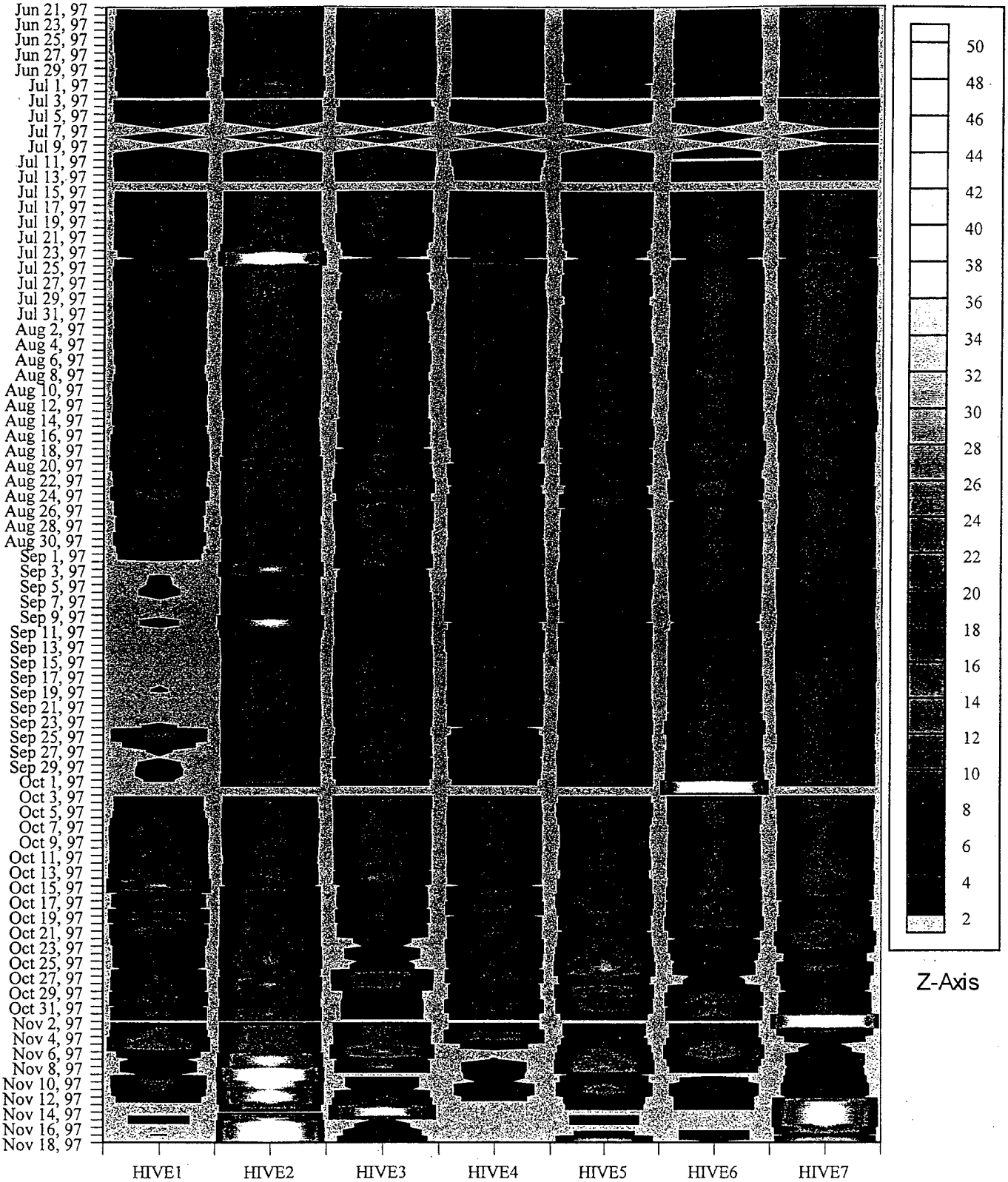


Figure 1.9. Normalized cumulative daily flight activity, Churchville, 1997. Blue to purple colors indicate a weak colony, while red to yellow indicate a strong colony.

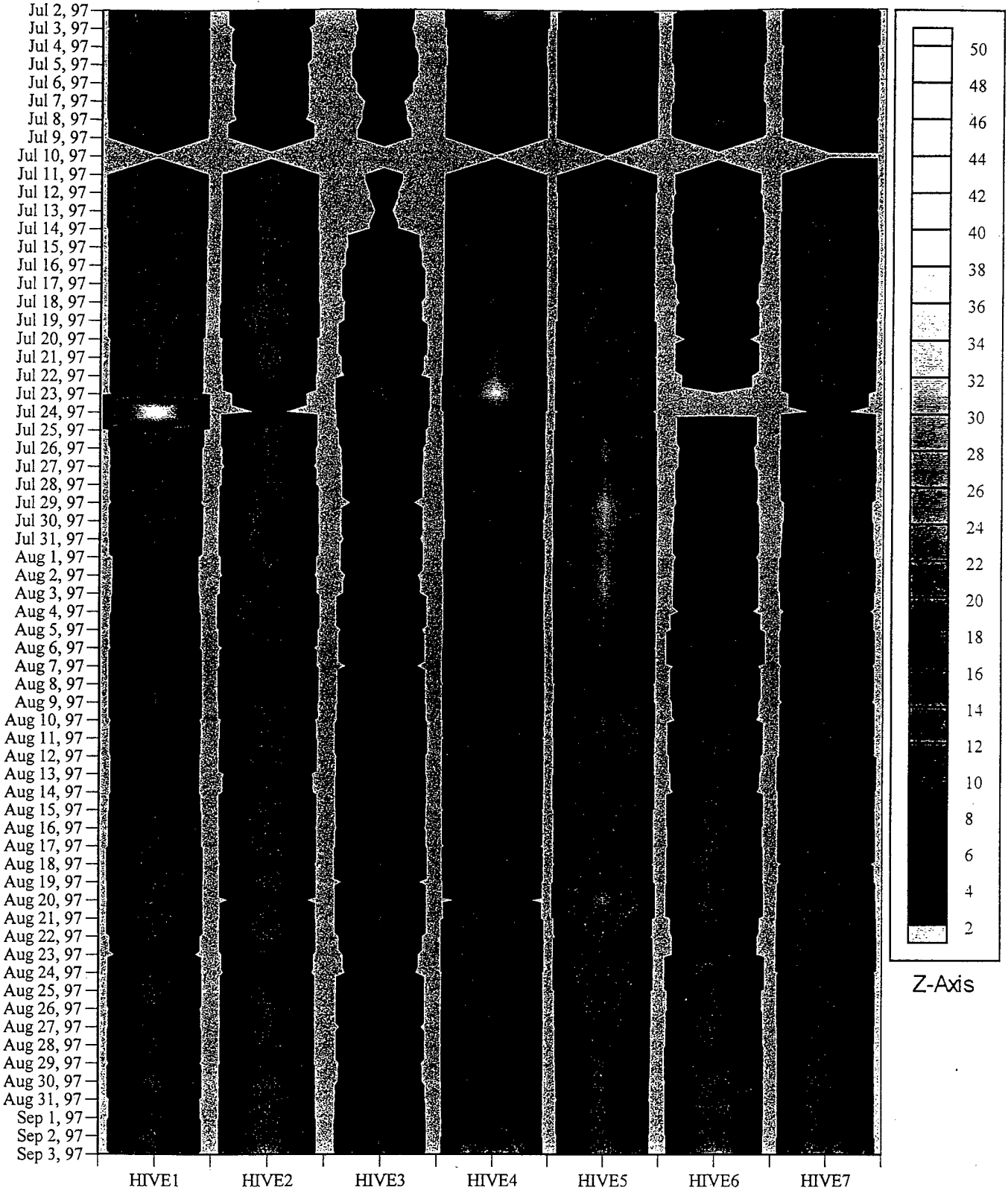


Figure 1.10. Normalized cumulative daily flight activity, West Branch Canal Creek, 1997. Blue to purple colors indicate a weak colony, while red to yellow indicate a strong colony.

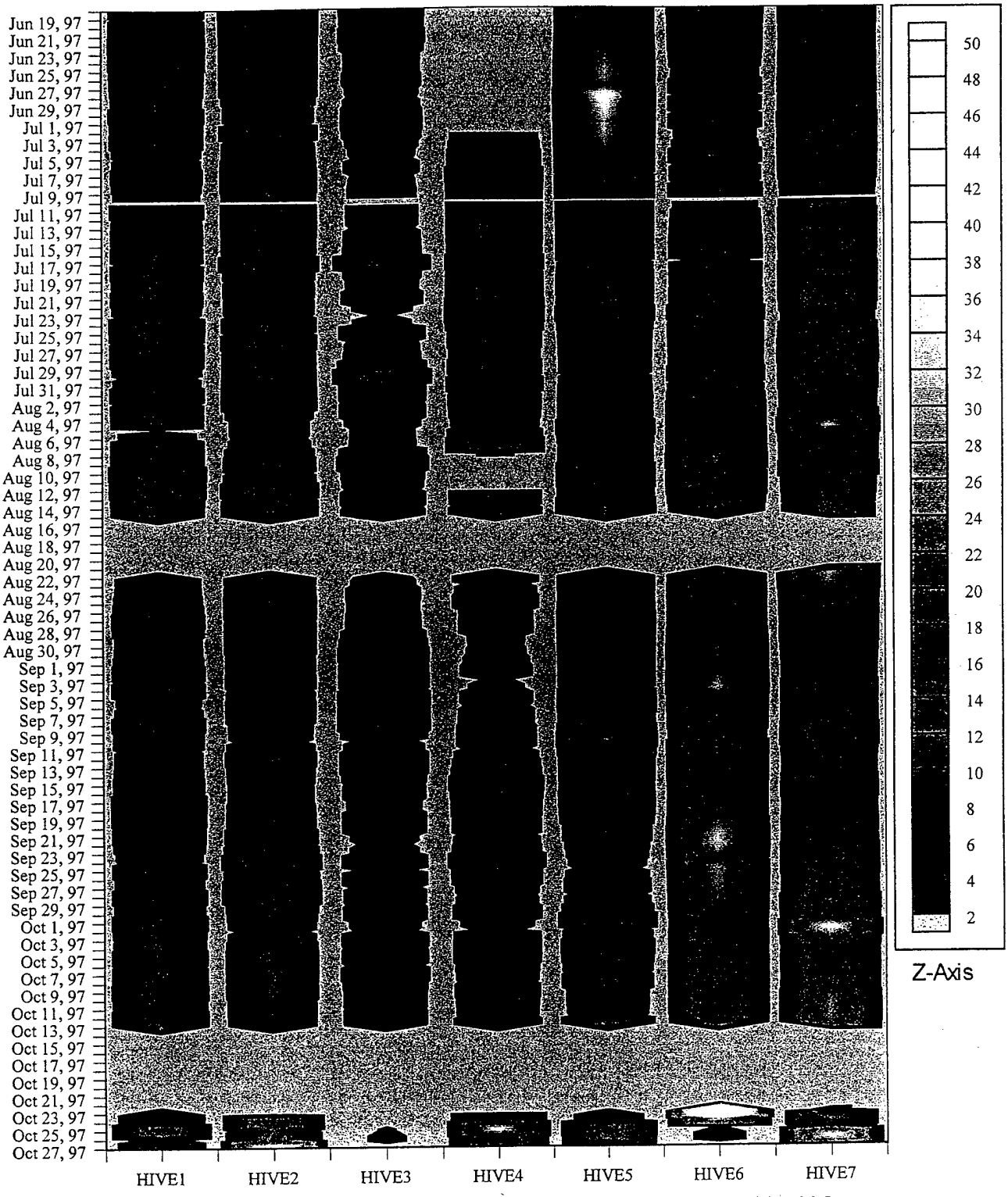


Figure 1.11. Normalized cumulative daily flight activity, Old O-Field, 1997. Blue to purple colors indicate a weak colony, while red to yellow indicate a strong colony.

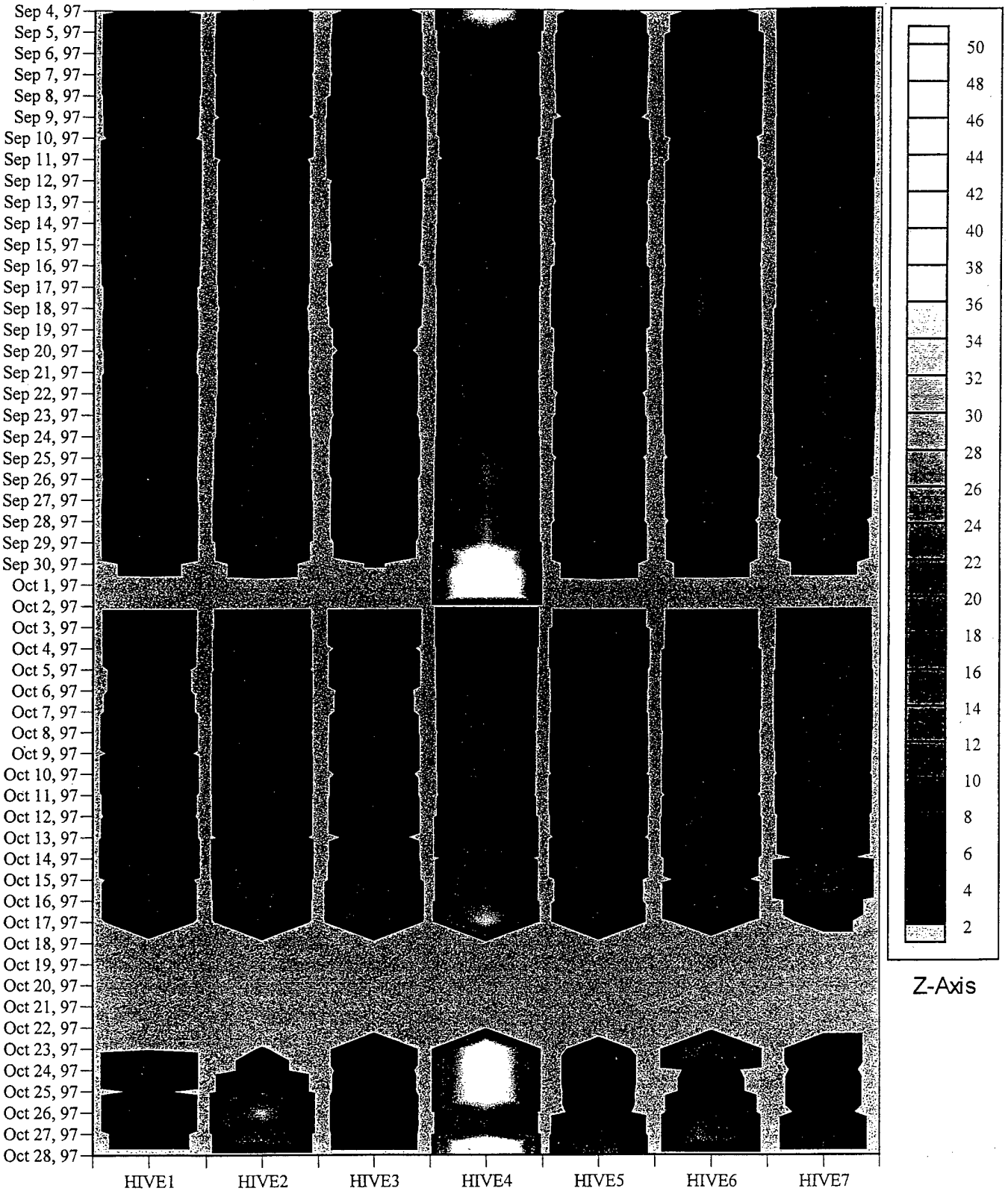


Figure 1.12. Normalized cumulative daily flight activity, J-Field, 1997. Blue to purple colors indicate a weak colony, while red to yellow indicate a strong colony.

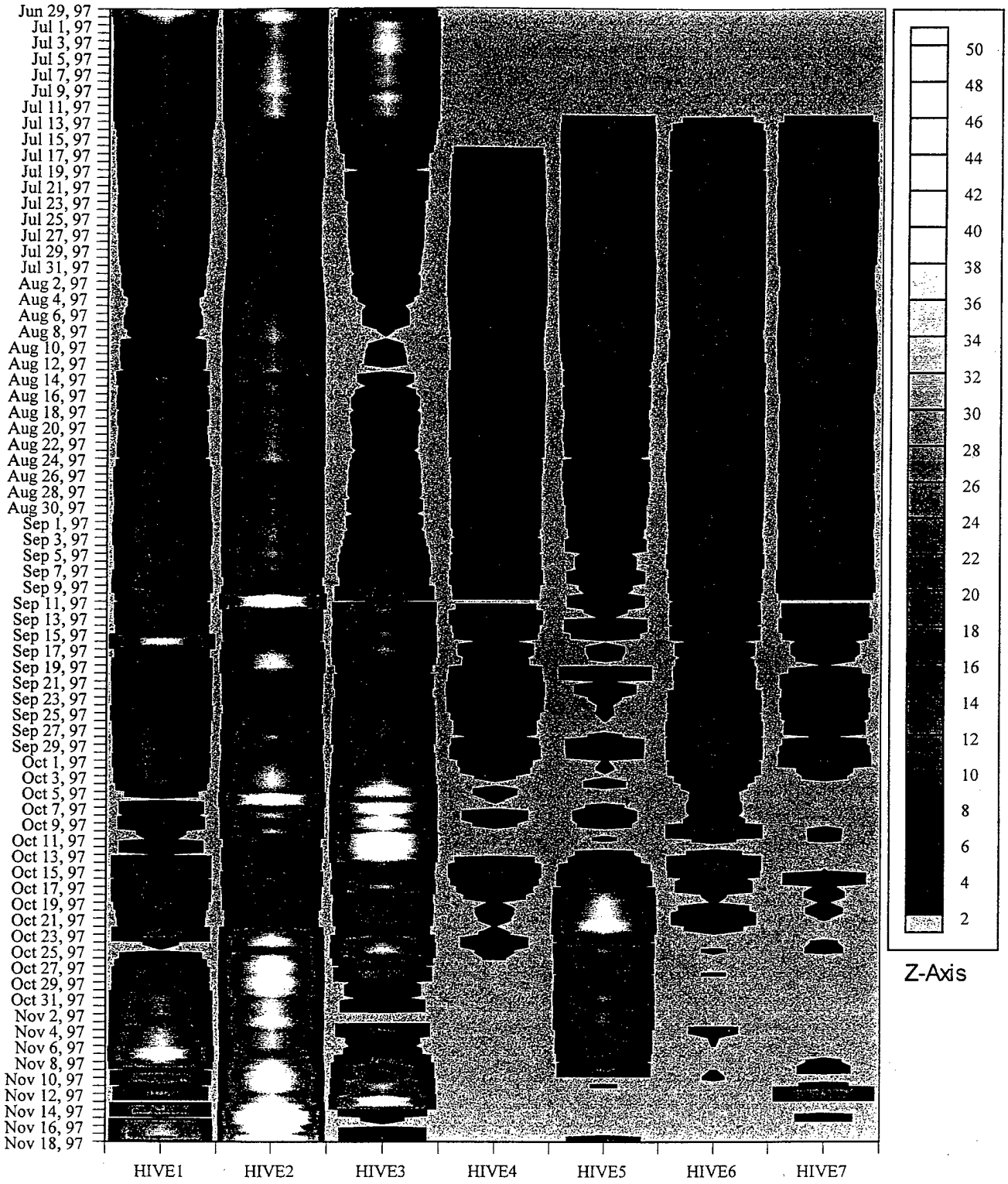


Figure 1.13. Normalized cumulative daily flight activity, Fort Missoula, 1997. Blue to purple colors indicate a weak colony, while red to yellow indicate a strong colony.

somewhat decreased levels of flight activity. Prior to transferring the West Branch Canal Creek colonies to J Field, the flight activity at colony #5 returned to more normal levels. This trend continued for the remainder of the flight 1997 season at J Field, as shown in Figure 1.12. During 1996, all but two colonies at West Branch Canal Creek showed slightly higher than expected contributions to site-wide total flight activity. In other words, strong, vigorously flying colonies were common at this site both in 1996 and 1997.

At Old O Field in 1997, as shown in Figure 1.11, colonies #5, #6 and #7 exhibited high contributions to the site-wide total flight activity for the entire season. Colony #4 demonstrated similar behavior until early-August, after which the queen was found to be laying drone cells.

Colonies at the Churchville reference site displayed little variance in total flight activity during 1997 as shown in Figure 1.9. Flight activity for colony #1 dropped significantly for a brief period after the swarm occurred during September. In general, no one colony demonstrated higher than average flight activity levels in comparison to the others.

Both Old O Field and the Churchville reference site showed a high degree of variability in total flight activity among the colonies at each site during 1996. The Churchville reference site exhibited much less colony to colony variability in total flight activity in 1997, compared to 1996. However, again in 1997, Old O Field continued to exhibit higher levels of difference in flight activity among the colonies at the site, compared to other sites.

Figure 1.13 displays the normalized flight activity map for the Fort Missoula site. This Figure demonstrates the importance of using related queens for environmental monitoring applications. Colonies #1-3 used sister queens, while the queens in colonies 4-7 were of mixed origins. Clearly, the sister queens were able to establish and maintain more uniform flight activity levels than the mixed queens.

1.6 Results of Tier 3 Evaluations of Flight Activity Data

A main focus of this year's data analysis efforts was the continued development of the data processing software (Section 1.3.3) and exploration of: (1) the data collected at thirty second intervals, and (2) of the results of daily sums used to provide event indicators. This was done in conjunction with field simulations and experiments such as weather events, hive maintenance activities, and chemical events/exposure performed at the Fort Missoula research and development site, with some parallel experimentation occurring at the Churchville reference site.

Flight activity response patterns documented for events at the reference sites can then be used to assess the probable cause of events occurring at other sites. For example, a swarm at one of the colonies at the Churchville reference site was used to demonstrate the capabilities of the bee-counter system under extremely high flight activity conditions.

Weather events such as approaching thunderstorms affected short-term flight activity patterns by causing bees to return to the hive en masse before the storm. The fast return of bees to the hive produced peaks in the thirty second flight activity plots that were indicative of the type of event that occurred. Rain events were characterized by a peak in bees returning to the hive and a sharp drop in the bees leaving the hive (Bromenshenk *et al.*, 1997).

With two years of data from multiple sites, we were able to track the passage of weather events across sites located within the same region. Figure 1.14 illustrates the passage of a rain event between two monitoring sites at APG and then on to the more distant Churchville reference site on 3 September, 1996. The ability to follow the progress of weather changes across a region suggests that a similar trend analysis could also be applied to monitoring the release of chemical contaminants from point and non-point sources, where gradients of exposure to the chemicals should be evident in the responses of a network of monitored colonies.

Honey-bee forager flight activity depends upon colony size and structure, ambient temperature, sunlight intensity, precipitation, other weather events, and water and forage resource availability. To the degree possible, all of the colonies in Maryland were started with sister queens, similar size populations, and housed in identical hive bodies.

Not unexpectedly, the sites at Maryland did not have exactly the same flight activity patterns under similar weather conditions. Whereas total flight activity is a function of colony size and status (e.g., presence of a laying queen and brood) and overall flight activity for a specific day is strongly influenced by weather conditions, between site seasonal differences in flight activity also are a function of different needs and availability of forage resources and water (e.g., for cooling during hot periods).

Changes in forage resources and local temperatures can dramatically alter flight activity patterns within a few days. This is demonstrated in Figure 1.15 by comparing the daily flight activity patterns for bees at West Branch Canal Creek between August 14 and August 17. On August 14 flight activity reached a steady level by 6:30 and maintained this level until a broad peak at 17:00. Flight didn't begin until 7:30 the next morning, and never reached a stable level for the rest of the day. August 16 began with a unusually large, broad flight activity peak that leveled off by 11:30 that morning. The large peak is not evident in the flight activity pattern for August 17. The peak occurring on August 16 could be due to a brief hot-spell beginning on August 15 and ending by August 17. Because flight activity depends on multiple variables, advanced methods of assessing complex data are required, such as the application of Artificial Neural Networks to discern response patterns and predict "expected" flight (discussed in Section 1.11).

However, the suite of tools provided with the numerical analysis and graphical display programs included on the CD-ROM (and described in Appendix A) proved to be sufficient to flag short-term disturbances. For example, on August 2, at Churchville, we found a consistent interval between the response peaks extracted by the DER*DIFF utility as shown in Figure 1.16. The

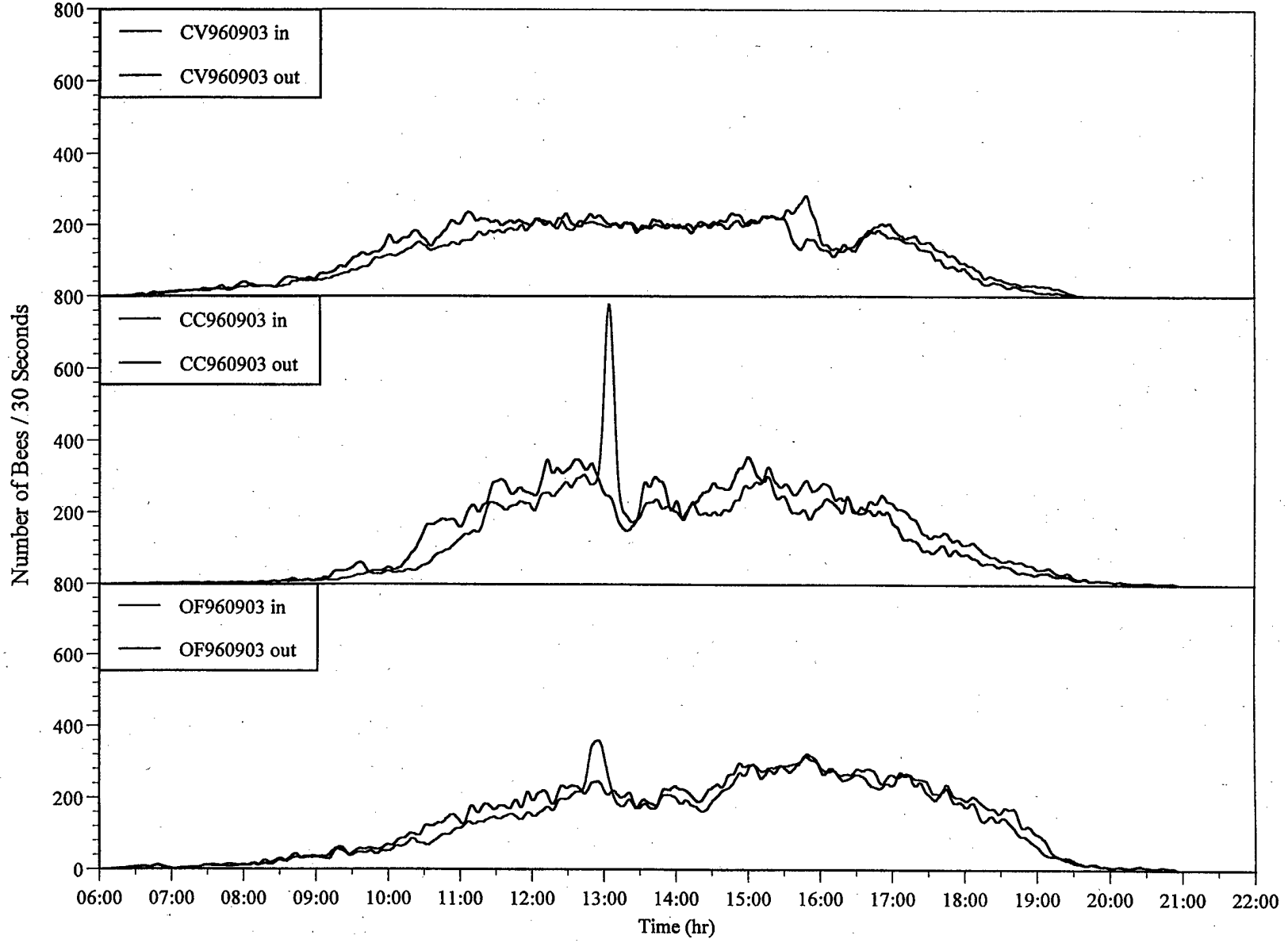


Figure 1.14. Total flight activity / 30 second at APG sites, 09/03/96. A rainstorm passed from Old O-Field to West Branch Canal Creek and then to the Churchville reference site.

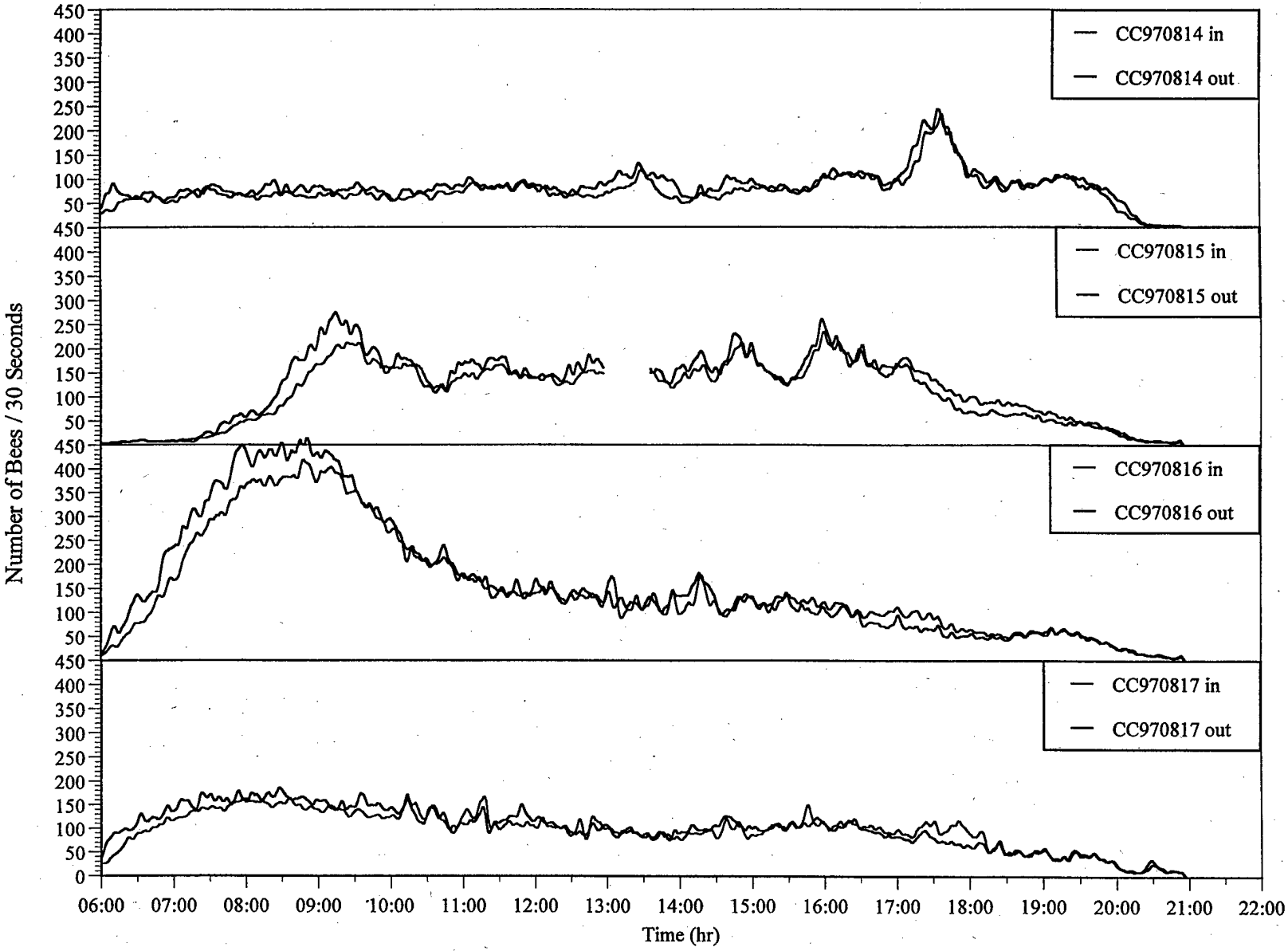


Figure 1.15. Total flight activity / 30 seconds at West Branch Canal Creek over a four day period.

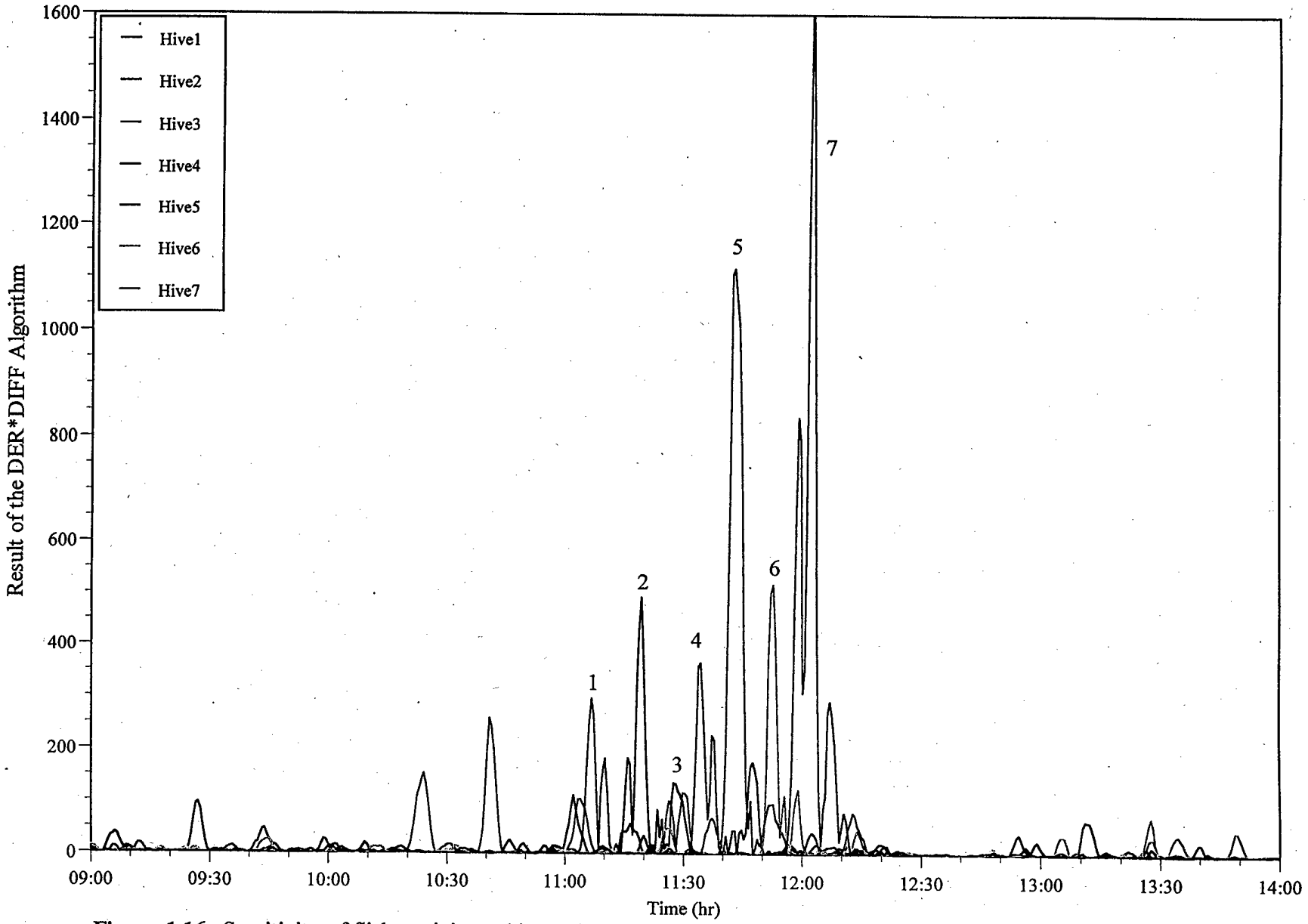


Figure 1.16. Sensitivity of flight activity to hive maintenance. Peaks indicate periods of hive maintenance as the beekeeper's colony check progressed from hive 1 to hive 7.

algorithm is described in the SITEVIEW program manual on the CD-ROM. Checking the log books, we found that one of our technicians was inspecting the hives. As this person moved from hive to hive, the plot reveals that the colony check disturbed colonies in order from #1 to #7, having the greatest impact on colonies #5 and #7.

On September 1, 1997 at the Churchville reference site, colony #1 swarmed. Strong colonies reproduce by swarming, although this swarm left the hive rather late in the season. While this was not the first time a swarm had been captured by bee-counters, it was unique in that the colony had been monitored over a long period of time, and that the swarm was a natural event, rather than one produced by beekeeper intervention. This colony's flight activity was monitored for several months both before and after the swarm occurred. Colony #1 began to reduce its flight activity five days prior to the swarm. The change could be seen both in an increased C.V. for flight activity among the colonies and by the Tier 2 graphical display of the data. The site-wide PRC dropped to 90% because the PRC for colony #1 dropped to 43% the day of the swarm. Normally, PRC is expected to be in the 94% to 98% range. The day after the swarm occurred, flight activity dropped below 5000 round trips per day and stayed at this level for the next 3 weeks until the hive was supplemented with more bees. The contribution to total site-wide flight activity from colony #1 remained below 4% for a three week period following the swarm.

The time resolution at which data is captured and integrated by the bee-counter system allowed close inspection of flight activity patterns during the swarm. Figure 1.17 demonstrates that extremely high levels of flight activity occurred during the swarm of colony #1. Over a 35 minute period, 10,068 bees left the colony, while the total flight activity for that time was 13,782 (i.e., while most bees were leaving the hive, others re-entered it). Over the next two hours, 438 bees returned to the colony with most returning within a period of only a few minutes. This return probably was due to bees that failed to move on when the swarm finally left the apiary. Typically, a swarm will leave a hive, regroup nearby, then later move to another site. Any bees left behind would be expected to return to the hive of origin.

The high level of flight activity attained during the swarm presented an excellent test to the capabilities of the electronics system and the physical limitations of bees traversing the counters. For a 35 minute period, a single counter is theoretically capable of detecting 1,176,000 bees. The number of bees traversing the counter during the swarm represented only 1.2% of the counting capability of the electronics. It is possible that the number and size of the bee-passageways could limit the amount of bees exiting at any given time. However, inspection of the counter passageway flight activity indicated that during the swarm all passageways were used at similar levels. A maximum of 469 bees traversed the counter during a 30 second period during the swarm. This indicated that each bee required just less than one second to traverse a counter passageway. Based on this rate of passage, the maximum number of bees that could traverse a bee-counter during a 15 hour monitoring period is 844,200. This number is far less than the theoretical limit of the device.

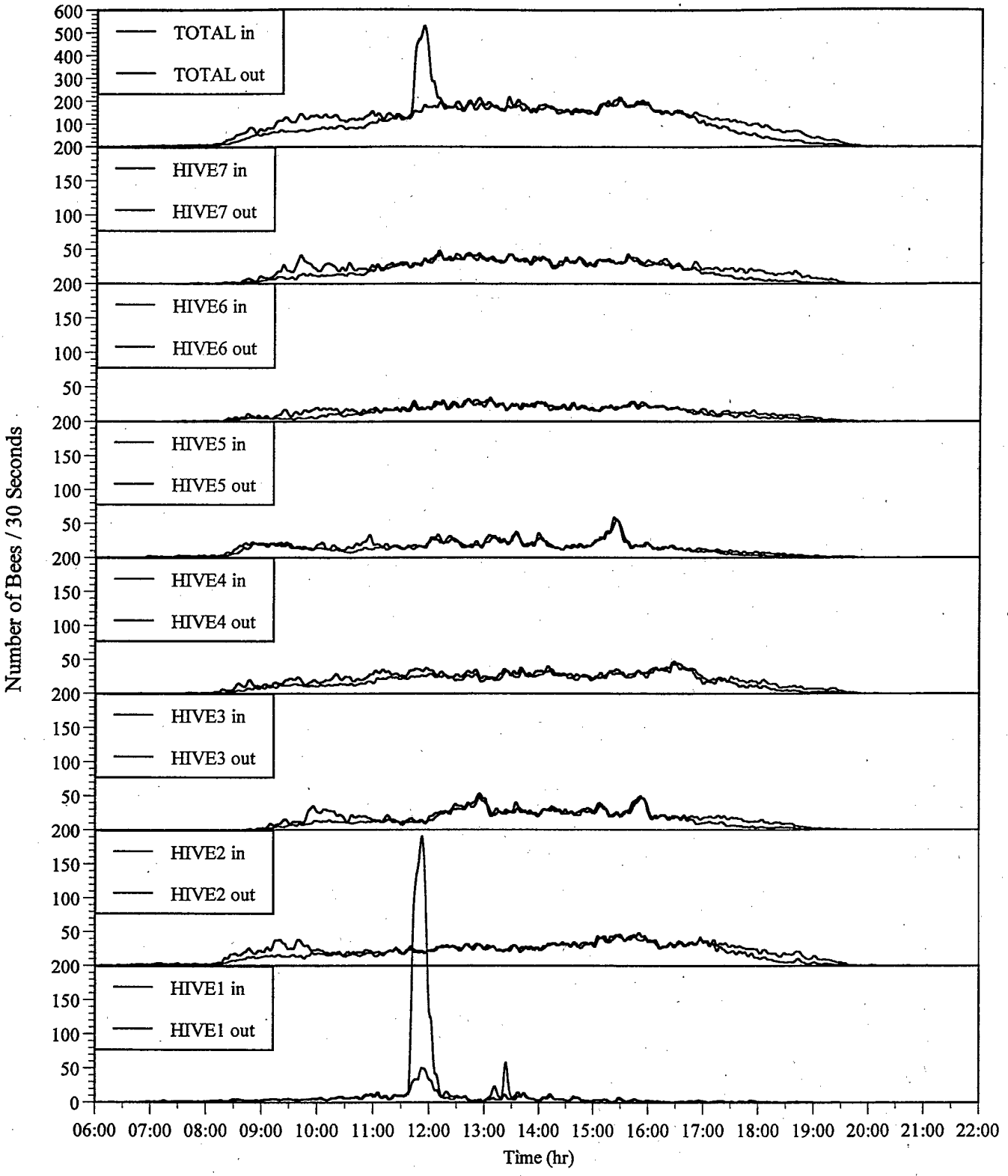


Figure 1.17. Total flight activity / 30 seconds, Churchville, 09/01/97. Colony 1 swarmed at 11:30. More than 10,000 bees departed, about 400 returned by 13:25.

Because day length, weather conditions, and the water and floral resource needs of colonies vary greatly between different geographical locations, we have included Figure 1.18 to demonstrate the differences between the start, duration, and end of forager flight on the same day at Fort Missoula, in Montana, and the APG sites in Maryland. Forager activity at Fort Missoula did not begin until about 9:00 hr in Montana, whereas all of the Maryland sites exhibited some flight activity by 6:30 hr. Flight activity continued at a high rate until 21:30 hr at Fort Missoula, while the Maryland sites shutdown between 18:00 and 20:30 hr. A sudden increase in bees returning to the colonies late in the day at the Churchville reference site was due to an evening rainshowers. Typically, light levels remain higher much later in the day in Montana than in Maryland, which probably accounts for bees still flying at 21:30.

The shape and variance of the Fort Missoula flight activity pattern in Figure 1.18 differs from that of the Maryland sites. The Fort Missoula site is adjacent to a field of knapweed, which the bees foraged extensively during the month of August. Figure 1.19 demonstrates the seasonal change in flight activity pattern brought about by the knapweed blossom. On July 23, bees were not foraging on knapweed. The flight activity pattern was characterized by a broad hump that covers the entire day. Less than two weeks later, when knapweed was in blossom, the flight activity pattern had a double-hump shape with little variance throughout the day. One month later, while knapweed resources were still sparingly available, the double-hump shape was barely discernable. Also, the flight activity variance had returned to the level observed before the onset of the blossom. The low variance in flight pattern during August and early-September is the result of results of bees being stimulated to actively forage plentiful resources in close proximity to the hives. Diminishing floral resources, more sparely distributed, would be expected to result in reduced and more variable flight patterns, such as were seen by the beginning of September.

An earlier example demonstrated that our analysis routines could track the disturbance of a beekeeper opening hives for inspection. A much less invasive experiment was designed to determine the impact, if any, of someone standing in front of the hive, blocking the entrance. A beekeeper, dressed in white coveralls, a hat and veil, blocked the flight path of incoming bees by standing 5 feet to the front of the counter entrance for 5 minutes and then stepping away.

Figure 1.20 demonstrates the impact of this experiment on normal flight activity patterns. Blocking the flight path was clearly characterized by a decrease in bees entering the counter, although it had little or no effect on the number of bees leaving the hive. When the beekeeper moved away from the flight path, a sharp increase in bees entering the counter occurred. Visual observations confirmed that a cloud of bees built up behind the beekeeper, then landed when the person moved away. In some cases, such as between hives #5 and #6, and #1 and #4, drifting occurred into adjacent hives.

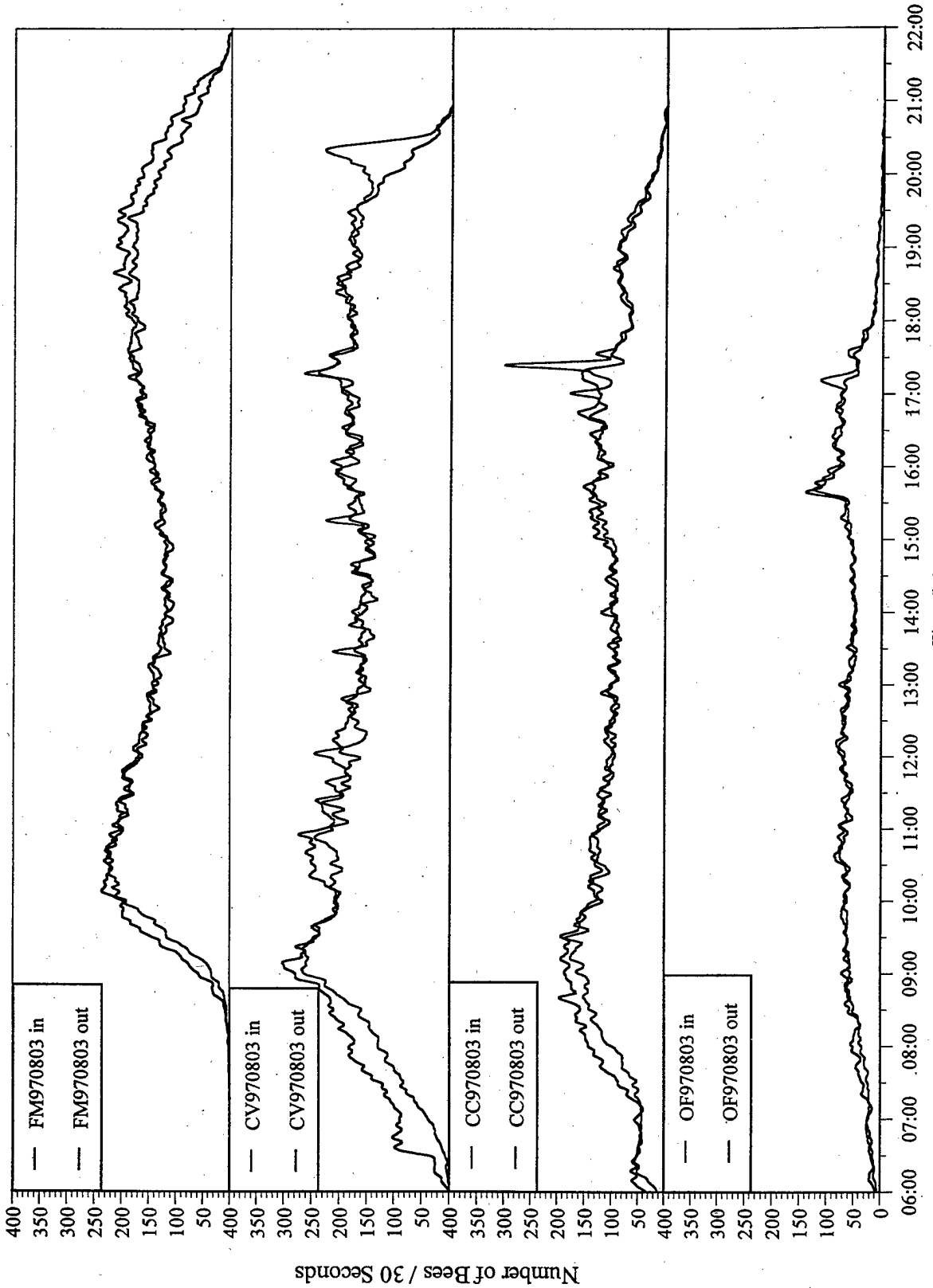


Figure 1.18. Comparison of Maryland and Montana forage patterns. Bees fly later in Montana.

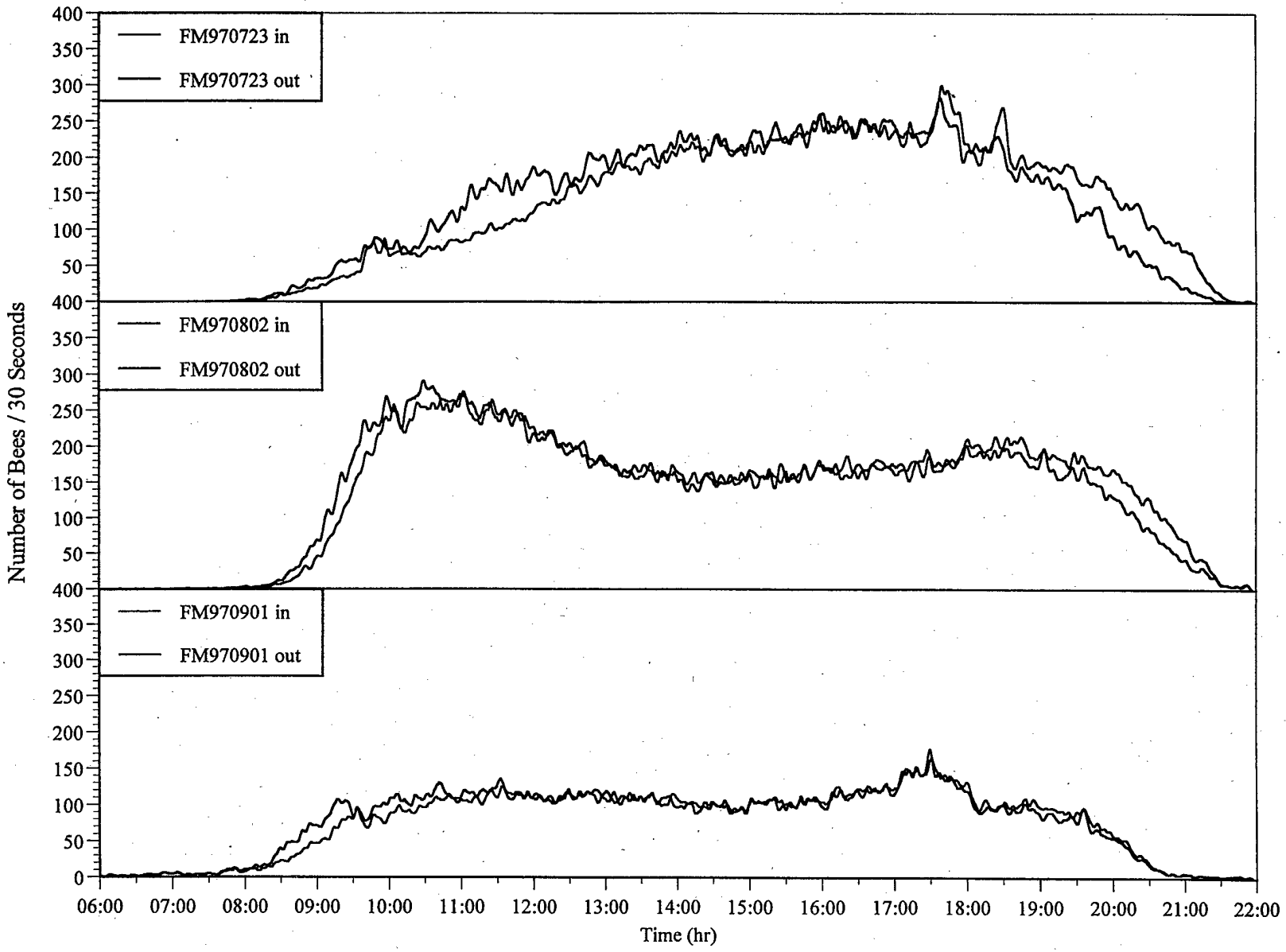


Figure 1.19. Comparison of Montana forage patterns relative to floral researce availability.

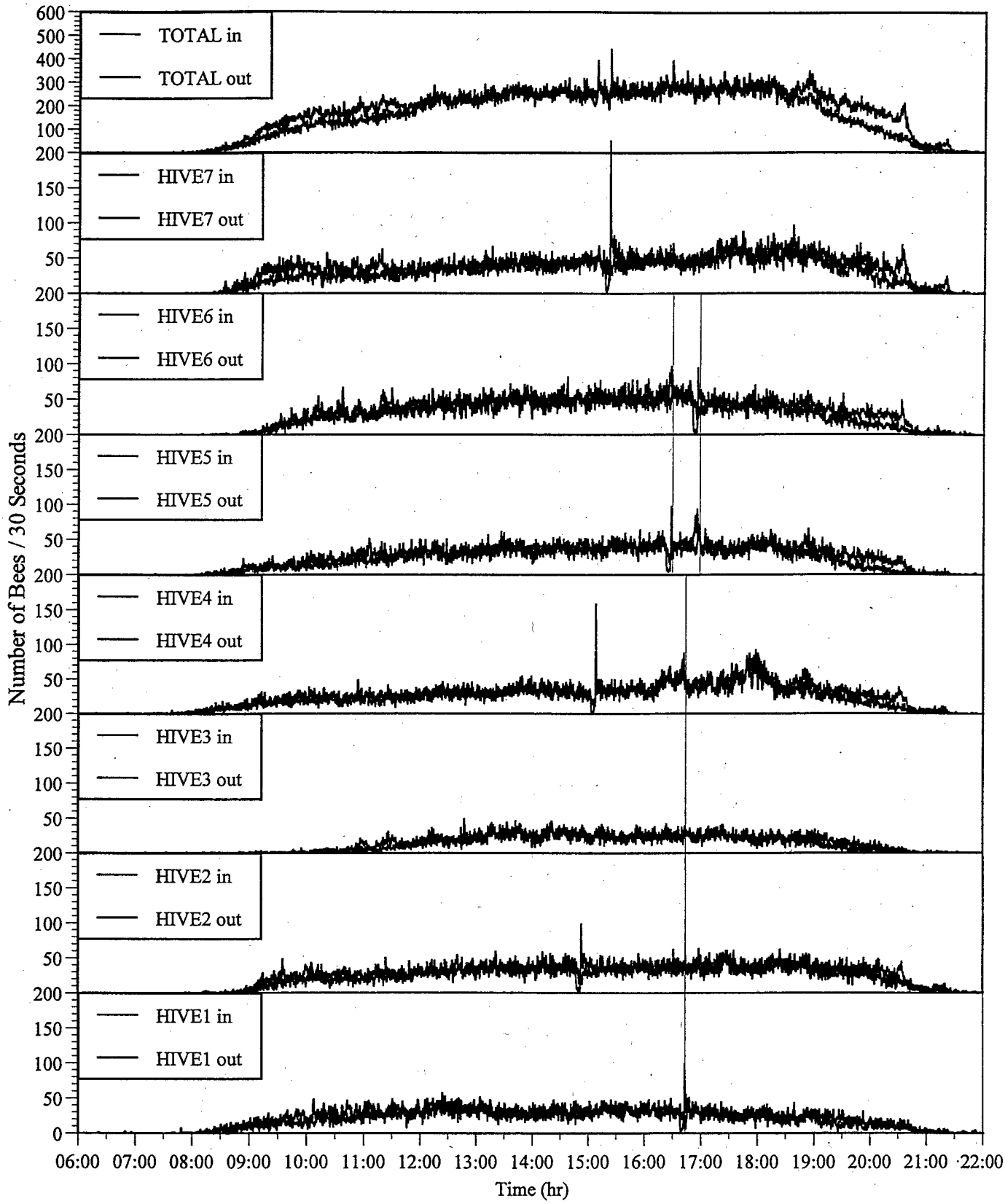


Figure 1.20. Total flight activity / 30 seconds, Fort Missoula, 07/21/97. Beekeepers blocked the entrance to selected hives during the afternoon causing spikes in activity. Thin lines denote drift between adjacent colonies 1 and 4, and 5 and 6.

Derivatives of flight activity patterns were used to better examine drifting. Drifting is defined as instances where bees leave one hive and return to another hive. The results of examining the filtered derivatives of flight activity patterns are shown in Figure 1.21. It is clear (indicated by a thin line) that drifting occurred from hive #6 to hive #5 as well as from hive #1 to hive #4. The total derivative (top plot) is calculated as the sum of the derivative from each hive. The derivative of the total activity (not shown) produced barely discernable peaks because the contribution to total flight activity from each hive was offset by the others. The DER*DIFF algorithm also was applied to this data to test its ability to discriminate short term events. Figure 1.22 indicates that drifting also occurred from hive #5 to hive #6 and that the drifting from hive #1 to hive #4 was not as intense as suggested by derivative analysis.

Total flight activity failed to produce readily discernible peaks via the DER*DIFF analysis, The brief changes in flight activity created by blocking the flight path of bees to single hives had little effect on the total site-wide flight activity. The top plot in Figure 1.22 is based on the DER*DIFF analysis of the total flight activity, not the sum of the DER*DIFF analysis for each of the hives.

The most striking difference between Figure 1.21 and 1.22 is the lack of noise in Figure 1.22. Filtered derivative analysis produces noisy results because of the variability of short term changes in flight activity. The DER*DIFF algorithm minimizes this noise by enhancing sensitivity to differences in bees entering and exiting the counter for a given period. The noise minimization is what makes the DER*DIFF analysis powerful for detection of short-term events.

Anomalous flight activity patterns were detected in 1997 on June 22 at Old O Field, July 28 at Fort Missoula, and August 5 at Fort Missoula. These patterns consisted of a sharp drop in the rate of bees leaving the counter, followed by a decrease in bees entering the counter. The lack of a sudden increase in the number of bees returning to the hive distinguishes these activity patterns from those induced by the approach of a rain storm. Because none of the regularly scheduled chemical sampling dates coincided with these unanticipated responses, these changes could not be evaluated with respect to potential exposures to chemicals.

Anomalous flight patterns were first detected on 28 July, 1997 at the Fort Missoula site as shown in Figure 1.23. This was the same day in which methanol was sprayed at the entrance of six of the seven counters, although the application of methanol preceded the response by several hours. A response was detected from all seven colonies, even though only 6 were treated with methanol.

Another event was detected on 5 August, 1997 at Fort Missoula, as shown in Figure 1.24. This event occurred after a cleaning agent Pine Sol™ was used near the apiary to clean materials. Neither of these changes in flight activity occurred at the time when the contaminant (i.e., methanol or Pine Sol™ was first introduced into the air in the apiary. A search of a USGS earthquake database indicated no seismic activity in the region. Also, there was no detectable change in weather patterns that should have produced the observed response.

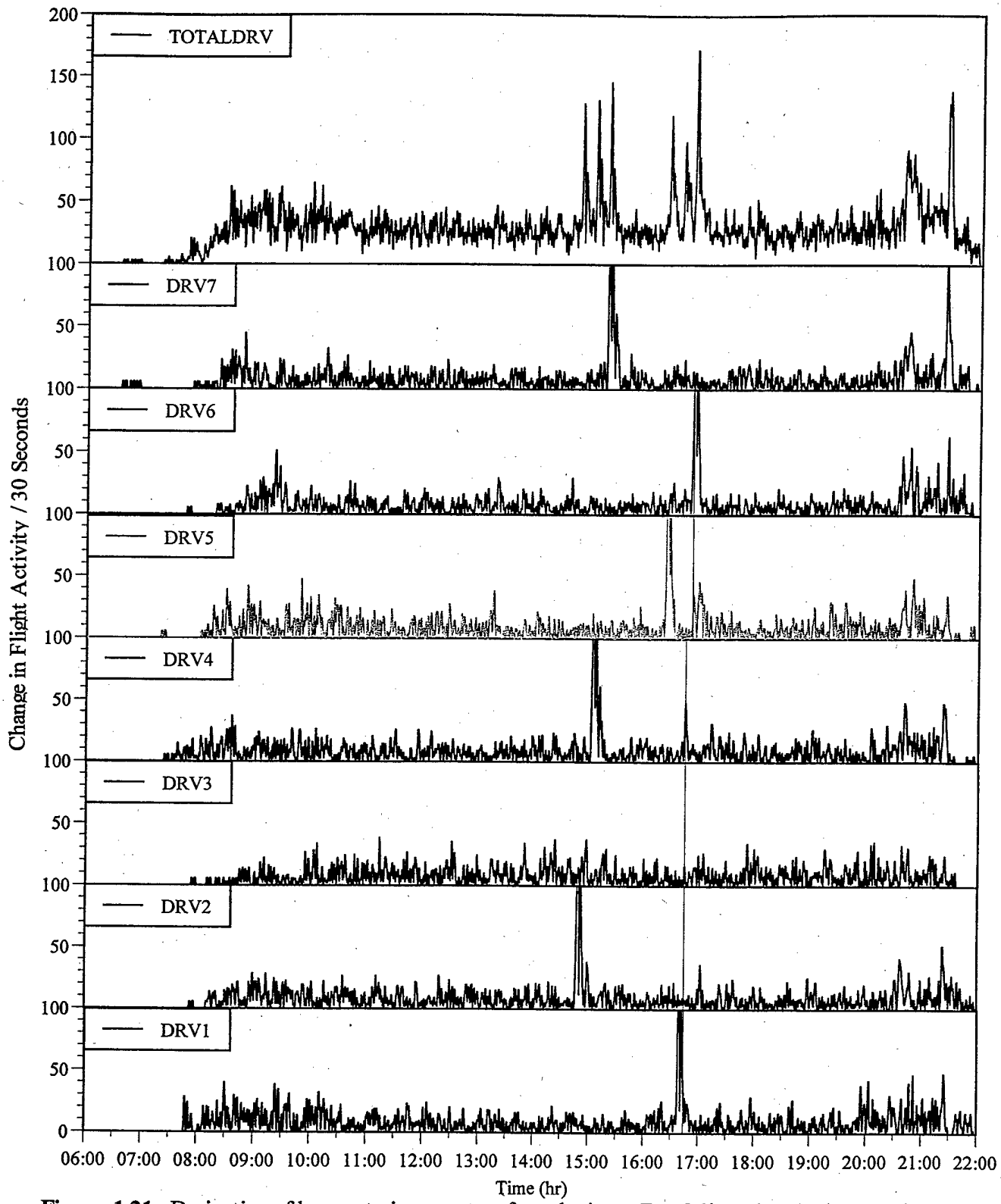


Figure 1.21. Derivative of bees entering counters for colonies at Fort Missoula, 7/21/97. Beekeepers blocked entrances by standing in front of selected hives at different times. The solid line indicates potential drift from colony 6 to 5 which are approx. 10 feet apart. The second vertical line indicates potential drift from colony 1 to 4. Colony 4 is approx. 20 feet behind colony 1. Sharp peak at end of day indicates rain event.

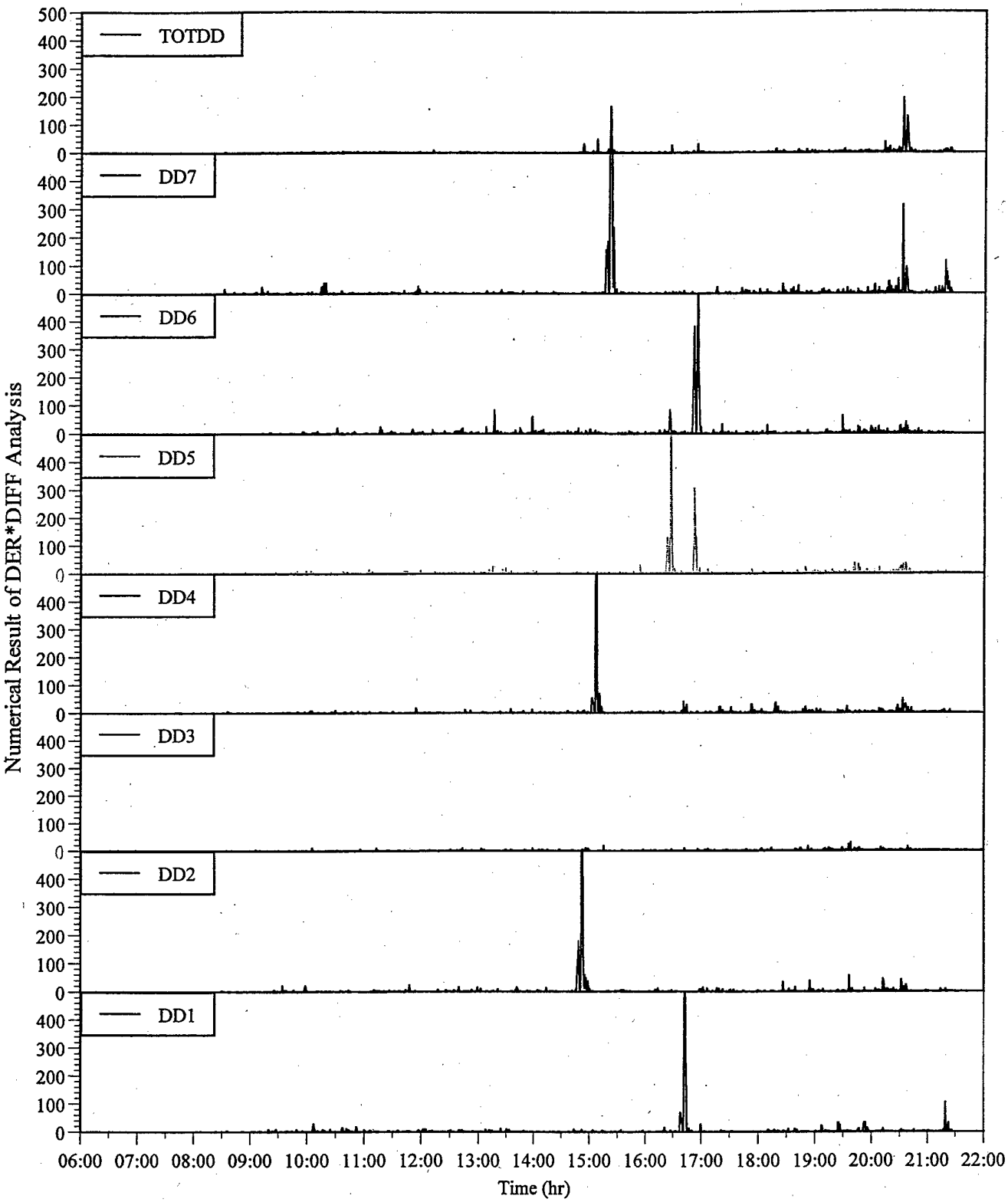


Figure 1.22. DER*DIFF analysis, Fort Missoula, 07/21/97. Large spikes are due to beekeepers standing in front of hive entrances. Note drifting between colonies 1 and 4 and 5 and 6.

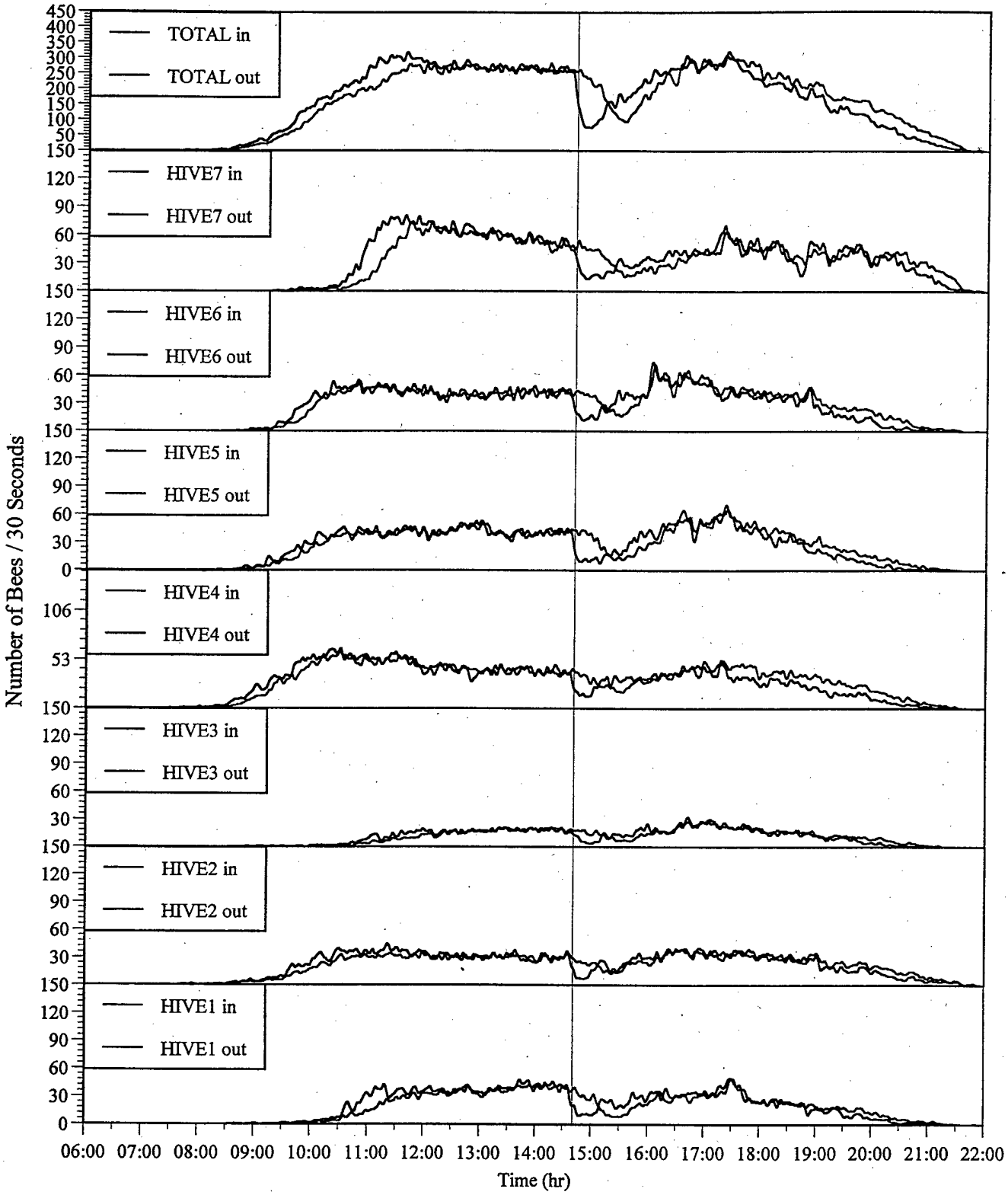


Figure 1.23. Total flight activity / 30 seconds, Fort Missoula, 07/28/97. Post-methanol treatment response.

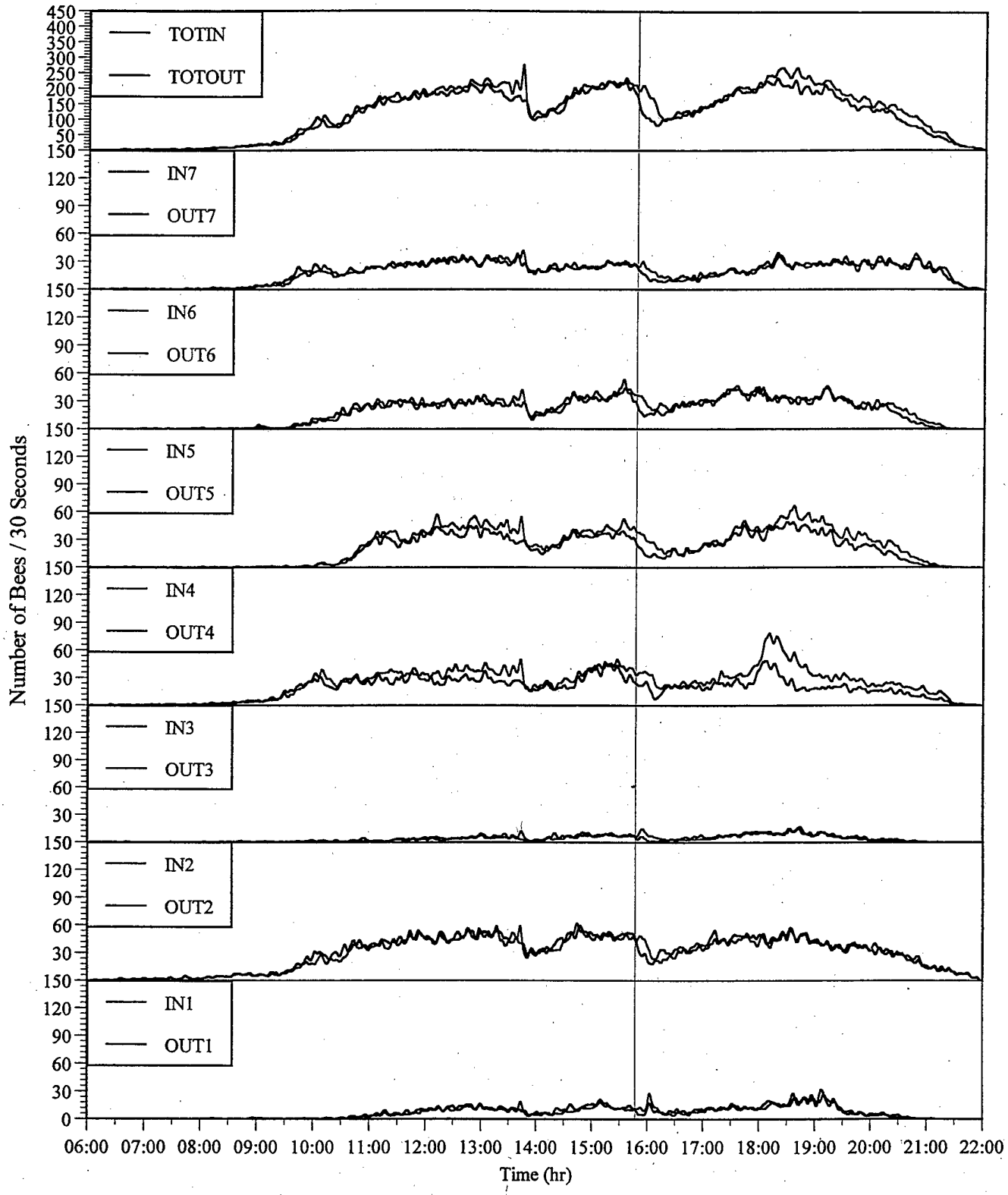


Figure 1.24. Total flight activity / 30 seconds, Fort Missoula, 08/05/97. Colony response to exposure to Pine Sol™.

These anomalous patterns were compared to the rest of the flight activity archives, and two similar events were found for APG sites. The first was the smoke event of 8 August, 1996 at the West Branch Canal Creek site, where beekeepers used smoke to calm the bees before working them. A plot of this data was included in the 1996 Annual Report. The second match occurred 22 June, 1997 at the Old O Field site as shown in Figure 1.25.

Figure 1.26 compares the responses garnered from events of known and unknown origin. The first plot shows the sitewide response to injection of smoke into hive entrances performed 8 August, 1996 at West Branch Canal Creek. The second plot demonstrates a response to an unknown stressor on June 22, 1997 at Old O Field. The third and fourth plots are from events that may have been caused by exposure to methanol (28 July, 1997) and Pine Sol™ (5 August, 1997), respectively. It is clear from the data that these responses are similar in nature.

With 3 of the 4 days following known releases of contaminants into the apiary, we believe that although the initial exposure may not have induced an immediate response, the exposures due to buildup of contaminants in the hive atmosphere caused some stress to the bees, which was manifested in the data. Without hive atmosphere chemical data for these subsequent days, it is difficult to assign the exact cause of the anomalous flight patterns. The potential for external stressors to induce a delayed response will be an important focus of our 1998 studies.

1.7 Results of Tier 4 Diagnostic Evaluations of Flight Activity Data

Tier 4 analysis of flight activity is a diagnostic tool. It is used to trouble shoot the counter equipment. The analysis uses suboptions 10-15 of Option 10 of the program SITEVIEW program (on the CD-ROM). This program allowed quick evaluation of counters and channels to find counters that were either defective or needed re-alignment. This provides the tools needed for systems and performance audits of the counters.

1.8 Summary of APG Results

The Tiered Analysis approach was applied to the monitoring trials at APG sites in 1996 and 1997. The results are discussed here in the context of other measures of colony performance and of the chemical exposure data (see Section 3). There was a general decrease in variability in the flight activity among hives at each of the Maryland sites in 1997 compared to 1996. Because of the presence of sister queens in all of the colonies in 1997, it is possible that the genetic makeup of the queen was partially responsible for this decrease in variability in flight activity. In a Fort Missoula (FM) test, some of bee colonies had sister queens and others had queens of mixed origin. The FM results indicated that the use of closely related queens reduces variability among the colonies at a given site. Based on these results, we believe that the use of sister queens in all of the Maryland colonies was partly responsible for reducing within site variability at APG sites in 1997. Also, we deployed the Maryland colonies in May and June of 1997, compared to July and August in 1996. By placing the colonies on-site earlier in the season, the bees were able to benefit

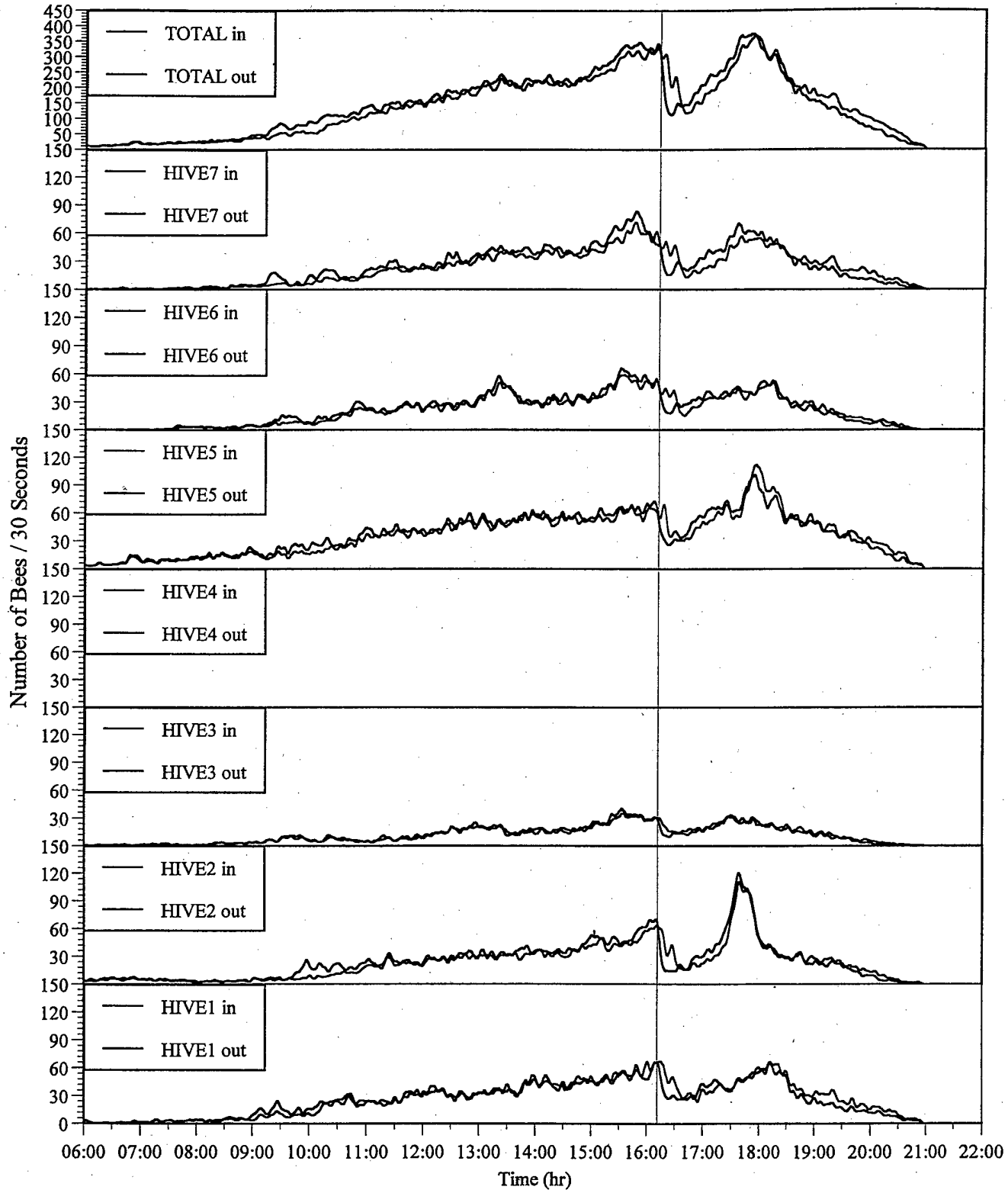


Figure 1.25. Total flight activity / 30 seconds, Old O-Field, 06/22/97. Anomalous event at 16:20 matches the response pattern found at Fort Missoula hives on 07/28/97 and 08/05/97 when elevated levels of contaminants where present in the apiary.

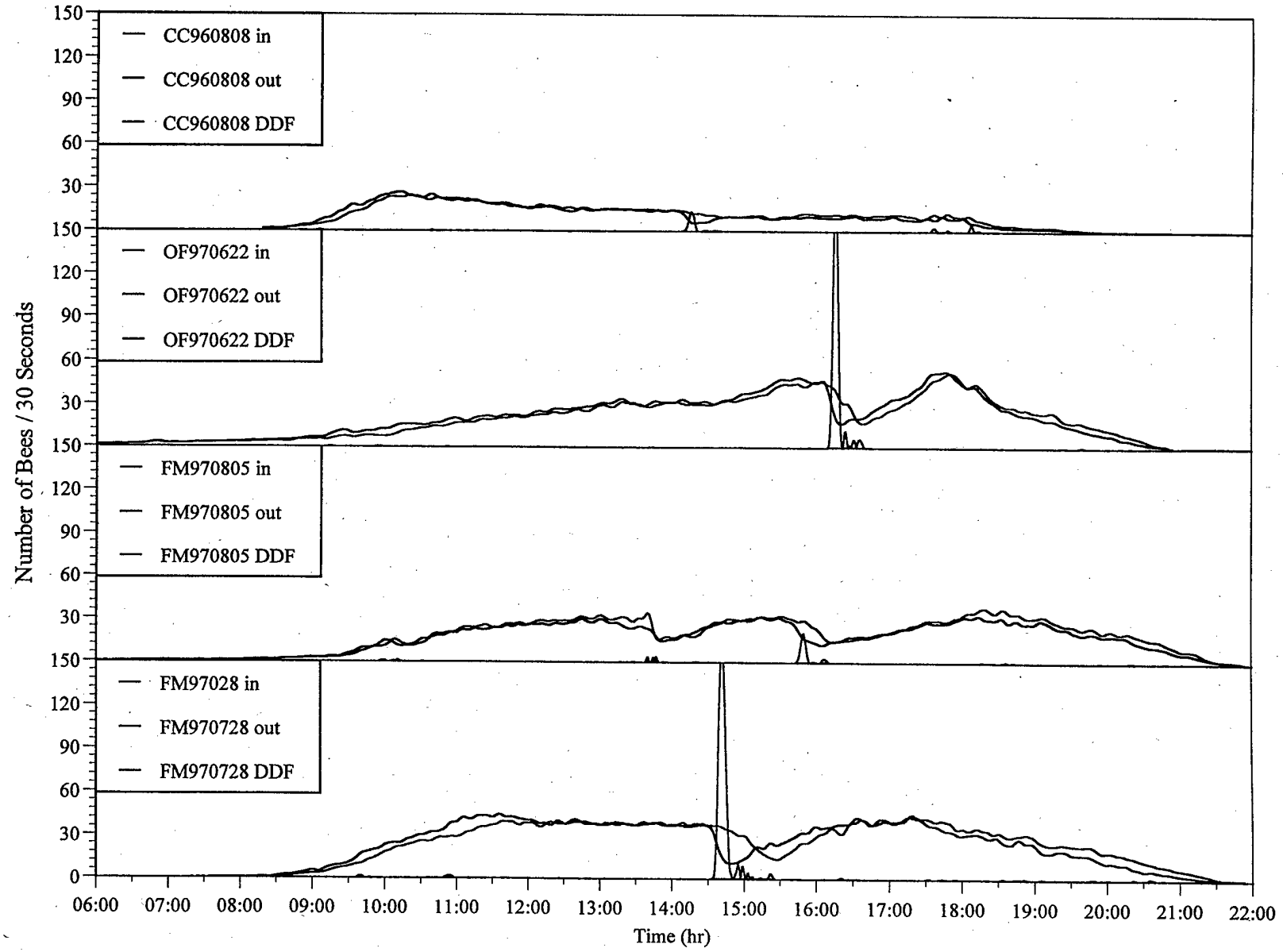


Figure 1.26. Detection of anomalous events using the DER*DIFF algorithm.

from the early season nectar flows that are characteristic of this geographical area. This allowed the populations to increase in size as well as to harvest and store more nectar and pollen.

In early September 1996 a hurricane swept through APG. Immediately after the storm, a sharp drop in activity occurred at Old O Field. A two day rain in late July of 1997 produced similar results, with decreased flight activity levels occurring at both West Branch Canal Creek and Old O Field. The duration and severity of the decrease in flight activity at Old O Field was much greater than that observed at West Branch Canal Creek. Flight activity at Churchville also dropped during the rain event, but quickly returned to normal. Section 3 of this report describes the exposures to chemical agents at all of the bee monitoring sites. In both 1996 and 1997, increased levels of volatile organics were found in the Old O Field hives during the periods of decreased flight activity. The levels of these chemical agents were about an order of magnitude higher in 1996. Sudden increases in the levels of chemicals inside the hives following rain storms suggest that the bees may be exposed to these agents via water, possibly as an oily film on the surface of puddles. Chemical uptake experiments conducted in Missoula in 1997 (Section 3) confirmed that this is a route of entry into the hive for organic solvents.

The Tier 1 indicators, TFA, C.V., PRC, and ANL (Table 1.3), were all used to provide metrics for comparing sites to one another. In 1996, all of these metrics showed Old O Field to be highly stressed. Although the colonies at Old O Field showed a marked improvement in C.V.s values in 1997 compared to 1996 (50-130%), the C.V. values for 1997 at Old O Field were still higher (25-75%) than those at West Branch Canal Creek (20-50%) and at the Churchville reference site (15-50%). The PRC metric shows improvement in 1997, compared to 1996, for both West Branch Canal Creek and Churchville. The changes were similar in nature to changes in the C.V. metric. In 1997 the percent of bees returning (PRC) each day to their hives ranged from 94% to 98% for both Churchville and West Branch Canal Creek. These levels were similar to those also observed for the sister queen colonies in Montana. We consider these to be acceptable levels of attrition in a healthy bee hive. The PRC for Old O Field in 1996 ranged from 90% to 102%. The 90% return levels were considered to be somewhat low. This metric improved to 94% to 104% in 1997. All of the Maryland hives exhibited return rates of 94% or better in 1997, compared to values as low as 90% in 1996. Percent return values were observed at Old O Field in both years, and at Old O Field and West Branch Canal Creek in 1996. Only Old O Field, among the Maryland sites, demonstrated values in excess of 98% in 1997. Values in excess of 100% indicate drifting among the hives at Old O Field. Our data suggests that drifting becomes more evident when population size and flight activity varies greatly among hives. The general decline of the bee populations at Old O Field, severe in 1996, and moderate in 1997, reflected a wider range of activity levels and colony sizes at Old O Field than at any of the other sites. The ANL results for Old O Field in both years were among the most variable for all of the APG area sites. Those for the Churchville reference site were the lowest.

Basically, the trends listed in Table 1.3 indicate improvements for colonies at West Branch Canal Creek and the Churchville reference site in 1996 compared to 1997. At Old O Field, some improvement occurred, but not to the extent as the other sites. Over both years, bees at West

Branch Canal Creek did nearly as well or as well as the colonies at the Churchville reference site; based on all of the Tier metrics, colony core temperatures (which address the colony's ability to thermoregulate), and levels of exposure to bioavailable environmental contaminants (see Section 3). Two of the four survey colonies were lost at J Field. These colonies were deployed in early June. The electronic hives were moved onto J Field toward the end of the season. This trial provided information about end of season variability and established that the electronic hives could be operated at this site. The trial was not of a long enough duration to adequately assess colony performance as affected by chemical agents at the site. However, the data provided a good basis for resuming the study at J Field in 1997. The loss of two of the four survey colonies in 1996 warranted deployment of the real-time monitoring system at J Field throughout the 1997 season.

The combination of abnormally high C.V., elevated PRC, and low flight activity in August, 1996, following a heavy rain event in late July, indicate that the colonies at Old O Field were exposed to external stressors. Exceeding any of these individual metrics is usually cause for a colony check. The colonies at Old O Field managed to exceed several metrics simultaneously, indicating that the hives were displaying symptoms of stress that could affect colony health.

1.9 Summary of Colony Responses to External Stimuli

Most of the biological responses to external stimuli were detected by the Tier 3 analysis. It can be concluded that most of the events that occurred in response to a biological stressor did so quickly. The timing of these responses varied from immediately after the stressor was introduced to several hours later, as was the case with methanol exposure.

Six different abiotic and biotic contributions to flight activity behavior were explored. These included weather events, seasonal changes, geographical differences, chemical exposure, hive maintenance, and swarming. Rain events have been characterized as having a large peak in the number of bees returning to the hive followed by an immediate drop to almost no bees entering or leaving the hive. Seasonal changes are primarily due to changes in temperature, rainfall, and forage resource material. Typically, flight activity increases during the warmest months and when forage material is abundantly available. Geographical differences are evident in the length of the forage period and the general daily patterns of flight activity; i.e., whether the activity remains at a constant level throughout the day, or variations in the activity occur.

Behavioral responses to chemical exposures have been characterized by smoking the hives to calm the bees, by experimental exposure to methanol, and the fortuitous Pine Sol™ incident. All of these events produced a drop in the bees leaving the hive followed by a matching drop in bees entering the hive. The DER*DIFF analysis method was devised to seek out these events by producing a large signal whenever the characteristic 'phase-shift' occurred. Hive maintenance affects flight activity by producing sharp, brief peaks in flight activity immediately after the event is concluded. Swarming produced a large enough change in flight activity for the swarm hive to

have been detected by almost all of the analysis methods. In fact, the swarm produced the most extreme conditions of flight activity over a 40 minute period.

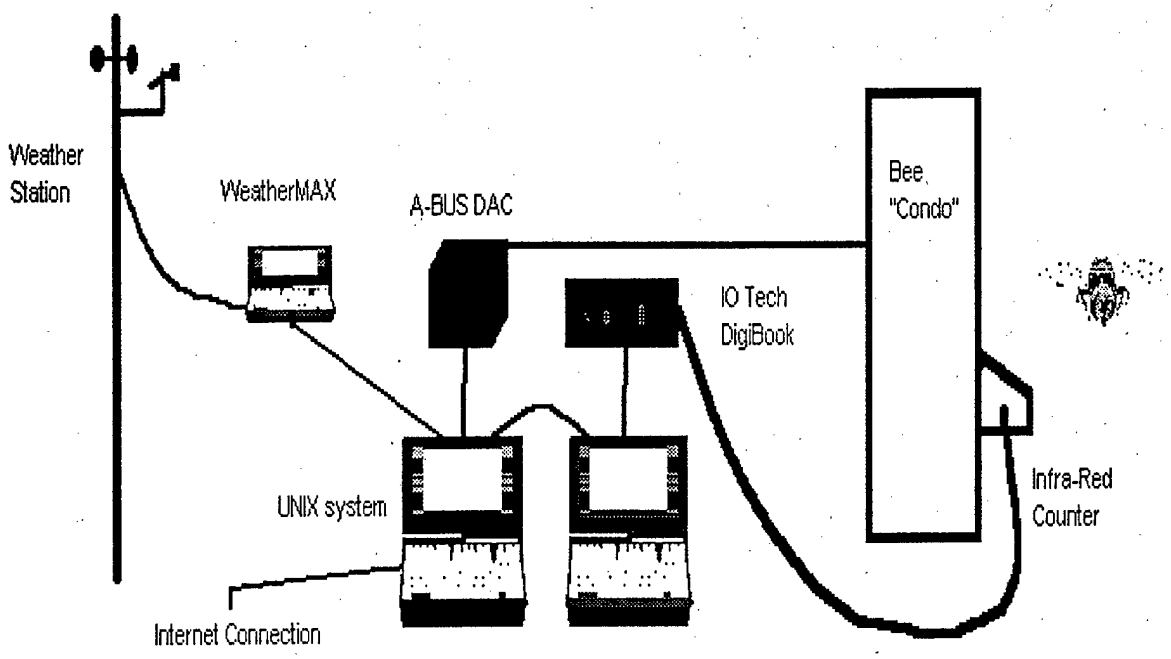
Because many external events induce rapid responses, detection of these responses and appropriate action such as initiating colony inspection or chemical sampling needs to be accomplished in real-time. To date, our systems have proven to be capable of monitoring colony behavior in real-time with the computers at each field site. However, transmission, access, and evaluation of the data at a remote site has relied upon physical exchanges of data storage devices such as shipping a data cartridge from Maryland to Montana. The ability to deliver data in real-time for rapid detection of colony reactions and for cause and effect analysis is crucial to the success of the concept. That capability was realized in our laboratory during the last six months.

1.10 Real-Time Data Delivery

In autumn of 1997, we achieved the ability to deliver all of our field collected data in real time through Internet or Intranet connections. The changes to the flight counter software that enabled this connection were described in the preceding sections. Connecting the rest of the electronic hive sensors was accomplished by: (1) porting the existing A-Bus data gathering system to Linux (Linux is the Free Software Foundation, Inc. release of a Unix clone that runs on an Intel architecture personal computer), and (2) replacing the existing weather gathering software (WeatherView©, ControlWare©) with an in-house developed program also running under Linux.

In addition to running in a more stable multitasking environment than Windows 95, the new Linux version performs aggressive error correction, archives data in a text format for easy use and automatically generates 15 second updates in HTML (the Hypertext Markup Language used by Web Browsers). A companion applet written in Java™ allows an interactive graphical display of current weather data. After the day's flight data has been collected, the counter computer sends a compressed data stream containing all the flight activity gathered during the day. A corresponding communication program running on the Linux system receives the data sent from the counter computer and archives it.

The result of all of these changes is that all of the electronic data collected at a site will now reside on a central computer, reducing the number of computers at a site from three to two (Figure 1.27). Due to the tremendous flow of data from the bee counters, one computer is required to manage the bandwidth, summarize flight data, and then transmit it to the central computer. This computer will also make the data available for real time analysis by a suite of decision making algorithms on the central computer. If the central computer is connected in some manner to a network (either by Ethernet or modem connection), archived and current data may be viewed using a web browser such as Netscape or Internet Explorer (e.g., see archived files from the Missoula Reference Site at **http://www.umt.edu/biology/bees**). If so desired, the network may be connected to the Internet so that data can be viewed on a browser running on a computer anywhere in the world. If for security or data privacy reasons this is undesirable or inadvisable, an



Intranet system can be established, preventing all but authorized clients to connect and download information from the system.

Figure 1. 27. Diagram of Linux-Based Data Acquisition/Decision-Making System

In addition to viewing the "raw" text data file, a custom Java applet has been written to allow the data (i.e., flight data, weather data, colony core temperature and other parameters) to be displayed in an interactive graphical format. This applet reads in the text data file and displays the data as a graph (Figure 1.28). The user may selectively turn graphed data on or off by clicking on check boxes. Additionally, individual series of data may be graphed on an alternate Y axis. For instance: in the weather data file the barometric pressure is saved in millibars, which ranges from 930 to 1066. Outside temperature, on the other hand, is saved in Celsius, which ranges from -40 to 50. If both attributes are plotted on the same Y scale, the outside temperature series becomes difficult to view compared to barometric pressure. By plotting the barometric pressure on the alternate Y axis, the user can more clearly and simultaneously view the data series for both of these parameters (Figure 1.28).

Currently, data from one acquisition system can not be overlaid on data from another acquisition system, such as comparing flight data and weather conditions on the same chart. Ongoing software development is focused on this area. This task will require synchronizing the time indexes from the three distinct acquisition systems in a real-time mode. This is also a necessary requirement for the real time rather than retrospective analysis of the data.

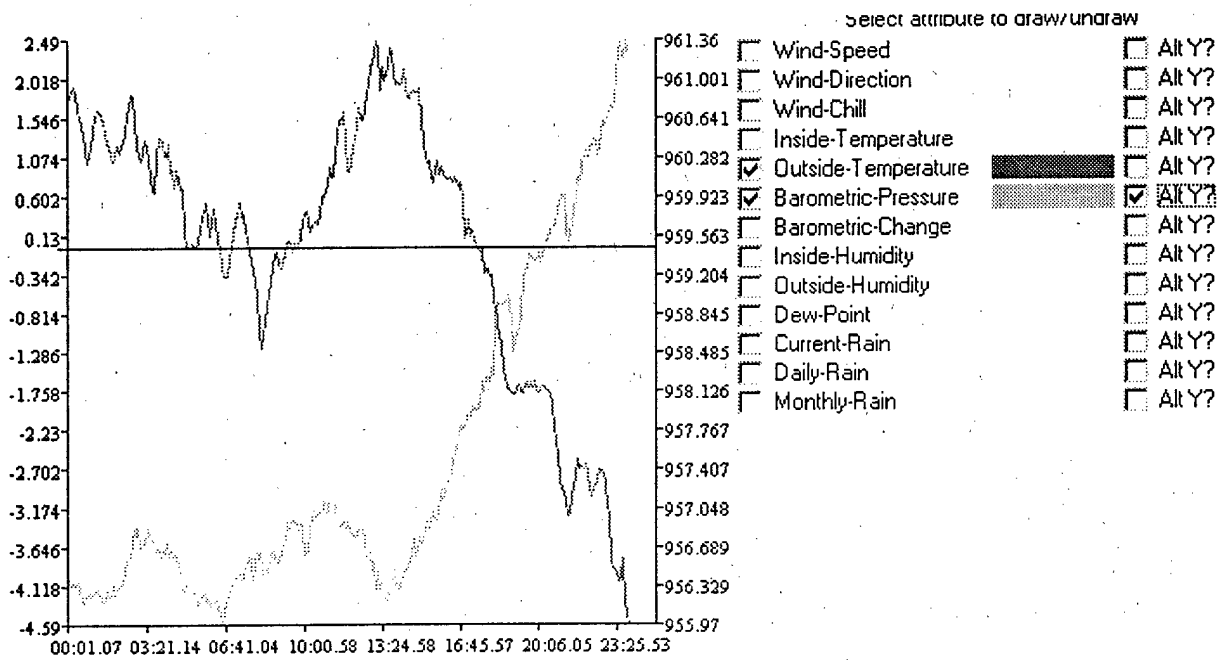


Figure 1.28. Example of Java graphed weather data in a WWW format. In this case, all weather variables except outside temperature and barometric pressure have been clicked off, and a second Y (Alt Y) axis activated to plot barometric pressure.

1.11 Artificial Neural Networks

Our Artificial Neural Networks (ANNs) continue to show great promise for a quick evaluation of colony dynamics. If we are to use the real-time behavioral data to flag events that warrant immediate investigation, including possible sampling for chemical exposures, we need software programs that will continually monitor the real-time data flows and provide rapid detection of unusual or anomalous behaviors. For flight activity, the C.V., PCR, and ANL values can be used in a form of Control Chart program to identify outlier values in quick time. How close that analysis comes to the real-time data collecting depends somewhat on the nature of the data. Distinct deviations in behavior, such as the peaks induced by a smoke event, a swarm, or robbing, can be flagged quickly, probably in less than 15 minutes. Other events, such as loss of forager bees during the day will be most evident at the end of the day when the PCR and ANL can be computed. Still other metrics such as increasing C.V. for flight activity among the hives at a site will be revealed over a period of days or weeks.

However, none of these procedures can directly determine whether the perturbation in behavior was due to a natural event such as a change in weather or to some other factor such as exposures to chemicals or a biological event such as robbing. This is where pattern learning and predicting programs such as ANN may have considerable utility. In essence, the ANN learns the behavioral responses of each colony to external driving factors such as weather. Depending on the colony, time of year, weather conditions, and food resource availability, this training period may require data from a few days up to two weeks. Once trained, the ANN can be used to retrospectively interrogate the data and produce a prediction of the expected colony behavior for any period for which weather data is available. The objective is to be able to use the ANN to review the real-time data and at 15 minute (or sometimes hourly) intervals, provide a plot of the expected behavior (e.g., flight activity) for the immediately preceding interval. In other words, if the ANN knows what the weather conditions were over the last 15 minutes, can it predict what the expected flight activity should have been for that 15 minute period, and can it accurately identify deviations from the expected colony performance that may warrant immediate inspection or sampling by the personnel conducting the real-time monitoring program?

Using data gathered over two summers from 21-28 colonies and a variety of sampling sites, a large database of activities and behaviors has been created. Most "normal" and some "abnormal" colony behaviors have been observed and documented, providing a criteria on which to judge the accuracy of ANN predictions. To provide a near real-time implementation of ANN processing, a "sliding window" of data processing has been used. Often, the sliding window requires only the weather data gathered over the previous two hour period to provide reasonable predictions of the expected flight activity for the next 15 minutes.

In some cases, accurate predictions require a retrospective period of an hour. Because bees can detect and respond to changing weather events before a weather station records the actual event, during stormy periods the weather data from the last hour is needed to make an accurate prediction of expected flight activity. It is hoped that this sliding window can be shrunk to require only the last one-half hour of weather data without losing predictive accuracy. Preliminary results of tests on this hypothesis appear promising, but more work and greater accuracy is required before continuing with the reduced window size.

Previously, we endeavored to predict exact flight activity using only the data gathered from the weather and colony core information. The premise was that when actual flight activity deviated from predicated activity, an alarm signal would be generated. Bee colonies are very dynamic systems and display rapid, minor changes in flight activity (e.g., phase shifts, magnitude changes, etc.). Some of these changes could generate false alarms.

Recently, under the direction of Dr. David Opitz, UM Department of Computer Science, we have explored the use of the Sum Squared Error (SSE) as a better indicator of general model accuracy. Using this metric has provided a reasonable indicator of the days when flight activity

was abnormal. We believe that a sufficiently accurate model can be created using this metric. That model can then be used to advance the near real-time analysis and prediction system.

The data collected to date provides only an indirect measure of pollen and nectar resource availability. Bee behavior is influenced by the presence of pollen and nectar producing plants. In Aberdeen, abundant native vegetation provide a mix of nectar flows, which can vary greatly from week to week. Many of the aberrations (i.e., unexpected) of flight activity, flagged by the ANN, could be traced to this factor. One such event was created artificially in the fall of 1996 at the Canal Creek site, when bees were allowed to "rob" an external food source. The sudden appearance of an abundant nectar source was displayed by the ANN as a large spike in the SSE for pattern day 33 (Figure 1.29). Our investigations indicate that other unexplained deviations (i.e., non-weather related) may be caused by similar biological events or by exposures to chemical agents.

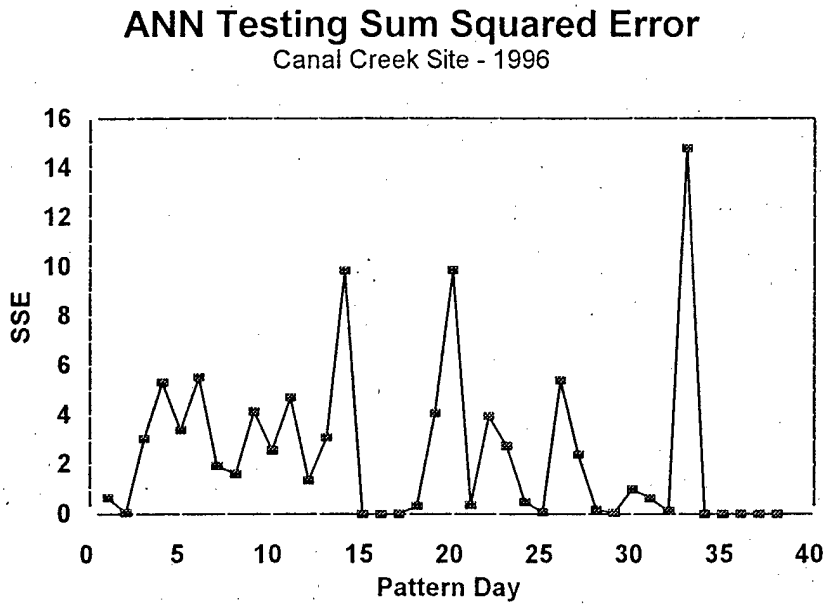


Figure 1.29. ANN sum of squared error applied to robbing event on day 33. Because the experiment was conducted at the end of season when little forager was available, flight activity was highly variable, and the SSE produced peaks on other days that reflect this natural "noise".

Further work is required to build a library to categorize those days when bee activities are disrupted by normal biological, but "unlearned" events, and which behavioral changes qualify as significant events (such as chemical exposures). Under an operational mode, these events would indicate immediate action and notification. Distinguishing these events will require: (1) additional

controlled dose response experiments, and (2) improved measures of nectar and pollen availability -- major research objectives for 1998.

Currently we are testing three different methods of training a neural network. Each uses pattern files containing data for a number of days. The network is first trained on a set of patterns representing consecutive, but not necessarily contiguous, days. Then the accuracy of the network is tested using the next consecutive pattern day. The 3 methods being tested are:

- Single day training. Each training pattern consists of only a single day's representation of data, using the trained network without reinitialization. This produces an adaptive network which prefers more recently observed behavior. Training time is fixed.

- Accumulated days training. After the network has been tested on a pattern day, that pattern is added to the training set. The network is not reinitialized. This method retains knowledge learned from previous training, with a bias toward older concepts learned. Training time increases linearly.

- Accumulated days training with reinitialization. Similar to number 2, but the network weights are randomly reset before training. This effectively "wipes out" any concepts previously learned. This method gives equal bias to all pattern days, which may prove more effective in detecting chronic behavioral changes. Training time is similar to method 2.

The SSE for a subset of data collected from hive #1 at the Churchville reference site is displayed in Figure 1.30. Each of the aforementioned training methods yielded a somewhat different response to the testing data, although the methods tended to converge with time. Predictions for the first week of data should be ignored, as the network has not built up a adequate enough model of basic bee behavior. Performance began to improve by the second week and then stabilized. Additional concepts (rain, wind, etc.) are learned as they are encountered. Thus, until the ANN has encountered the event, it doesn't know how the bee colony will respond. This results in SSE spikes which are not indicative of a harmful bio-alert. The number of these "unknown" events decreases with time (as can be seen in Figure 1.30) and the ability to flag events of interest greatly improves.

Before a predictive ANN system can be used in a routine manner, we need to provide the ANN with "library" of basic bee behaviors which can be used to teach the general neural network. Provisions will be made to adapt the network model to individual hives without losing the essence of basic bee behavior. The use of one or more of the training methods to "custom fit" a model to a particular hive, along with other knowledge transfer ideas, are being explored.

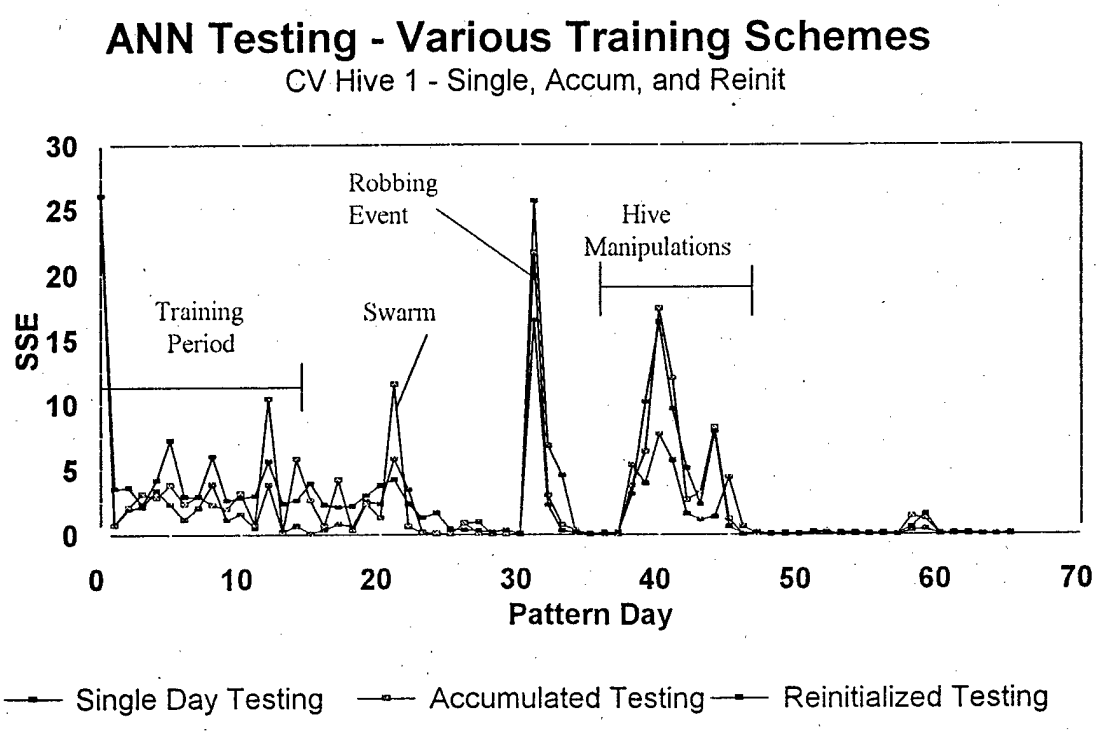


Figure 1.30. Comparison of single day, accumulated testing, and reinitialized testing of the ANN. After the first two weeks of training, each of the three methods exhibit convergence and intervention events (e.g., swarms, robbing, hive manipulations) can be readily discerned.

1.12 Focus Areas for 1998 Field Season

Areas of real-time monitoring of honey bee colony population behaviors for the 1998 field season include:

- Development of an extensive library of basic bee colony behaviors in both pristine and contaminated areas.

  We have already been archiving an extensive database from our Maryland and Montana sites. Preliminary datasets that have been processed using the SITEVIEW and ANN analysis routines have proved to be reliable and can be applied to next year's samples.

- Conduct of an extensive set of dose-response trials to document colony behaviors in response to chemical agents.

We need to expose colonies to chemicals as both as agents encountered outside the colonies (e.g., in water, on plants, in the air) and inside the colony (e.g., in nectar, pollen, hive atmospheres).

- Addition of new sensors to monitor nectar and pollen resource availability.

  Currently, we monitor pollen with a clock driven trap that must be manually weighed and plotted to ascertain how much pollen came into each hive for any given time of the day. We have no good way of determining which bees are bringing in nectar. We are working on availability (i.e., by the gain or loss of biomass).

- Delivery and distribution of real-time monitoring data from remote locations to central data acquisition and processing facilities.

  We have achieved the ability to transfer the field data from each site to a decision-making computer located either locally (i.e., the USACEHR Administration Trailer) or remotely (i.e., the UM laboratory in Missoula, MT). Appropriate safeguards can be put in place to secure the data and provide access only to authorized individuals. An example of this capability can be seen on our WWW site **(http://www.umt.edu/biology/bees)** by viewing the on-line weather station and the archived data files from the Montana reference site.

- Refinement of our SITEVIEW and ANN tools for real-time data processing and predictions.

  We are building a set of software routines that will flag behavioral metrics that exceed pre-determined thresholds (based on our ever expanding database of colony behaviors) and ANN programs that will continuously monitor and detect behaviors that are inconsistent with ANN predictions. For example, the ANN has already proven to be capable of detecting changes in flight activity that are inconsistent with the behavior expected from a colonies previous day's flight and current weather conditions. The objective is to be able to quickly recognize anomalies and alert an observer to inspect the colonies, and if warranted, initiate chemical exposure monitoring.

- Continue applications of real-time monitoring of potential environmental hazards at APG sites.

  The monitoring activities initiated at J Field in the autumn of 1997 will be conducted for a full field season at J Field. This will provide a reliable database for assessing current conditions at the site and the effectiveness of phytoremediation, removal actions, and any capping activities that may occur in the future at this site. As before, reference sites at Churchville and Montana will be used for comparative purposes.

1.13 REFERENCES

Bromenshenk, J.J.; Smith, G.C.; King, B.E.; Seccomb, R.A.; Alnasser, G; Henderson, C.B.; Loeser, M.R.; and C.L. Wrobel. 1997. New and Improved Methods for Monitoring Air Quality and the Terrestrial Environment; Applications at Aberdeen Proving Ground—Edgewood Area. Contract No: DAMD17-95-C-5072. Performance Organization Report # GESL-96-01-ANN/DEF for U.S. Army Medical Research and Materials Command, Fort Detrick, Frederick, MD 21702-5012. 176 pp.

SECTION 2

ELECTRONIC HIVE AND SITE DESCRIPTIONS

2.1 Electronic Hives

The electronic beehives (condos) developed at the University of Montana were described in detail in our previous annual report. Each of the hives is fitted with a bi-directional bee counter (Figure 2.1) to determine the numbers of bees entering and leaving the hives, in-hive sensors to measure metrics such as the temperatures in the brood nest, and chemical probes for sampling the air inside the hive. A trap underneath each hive collects the dead and dying bees removed from the hive by housekeeping bees. A clock-driven trap (Figure 2.2), in conjunction with a scraper screen placed under the hive, collects pollen dislodged from incoming foragers. The clock provides a time record of when the pollen was collected.

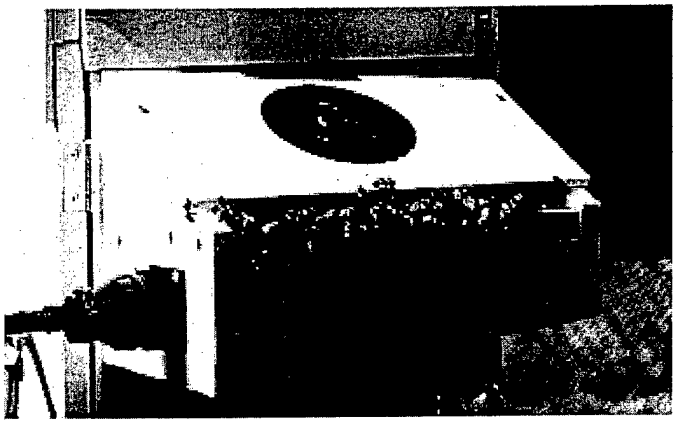


Figure 2.1. Bi-Directional Entrance Bee Counter

Figure 2.2. Detail view of condo internal workings: A, polyethylene support plates; B, clock driven pollen collector. The pollen scrapper screen fits under the support plates.

The condos were altered during 1997 to permit easier access to the hives for inspection. A flip-top outer enclosure (Figure 2.3) both permits easy access and protects the internal hives and electronic systems.

Our initial versions (1995) of these systems had front access doors. Our 1996 systems added ports at the back for the chemical sampling probes and for the electronic sensors that measure brood nest temperatures, relative humidity, and hive biomass. The relative humidity data proved to be of little use in 1996. Levels inside the hive equaled or exceeded those of the ambient air. The strain gauges also proved to be too insensitive to provide much useful information. However, core temperature proved to be a critical metric, reflecting the presence or absence of the queen, and of eggs, larvae, or pupae. In 1997, we replace the relative humidity probes with additional temperature probes and initiated additional research

and development of a strain gauge assembly. The revised strain gauge system for tracking changes in the weight of the hives will be implemented in the 1998 studies.

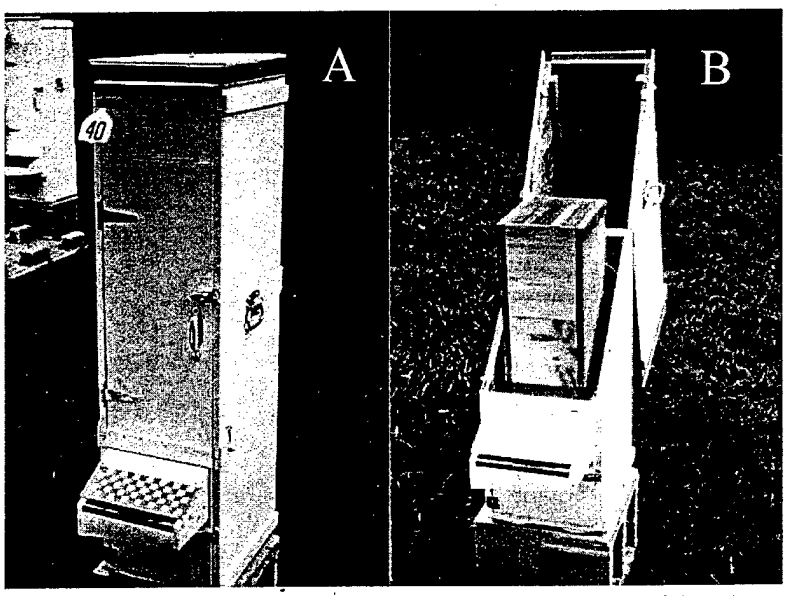


Figure 2.3. Bee Condo: A, operational mode; B, with hinged top swung open to reveal two-story mini hive.

In 1996, we placed the electronic hives in a row, using colors on the doors to help the bees find their respective hives. The hives at West Branch Canal Creek were mounted on a flatbed trailer. Those at other sites were placed on wooden stands.

The hives were placed farther apart and were distributed at each site in a more scattered arrangement in 1997. In addition to colors, symbols were used to mark the entrances (such as the circle shown in Figure 2.1). At each site, a tool box or shed housed three data acquisition interfaces and three computers. A digital weather station also was employed. The mast was attached to either the trailer (at West Branch and J Field) or to the storage shed (at Old O Field and at the Churchville reference site. The temperature and relative humidity probes were positioned about 3 meters above the ground. The wind speed, direction and frequency measurement devices and a photocell to determine light levels were positioned about 4 meters above the ground. The masts were kept short to position the measurement devices at about the height of the main flight path of the bees leaving and entering the apiary. Figure 2.4 shows the layout of the hives and storage shed at the Churchville reference site.

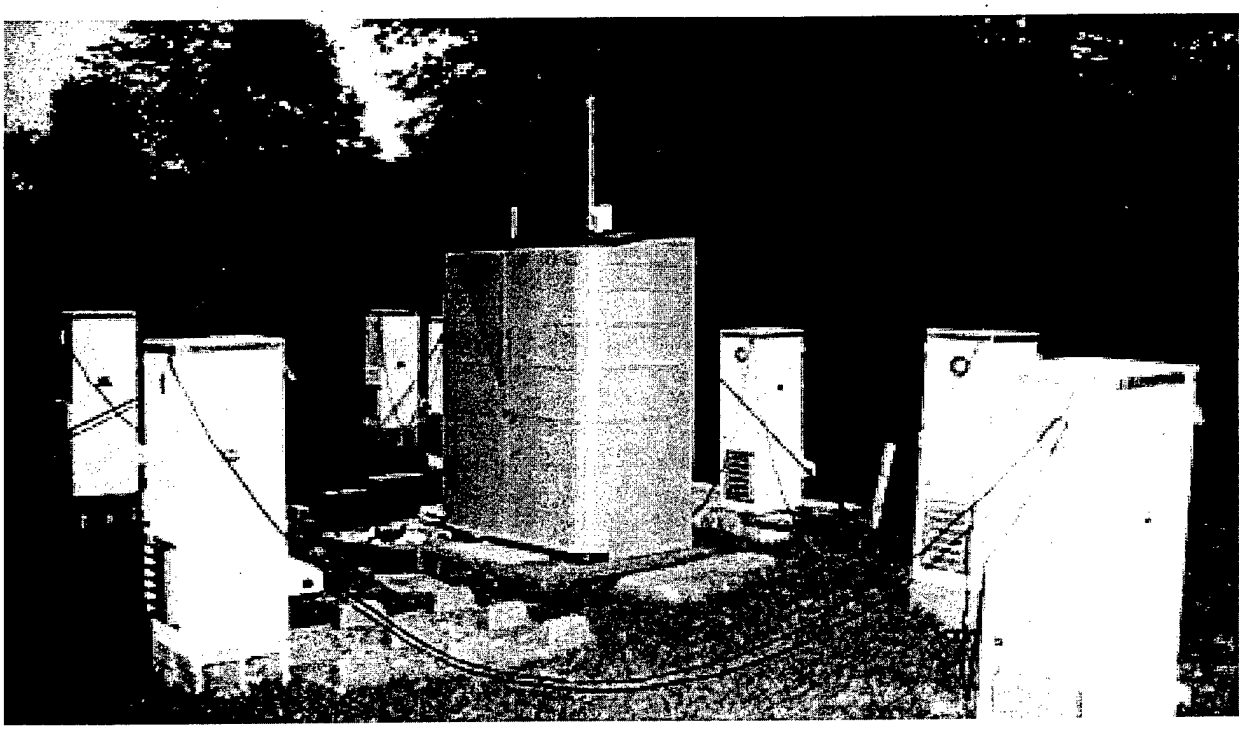


Figure 2.4. Electronic hives at Churchville Reference Site, 1997. Each of the "condo" hives contains a mini-hive with a colony of bees. The small storage shed houses a bank of three computers. The weather station mast can be seen extending above the roof of the shed.

2.2 APG—Edgewood Sites

Under funding from USACEHR (formerly USABRDL), at 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. The study also showed that this method held 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 1997, these studies were again expanded to include locations within the Bush River area and at J Field.

2.3 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 landfills at Old O Field (1996 and 1997) and at J Field (1997). In 1997, 12 additional locations, mostly landfills, were monitored at the Bush River area. 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.

2.4 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 arsenic (As), beryllium (Be), and strontium (Se), and a wide array of chlorinated aliphatic hydrocarbons have been found across the site. The Old O Field landfill on the peninsula was being capped during the period of these studies, and capping of the J Field landfill was planned. Both of these sites (Figure 2.6) contain a mixture of chemical agents. The Bush River area has dumps, landfills, a chemical depot site, chemical disposal sites, and sites where materials were burned. Chemical agents of concern included volatile and semi-volatile organics, metals, trace elements, polychlorinated biphenyls, and radionuclides.

However, most of the monitoring and remediation conducted to date at APG 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.

During both summers, clusters of seven instrumented colonies (i.e., "condos") were deployed at:

- West Branch Canal Creek near the block of trailers belonging to the U.S. Geological Survey (USGS) and USACEHR (Figure 2.5).
- Old O Field hazardous waste landfill (Figure 2.6)
- Off-post reference site near Aldino road east of Churchville.

In 1997, the electronic colonies at West Branch Canal Creek were moved to J Field in late summer (Figure 2.6). Also in 1997, another set of seven condos were assembled and deployed at the University of Montana's new research apiary on the west campus in Missoula. The results of the trials and experiments conducted with 21 electronic units in 1996 and 28 units in 1997 are

described in Section 1 of this report. The real-time data for the two field seasons can be provided on a CD-ROM as a supplement to this annual report. Please direct requests for copies of the CD-ROM to The University of Montana.

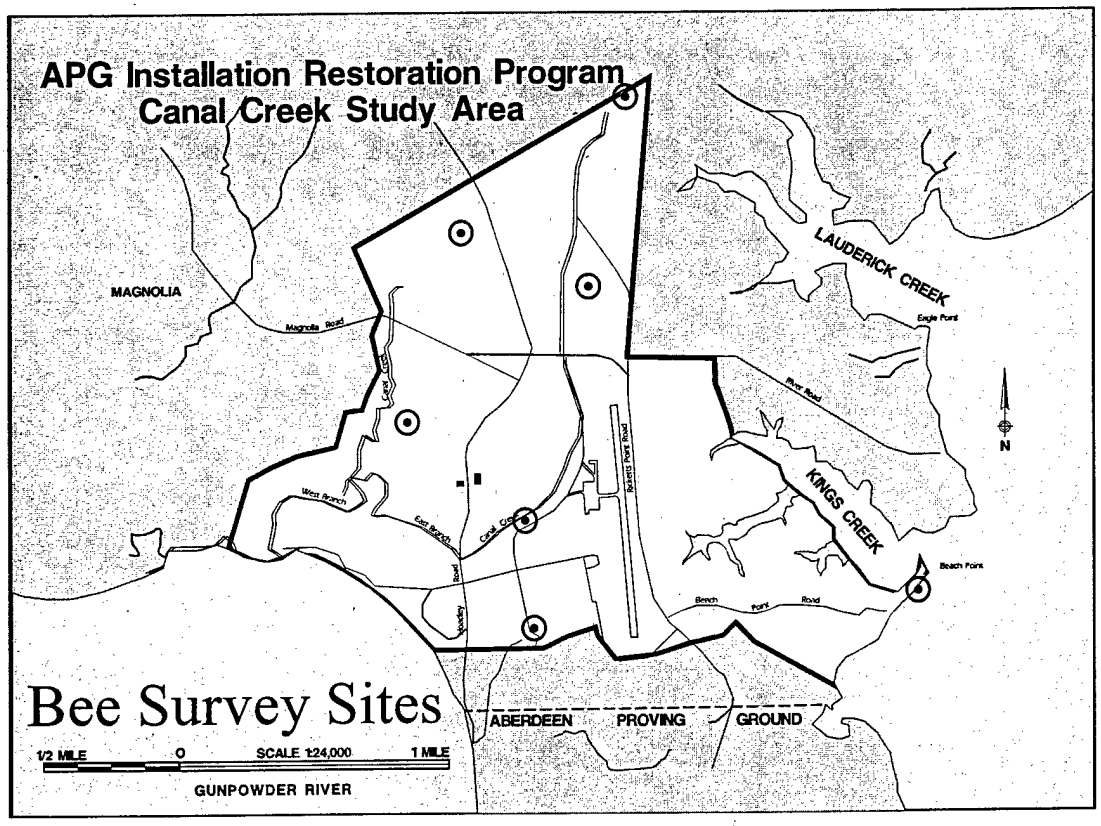


Figure 2.5. Canal Creek Area Honey Bee Survey Sites. The red circle identifies the location of the instrumented (real-time monitoring) hives at West Branch Canal Creek.

Additional groups of two or more nucleus colonies were placed (Figures 2.5 and 2.6) 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
- Abandoned railroad tracks, northwest of the G Street area
- Lauderick Creek area, adjacent to the golf course
- East Branch Canal Creek, southeast of the horse stables

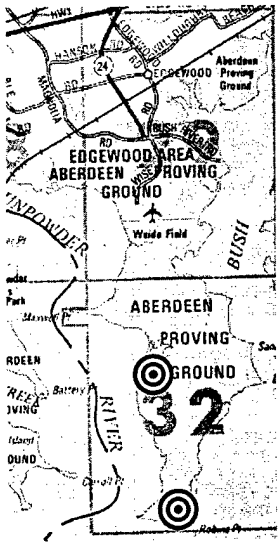


Figure 2.6. Aberdeen Peninsula. The red circle represents the Old O Field Instrumented Hive Site, the yellow circle identifies J Field.

Both the Old O Field and J Field locations were on a restricted access area of the Edgewood peninsula. A water treatment plant also is located at the Old O Field landfill. Old O Field was capped in 1996, with the final work completed in 1997. A phytoremediation project has been established at J Field, and plans have been made to cap J Field. At Old O Field, a set of seven electronic hives and another 4 to 6 survey hives were deployed in 1996 and 1997. The survey hives were used to examine gradients of exposure, with the hives set out on opposite sides of the landfill. Similarly, at J Field, pairs of survey hives were placed on each side of the phyto-remediation area, and a set of electronic hives was placed nearby. The electronic hives at J Field had been used at West Branch Canal Creek in 1996 and again through most of the summer in 1997, before being moved to J Field at the end of the season.

At Bush River (Figure 2.7), pairs of survey hives were placed at 12 locations. Two of the sites were inside the fence and one just outside of the fence at Cluster 3. Five sites were behind the security fence of the Southern Bush River Area. The other four sites were just outside the security fence for the Southern Area.

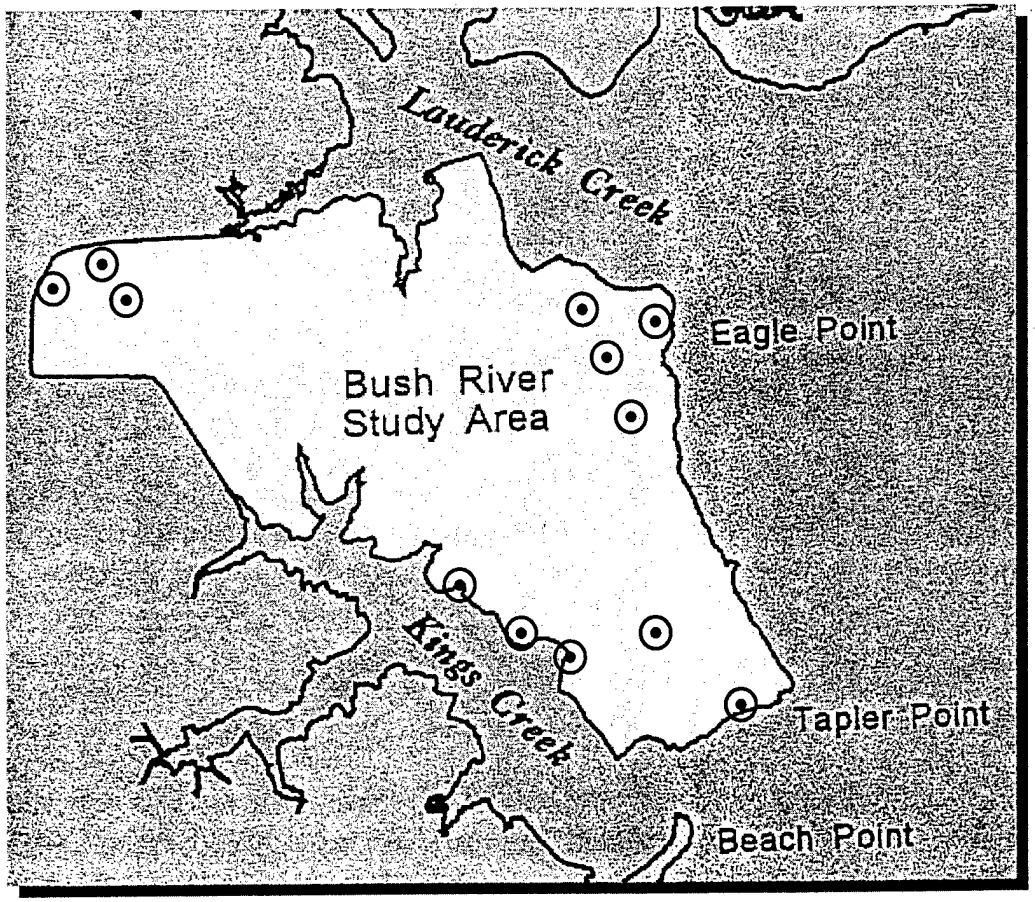


Figure 2.7. Bush River Honey Bee Colony Survey Sites, 1997. Red circles indicate locations with higher than average levels of chlorinated hydrocarbons (see Section 3).

In addition to the sites shown on these maps, the Churchville Reference site is located northwest of APG-Edgewood, about 1.6 km west of a small rural airport on Aldino Road. The reference apiary in Missoula, Montana is about 6400 km from APG. The climate, geography, and floral resources in Montana are distinctly different from Maryland. The Montana site serves as a good test bed to compare the degree to which healthy colonies act the same or behave differently due to habitat and climate. Many of the chemical agents seen in colonies in Maryland, both at APG sites and at the Churchville reference site, are not found in the Montana bees. Therefore, we can use the Montana site as a control to study uptake dynamics and chemical effects.

SECTION 3

MONITORING OF EXPOSURES TO BIOAVAILABLE CONTAMINANTS

3.1 Chemical Sampling and Analysis

Chemical sampling and analysis for this project are guided by two overall objectives:

1) Establishing the biomonitoring relationship between acute exposures to specific chemical agents and measured behavioral endpoints in honey bees; and

2) Assessing the bioavailability of chemical agents to honey bees in order to compare sites with respect to chronic exposures to chemical agents.

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). Selected forager samples were prepared for radiochemical analysis by gamma scans, but results are not yet available.

Three new aspects of chemical sampling are presented in this year's report. First, we address quantification of the TD/GC/MS results. Factors have been generated for converting ions per liter (ipl) data, as reported in last year's comparative studies, into conventional concentration units of parts per trillion by volume (ppt), and nanograms per cubic meter (ng/m^3). It should be remembered, however, that these represent time-weighted averages since they were accumulated over an extended pumping period - some more than 24 hours. Second, we briefly assess the factors that limit the precision with which TD/GC/MS concentrations can be measured. Our "constant flow" pumps drift about 9% in their pump factors over the course of collecting a sample -- about the same precision with which our GC/MS is tuned prior to each run. Finally, we present the results of dosing experiments conducted in Missoula. The results support a mechanism through which honey bees transport aqueous-borne contaminants into the hive. Bees were trained to obtain water from a specific location that was subsequently contaminated with a small quantity of perchloroethylene (PCE).

PCE was measured in hive atmospheres during the sampling period immediately following its addition.

Analysis of samples from the 1997 field season indicate that levels of organic contaminants in the Old O Field area were significantly reduced in their bioavailability compared to 1996. No colonies became queenless at the Old O Field locations this year, as compared to six out of 12 leaving during the 1996 field season. None of the 61 colonies showed chemical levels suggestive of an acute toxicity exposure scenario. Measurable levels of tear gas breakdown products (acectophenone and benzaldehyde) were noted in some Bush River storage areas.

3.2 Experimental Methods and Materials

3.2.1 Sampling Design/Frequency - 1997 APG Field Applications

Samples were collected from a total of 69 honey bee colonies. Three sets of instrumented condo units (7 colonies each) were initially returned to the same sites as in the 1996 APG field applications: 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. During August, the instrumented condo cluster from West Branch Canal Creek was moved to J Field. Twelve pairs of free-standing colonies were distributed at new sites throughout the Bush River peninsula -- 5 pairs within the secured portion, 3 pairs in and around the Cluster 3 area and 4 pairs outside of the fence on the southern boundary of the secured area. Other free-standing colonies were placed on range sites -- at Old O Field (4 hives) and J Field (4 hives); at locations previously monitored in the office, residential and recreational portions of APG's Edgewood area -- Beach Point (2 hives), National Guard Armory (2 hives), G Street (2 hives), Lauderick Creek (2 hives), Youth Center (2 hives), East Branch Canal Creek (2 hives) and West Branch Canal Creek (2 hives); and at the Churchville farm reference site (2 hives).

A final round of sampling was performed on many of the colonies at the end of the 1997 field season. Colonies entrances were screened and the hive boxes moved to a holding area on G Street. With entrance screens in place, no foraging activity was allowed. Residues seen in these samples represent contaminants that had accumulated within the hive prior to screening.

3.2.2 Air Sampling

Sampling of hive atmospheres and outside ambient air took place periodically between May 7, 1997 and October 31, 1997 (Table 3.1). The number of samples on any date was limited to 21, the number of constant flow pumps in our 1997 equipment inventory. Sites in a group with samples missing on a given day were generally due to plugged copper tubes, wet desorption beds, tube breakage or pump power failure. Analytical results from May and June samples are limited and of questionable use. A malfunction in our thermal desorption unit

TABLE 3.1

Hive Atmosphere Sampling
1997 APG Field Applications
(through October 31, 1997)

Site (Colony ID)	Sampling Dates
Old O Field	
OF (#323)	6/06, 6/19, 7/15, 7/31, 9/19, @10/29
OF (#399)	7/15, 7/31, 9/19, @10/29
OF1 (#50)	7/31, 8/26, 9/19, @10/29
OF2 (#49)	7/31, 8/26, 9/19, @10/29
OF3 (#34)	7/31, 8/26, 9/19, @10/29
OF4 (#41)	7/31, 8/26, 9/19, @10/29
OF5 (#26)	7/31, 8/26, 9/19, @10/29
OF6 (#30)	7/31, 8/26, 9/19, @10/29
OF7 (#35)	7/31, 8/26, 9/19, @10/29
OFW (#322)	7/15, 9/19, @10/29
OFW (#398)	6/6, 6/19, 7/15, 9/19, @10/29
OF air	6/19, 7/15, 7/31, 8/26, 9/19
Bush River	
BR1 (#345)	5/7, 5/19, 6/6, 6/19, 7/29, 8/29, 10/9, @10/31
BR1 (#391)	7/29, 8/29, 10/9
BR1 air	6/19, 7/29, 8/29, 10/9
BR2 (#314)	5/7, 5/19, 6/6, 6/19, 7/29, 8/29, 10/9, @10/31
BR2 (#392)	7/29, 8/29, 10/9, @10/31
BR2 air	5/19, 7/29, 8/29, 10/9
BR3 (#341)	5/7, 5/19, 6/6, 6/19, 7/29, 8/29, 10/9, @10/31
BR3 (#316)	7/29, 8/29, 10/9, @10/31
BR3 air	7/29, 8/29, 10/9
BR4 (#337)	5/7, 5/19, 6/6, 6/19, 7/29, 8/29, 10/9, @10/31
BR4 (#374)	7/29, 8/29, 10/9
BR4 air	7/29, 8/29, 10/9
BR5 (#343)	5/7, 5/19, 6/6, 6/19, 7/29, 8/29, 10/9, @10/31
BR5 (#362)	7/29, 8/29, 10/9, @10/31
BR5 Air	6/6, 7/29, 8/29, 10/9
BR6 (#342)	5/7, 5/19, 6/6, 6/19, 7/27, 8/19, 9/7, 10/8, @10/31
BR6 (#382)	7/27, 8/19, 9/7, 10/8
BR6 air	7/27, 8/19, 9/7, 10/8

TABLE 3.1 (Cont'd.)
1997 APG Field Application Samples

Site (Colony ID)	Sampling Dates
Bush River (Cont'd.)	
BR7 (#371)	5/7, 5/19, 6/6, 6/20, 7/27, 9/7, 10/8, @10/31
BR7 (#329)	7/27, 8/19, 9/7, 10/8
BR7 air	5/7, 5/10, 6/20, 7/27, 8/19, 9/7, 10/8
BR8 (#368)	5/10, 5/19, 6/6, 6/20, 7/27, 8/19, 9/7, 10/8
BR8 (#334)	7/27, 8/19, 9/7, 10/8, @10/31
BR8 air	5/19, 6/6, 7/27, 8/19, 9/7, 10/8
BR9 (#384)	5/10, 5/19, 6/8, 6/20, 7/27, 8/31, 10/8, @10/31
BR9 (#387)	7/27, 8/31, 10/8, @10/31
BR9 air	6/8, 7/27, 8/31, 10/8
BR10 (#385)	5/10, 5/19, 6/8, 6/20, 7/27, 8/31, 10/8, @10/31
BR10 (#321)	7/27, 8/31, 10/8, @10/31
BR10 air	5/10, 5/19, 7/27, 8/31, 10/8
BR11 (#366)	5/10, 5/19, 6/8, 6/20, 7/27, 8/31, 10/8, @10/31
BR11 (#344)	7/27, 8/31, 10/8, @10/31
BR11 air	6/20, 7/27, 8/31, 10/8
BR12 (#359)	5/10, 6/8, 6/20, 7/27, 8/31, 10/8, @10/31
BR12 (#386)	7/27, 8/31, 10/8
BR12 air	5/10, 6/8, 7/27, 8/31, 10/8
West Branch Canal Creek	
CC1 (#27)	7/25, 8/19, 8/23
CC2 (#31)	7/25, 8/19, 8/23
CC3 (#48)	7/25, 8/19, 8/23
CC4 (#37)	7/25, 8/19, 8/23
CC5 (#46)	7/25, 8/23
CC6 (#42)	7/25, 8/19, 8/23
CC7 (#47)	7/25, 8/19, 8/23
CC (#357)	5/18, 6/15, 7/15, 8/23, 10/4
CC (#107)	10/4
CC air (sign level)	5/18, 6/15, 7/25, 8/19, 8/23, 10/4
CC air (ground level)	7/25
J Field	
JFN (#307)	5/11, 6/6, 6/19, 7/15
JFN (#356)	5/11, 6/6, 6/19, 7/15, 7/31, 9/15, 10/23
JFN air	5/11, 6/6, 7/31, 10/23

TABLE 3.1 (Cont'd.)
1997 APG Field Application Samples

Site (Colony ID)	Sampling Dates
J Field (Cont'd.)	
JFS (#396)	6/19, 7/15, 7/31, 9/15, 10/23
JFS (#306)	6/19, 7/15, 7/31
JFS air	7/15, 7/31
JF1	9/15, 10/23
JF2	9/15, 10/23
JF3	9/15, 10/23
JF4	9/15, 10/23
JF5	9/15, 10/23
JF6	9/15, 10/23
JF7	9/15, 10/23
JF air	9/15, 10/23
Other APG Edgewood Areas	
G Street 1 (#348)	5/18, 6/15, 7/20, 8/12, 10/4
G Street 2 (#355)	5/18, 6/15, 7/20, 8/12, 10/4
GS1	6/1
GS2	6/1
GS3	6/1
GS4	6/1
GS5	6/1
GS6	6/1
GS7	6/1
G Street air	5/18, 6/1, 6/15, 7/20, 8/12, 10/4
Lauderick Creek 1 (#365)	5/18, 6/15, 7/18, 7/25, 8/12
Lauderick Creek 2 (#312)	6/15, 7/18, 7/25, 8/12
Lauderick Creek air	5/18, 6/15, 7/18, 7/25, 8/12
National Guard 1 (#352)	5/18, 6/15, 7/20, 8/12, 10/4
National Guard 2 (#328)	6/15, 7/20, 8/12, 10/4
National Guard air	5/18, 6/15, 7/20, 8/12, 10/4
Beach Point 1 (#319)	5/18, 6/15, 7/20, 7/25, 8/12, 10/4
Beach Point 2 (#324)	6/15, 7/20, 7/25, 8/12, 10/4
Beach Point air (sign level)	5/18, 6/15, 7/20, 7/25, 8/12, 10/4
Beach Point air (ground level)	7/25

TABLE 3.1 (Cont'd.)
1997 APG Field Application Samples

Site (Colony ID)	Sampling Dates
Other APG Edgewood Areas (Cont'd.)	
Youth Center 1 (#303)	5/18, 6/15, 7/18, 7/25, 8/12, 10/4
Youth Center 2 (#388)	6/15, 7/18, 7/25, 8/12, 10/4
Youth Center air (sign level)	5/18, 6/15, 7/18, 7/25, 8/12, 10/4
Youth Center air (ground level)	7/25
Youth Center air (culvert level)	7/25
East Branch Canal Creek 1 (#383)	5/18, 6/15, 7/20, 8/12, 10/4
East Branch Canal Creek 2 (#393)	6/15, 7/20, 8/12, 10/4
East Branch Canal Creek Air	5/18, 6/15, 7/20, 8/12, 10/4
Churchville Reference Site	
CV1 (#43)	6/1, 8/9, 8/24, 9/13
CV2 (#36)	6/1, 8/9, 8/24, 9/13
CV3 (#29)	6/1, 8/9, 8/24, 9/13
CV4 (#44)	6/1, 8/9, 8/24, 9/13
CV5 (#40)	6/1, 8/9, 8/24, 9/13
CV6 (#28)	6/1, 8/9, 8/24, 9/13
CV7 (#38)	6/1, 8/9, 8/24, 9/13
CV (#127)	6/1, 7/10, 8/9
CV (#159)	6/1, 7/10
CV Air	6/1, 7/10, 8/9, 8/24, 9/13

@Hives sampled at G Street site, no bee foraging allowed

caused residues desorbed after 1 minute to be vented; the normal desorption period is 10 minutes. Thus, about 90% of each early season sample was not retained for introduction into the GC/MS. Values reported for May and June dates represent only those contaminants present at the highest levels and are strictly qualitative. They cannot be related to their actual concentrations. The desorption equipment problem was corrected before any of the July - October samples were processed.

Each time that the condo clusters were sampled, an ambient air sample was collected in the same vicinity and over the same duration. The sampling pump and tube were attached to the top of the metal warning signs at a height of about 2 m. Ambient air samples from the free-standing Edgewood colonies were gathered in a similar manner unless otherwise noted. After some elevated levels of contaminants were noted again at the Youth Center, ambient air was collected at three levels for this site -- sign level, ground level and in the throat of a nearby culvert. Because of the limited number of pumps, we were not able to collect an ambient air sample at each colony on every sample date. The dates on which colony ambient air was sampled are incorporated with the colony sample schedule in Table 3.1.

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.

Air samples were 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.

or four-phase Carbotrap 400 tubes, designed to perform better under humid conditions:

-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 about 125 ml/min. This allowed larger volumes of air to be sampled. We achieved far better limits of detection in this manner and were successful in seeing a variety of contaminants.

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.

Since we noted a number of samples in 1996 with small pump volumes, we suspected that the copper tubes were sometimes plugged by debris. In 1997, we added an extra step to our field procedures. As each sorption tube was collected, the copper tube was examined for blockage. On some occasions, the blockage was a propolis plug placed by hive workers. With others, the tube plugged as it was inserted through hive stores (comb, honey or pollen). Pump log sheets were annotated so that the significance of the results could be evaluated.

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.

3.2.3 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.

A system blank or a trip blank was inserted after every two samples. 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 generally covered a range of 35 to 260 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 as shown in Table 3.2.

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%.

Table 3.2
Mass Spectrometer Calibration Parameters

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%

3.2.4 Quantification of TD/GC/MS Analytical Results

One goal of the 1997 activities was quantification of the TD/GC/MS analyses into units other than relative concentrations. Since there are two quantities that comprise a concentration calculation -- 1) the quantity of a contaminant, and 2) the volume of air pumped -- studies were conducted on each quantity to ascertain how their accuracy and precision affected calculated concentrations. First, we constructed a new apparatus to quantitatively calibrate the GC/MS response for known quantities of specific contaminants. In a second study, we

monitored pump performance with flowmeters to determine their constancy and factors that influenced pumping rates.

3.2.4.1 Calibration with Analytical Standards

The quantity of a contaminant represented by an absolute ion abundance was determined by volatilizing and sorbing known quantities of certified analytical standards into Carbotrap tubes. The sorption was conducted in an apparatus designed to mimic, as much as possible, the same conditions found in field sampling (Figure 3.1). The design, testing, and placing into operation of an appropriate calibration system for sorption tubes was a major objective of our 1997 chemical work. Ultrapure helium (Liquid Air) was metered through a 15- mm variable area flowmeter (Cole Parmer, E-03217-50) into a silanized quartz T housed in an insulated block of ceramics. The flow rate was set to 120 mL/min to match the draw rate of the cassette

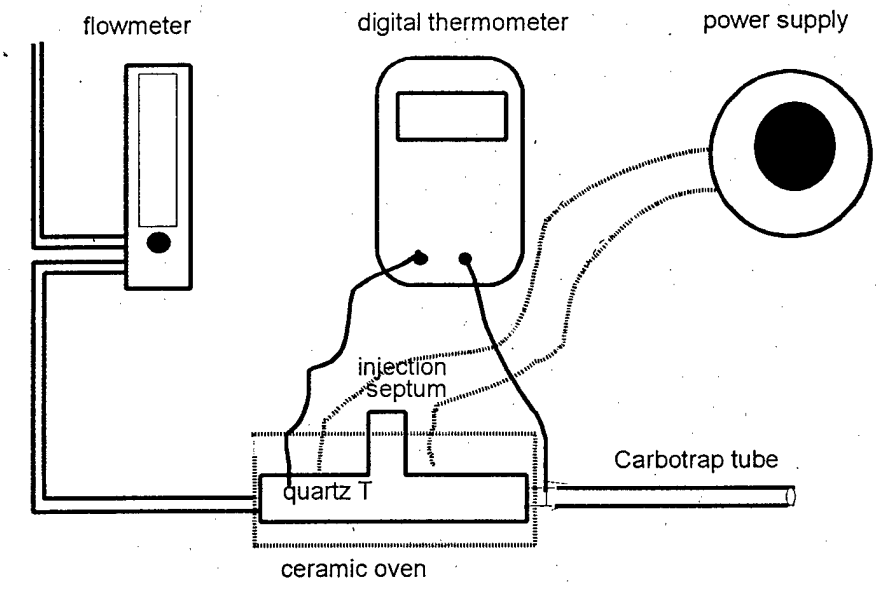


Figure 3.1. Calibration device for the TD/GC/MS sampling tubes. Samples are "pushed" onto the trap by a helium carrier gas flow from the flowmeter, but beyond that, sorbed in an analogous manner to field samples.

pumps. Solutions containing known amounts of volatile organic analytes from EPA Method 524.1 (Supelco, VOC Calibration Standards Kit 4-8804) were injected through a GC septum in a second branch of the quartz T.

A resistance heater was used to volatilize the injected aliquot. The quartz T was maintained at a temperature of 100 °C, adjusted with a Calrad VC-5 variable power supply and monitored via a dual input digital thermometer (Omega HH12), to assure that the entire sample was volatilized. A Carbotrap tube to sorb the sample was attached through Teflon fittings to the final branch of the quartz T that placed it outside the ceramic block. A second temperature probe, outside the oven outlet where the sorption tube connected. This was also monitored by the HH12 and assured that heat from the analyte volatilization process did not unduly warm the Carbotrap tube above temperatures experienced in field sampling.

A series of solutions was made such that 50, 100, 200, 400, 600, 800 and 1000 ng of each analyte could be easily dispensed from a gas-tight syringe into the heated quartz T of the calibration apparatus. An internal standard consisting of 250 ng 1,2-dichlorobenzene-d_4 (Supelco, 4-8948) was present in each final solution of the calibration procedure. Also added, as surrogates, were 250 ng each of fluorobenzene and 4-bromofluorobenzene (Supelco, 4-8083). Ion abundance *vs.* ng analyte data were plotted to evaluate linear response of the MS unit over the quantitation range chosen. Once linearity was confirmed, linear regressions that forced the best fit line through the origin were performed to determine a factor through which absolute ion abundances could be transformed into ng of analyte present in a sample. Because such good linear response was obtained for the majority of samples, we discarded data from occasional samples where non-quantitative transfer of analyte from the syringe occurred.

Good linear response was observed for all eight contaminants quantified. Coefficients of determination (r^2) varied from 1.00 for perchloroethylene (PCE), toluene (Tolu), ethylbenzene (Etbz) and *p*-dichlorobenzene (DCB) to 0.91 for benzene (benz). As an example, data from the PCE calibration are shown in both tabular (Table 3.3) and graphical form (Figure 3.2) below. Replicate measurements at 50 and 200 ng were included to demonstrate reproducibility of the injections.

The regression parameters were subsequently used to generate numeric factors to convert ion per liter (ipl) values into units that are often used to express exposure limits for atmospheric contaminants, namely, parts per trillion (ppt) by volume and ng per cubic meter (ng/m3). Both regression parameters and the corresponding conversion factors are summarized in Table 3.4.

3.2.4.2 Evaluation of Pump Flow Rates

As noted above, two quantities must be measured before a contaminant concentration can be calculated. The first, the quantity of contaminant, was addressed in Section 3.2.4.1. The second, examined here, is the volume of air sampled. Two questions regarding our pumping system were evaluated: 1) How constant were pump flow factors (mL pumped/pump-cycle count) over the sample collection period? and; 2) Does the length of Tygon tubing connecting the sorption tube to the pump affect its flow rate?

Table 3.3

Calibration Data for PCE Quantitation

Analyte Quantity (ng PCE)	Absolute Ion Abundance
50	1,800
100	2,930
200	6,995
400	13,800
600	20,620
800	28,600
1000	34,790

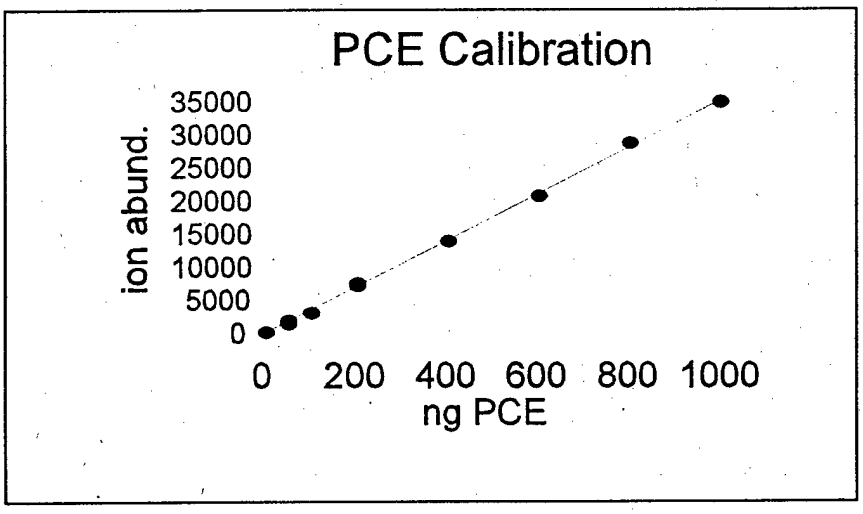


Figure 3.2. Perchloroethylene (PCE) calibration plot

Table 3.4
Regression Parameters and Conversion Factors for TD/GC/MS Quantification

Contaminant	Regression Slope	r^2	A ipl * A = ppt	B ipl * B = ng/m^3
PCE	35.0 (± 0.3)	1.00	0.00420	28.6
TCM	41.9 (± 1.0)	0.99	0.00379	23.9
TCE	39.1 (± 1.5)	0.98	0.00474	25.6
Benz	124 (± 8)	0.91	0.00250	8.06
Tolu	121 (± 1)	1.00	0.00219	8.26
Etbz	104 (± 2)	1.00	0.00221	9.62
DCB	68.3 (± 1)	1.00	0.00243	14.6
Naph	189 (± 4)	0.99	0.00101	5.29

The constancy of pump flow factors was tested by attaching a pumping system (copper tube, sorption tube, 93-cm (3-ft) Tygon tube and constant flow pump as shown in Figure 3.3) to a bubble film flowmeter. A typical example is provided here for Pump M whose flow rates and pump cycle counts were recorded periodically over an 11-hour period (Figure 3.4). The flow rate varied over a range from 175.8 mL/min to 186.6 mL/min. When compared to pump-cycle counts, these yielded pump factors that ranged from 0.368 mL/count to 0.403 mL/count, a variation of about 9% between the two values.

The flow rate imparted by the constant flow pumps proved to be a function of both the length of Tygon tubing used to connect the sorption tube to the pumps and the type of sorption tube -- a three-phase Carbotrap 300 tube versus a four-phase Carbotrap 400. Lower pump flows were observed with very short tube lengths (Figure 3.5). We suspect that a larger pressure drop was created by a pump cycle and led to greater turbulent friction. A standard 93 cm tube was used on all pumps for the 1997 field season. We also noted that flow rates were affected by the type of sorbent tube on which we were pumping. The Carbotrap 300 tubes showed a flow rate that was consistently 7% lower than flow rates through Carbotrap 400 tubes. When sample tubes were desorbed, the type of trap was noted in the instrument log book.

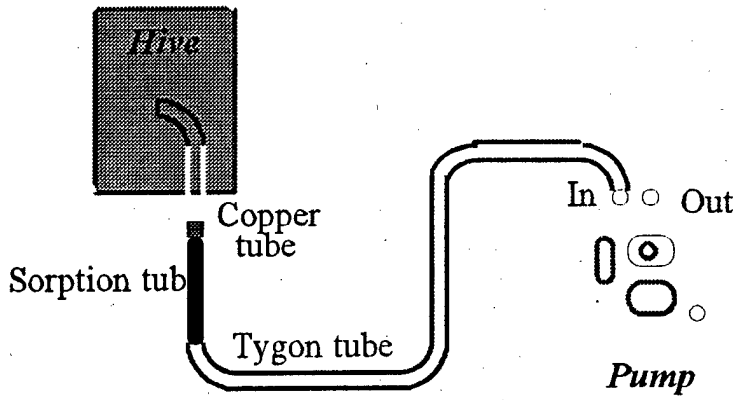


Figure 3.3. Sample pumping system

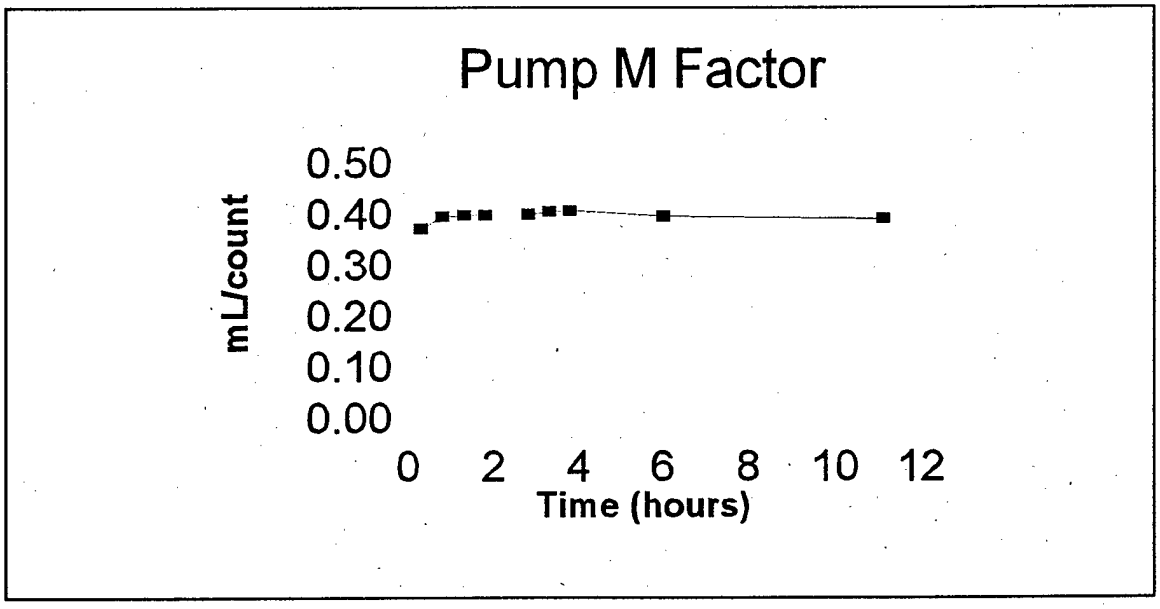


Figure 3.4. Pump factor constancy over 11-hour period

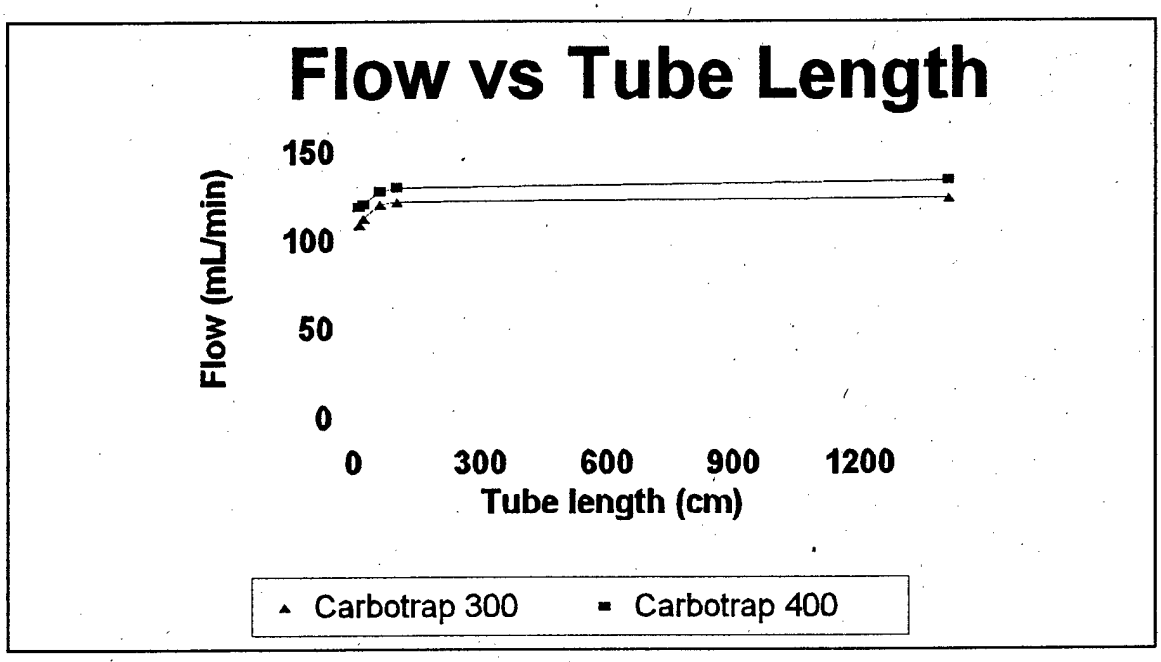


Figure 3.5. Pump flow as a function of Tygon tube length and trap type.

3.2.4.3 Summary of Limitations on Reported Concentrations

Our ability to quantify the concentration of a contaminant in a given sample is limited to an uncertainty of about $\pm 10\%$ for contaminants above 100 ppt (see propagation of error analysis, Appendix B. The major factor that proves most difficult to control is the variation in pump flow rate. As the flow rate changes, so do the pump factors used to compute the volume of air sampled -- the quantity used in the denominator of our concentration calculation. The device built to quantitatively sorb a known amount of analyte gave excellent linear response curves with the exception of benzene. Regular calibration of the GC/MS was done to maintain performance within a $\pm 10\%$ window.

All concentrations contained in this report have been generated from the numeric factors of Table 3.4. While a series of calibration runs were done over the course of the summer, we did not adhere to a strict schedule of running a standard at the start of every set of samples. This was especially true with samples from the latter part of the season. We could tell from the voltages needed to achieve a good tune, that our mass spectrometer's detector filament was close to the end of its service life and that "sample varnish" had built up in transfer lines. Rather than dismantle the instrument, rebuild it and change its operating characteristics for part of the sample set, we chose to run the remaining samples in as efficient a queuing as possible. Even at that, the filament burned out with some samples from tests conducted in Montana still unanalyzed. No significant departures were noted between any of the calibration runs, so we feel confident that the concentrations still fall within a $\pm 10\%$ range.

In the same vein, we retroactively used the numeric factors of Table 3.4 to back-calculate and estimate the concentrations from the 1996 field season. We realize that these numbers must be considered to be estimates of the actual values, but this at least provides an approximation of the real values. This is particularly important because of the relatively high peaks for organics such as PCE that we saw at Old O Field in 1996. We felt justified in doing so because the operating parameters of the mass spectrometer were carefully tuned, standardized against a "surrogate" standard, and monitored before each run. The only factor not accounted for in this process is that of the thermal desorption step. Since that is completely automated, short of changing the program, we believe that variations instilled by this procedure will not be outside the $\pm 10\%$ range. Concentrations noted in the cleaner sites are comparable between the two field seasons when the retroactively calculated 1996 concentrations are compared to the 1997 levels.

With a fully functional calibration apparatus in hand and with a refurbished instrument in 1998, we will assume a regular schedule of calibration standards that includes a wider range of compounds. During the 1997 field season, for example, we noted the presence of both acetophenone and benzaldehyde in samples. These are potential precursors and break down products of tear gas, so it would be pertinent to quantify them in future applications.

Because of interest stimulated by our past reports and presentations, several other laboratories will begin collaborative analytical efforts with us next year. They will be making measurements on hive atmospheres using a direct-sampling, ion-trap mass spectrometer (Oak Ridge National Laboratory-ORNL) or immunochemical techniques (US Environmental Protection Agency-Human Exposure Research Branch). The ORNL investigators also have been working on developing appropriate calibration devices for TD/GC/MS with sorption tubes. In addition, they are one of the primary developers of the actual sorption tubes employed. We have had site visits and conference calls with these scientists, and we have obtained some additional funding for this effort from the Defense Advanced Research Projects Agency. Both UM and ORNL are committed to working together toward thoroughly testing this approach and producing rigorous quality assurance and quality control protocols for these new systems. This will provide an opportunity to conduct interlaboratory comparisons to assure the accuracy of the determinations.

A final comment about the nature of reported concentrations is also warranted. The concentrations reported for hive atmospheres are not necessarily relatable to concentrations that would be encountered in the environment. First, they are time-weighted averages since they are accumulated over an 8- to 24-hour pumping period. They carry no indication of how the contaminant was sorbed over time. It could have been a constant low level or a series of spike episodes. Second, we have no certain knowledge of how the contaminant made its way into the hive -- whether it wafted in from an ambient air load, was carried in by a couple of highly contaminated bees or was sorbed uniformly by the entire foraging population. Third, we do not yet know the efficiency with which various contaminants are transported by honey bees. It is possible that some contaminants are retained to a greater degree than others. Certain

lipophilic compounds may dissolve into the chitin of the bees' exoskeletons while more polar compounds roll off after direct contact.

3.2.5. Trace Element Determinations by ICP-MS

Honey bees and pollen samples were analyzed for trace metals and selected inorganics by inductively coupled plasma-mass spectrometry. Elements determined were Be, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Cd, Cs, Ba, Tl, Pb, Bi and U. Bee and pollen samples were oven-dried in acid-washed, covered glassware at 45°C for about two weeks. They were then homogenized by grinding in a Wiley mill to pass a 20-mesh screen. The dried, ground samples were stored in high-density polyethylene and shipped to USACEHR laboratories at Ft. Detrick, MD for ICP-MS analysis.

A 0.5 gram portion of each sample was transferred to a digestion vessel and 10 mL of trace metal grade nitric acid (Fisher Chemical Co.) was added. The digestion vessel was sealed and placed into a CEM MDS-2000 microwave digestion oven. It was then subjected to the following digestion program:

40% power to 20 PSI in 10 minutes, time at pressure 5 minutes
50% power to 40 PSI in 10 minutes, time at pressure 5 minutes
50% power to 80 PSI in 10 minutes, time at pressure 5 minutes
40% power to 100 PSI in 10 minutes, time at pressure 5 minutes

Following digestion, 40 mL of deionized water were added.

A Hewlett Packard 4500 Inductively Coupled Plasma-Mass Spectrometer was used to determine the trace metal concentrations. Peak intensities were measured against internal standards. A 1 ppm multi-element standard was used as a laboratory control spike. Recovery was evaluated from 1-mL additions of the standard to 0.5 gram dried samples prior to the acid digestion step.

3.3 Chemical Results and Discussion

For purposes of reporting and discussing the results of the chemical sampling, sample locations will be broken into the following groups: Old O Field, J Field, Southern Bush River area, Cluster 3, West Branch Canal Creek, Upper Post area (East Branch Canal Creek, Beach Point, G Street, National Guard Armory, Lauderick Creek and Youth Center) and the Churchville reference site. Since we conducted sampling at many sites during both the 1996 and 1997 field applications, we present some comparative summaries in this report. Only 1997 results are available for the J Field, Southern Bush River and Cluster 3 sites, so no comparisons can be drawn for these three study areas.

This report contains only a summary of the chemical results. For each location we present a table that indicates the most **acute exposure** for each contaminant noted within a hive at a given location and the highest ambient air level that was observed. These are tabulated in columns labeled with **"Max."** Again, it should be emphasized that levels reported are time-weighted averages -- concentrations accumulated over the entire pumping period. In addition, levels reported in the hive are difficult to relate to levels encountered in the ecosystem because of the uncertainties surrounding how many bees were in contact with a contaminant and how efficient the uptake was. An indicator of **chronic exposures** encountered is tabulated in columns labeled **"Mean."** These values represents composite means over all hive or ambient air samples gathered at a site. A complete database of chemical results on a hive-by-hive basis for each date is available in Excel format on the CD ROM that accompanies this report. Unless otherwise noted, concentrations of organic contaminants are given in parts per trillion (by volume).

3.3.1 Volatile and Semi-Volatile Organic Contaminants

Hive atmospheres during the 1997 field season contained the same general types of volatile and semi-volatile organic contaminants as were seen in 1996. Chlorinated hydrocarbon solvents found included perchloroethylene (PCE), trichloroethylene (TCE) and tetrachloro-methane (TCM). Hexachloroethane (PCA), the smoke obscurant component found only at Old O Field in 1996, was completely absent from hive atmospheres. Dichlorobenzene (DCB), a solid pesticide often used to repel moths, was seen in many locations. The BTEX group of petroleum and gasoline residuals was almost ubiquitously present. We quantified benzene (Benz), toluene (Tolu) and ethylbenzene (Etbnz) from the BTEX group. The xylenes co-eluted with major terpene peaks and were not easily quantified. We also found naphthalene (Naph), a component of diesel, present at most sites. Finally, we noted the presence of acetophenone (AcPh) and benzaldehyde (Bnzald), both of which are associated with tear gas.

PCE and TCE were often at levels significantly in excess of ambient air, suggesting they were contacted as an organic film on standing water. TCM, BTEX and Naph occurred in ambient air at levels near those in the hive atmospheres. Since BTEX and naphthalene are known components of gasoline and diesel exhaust, they likely originate from vehicles or equipment in the vicinity of our sampling units.

3.3.1.1 Old O Field

The most dramatic result from the 1997 field application was a reduction in the bioavailable chlorinated hydrocarbons at Old O Field, achieved through installing an earthen cap over the body of the landfill and eliminating other likely exposure routes (Table 3.5). PCE levels were so high during the 1996 season that queens disappeared from six of twelve hives located around the landfill body. There were no queen losses in 1997 and levels of chlorinated hydrocarbons were an order of magnitude less with respect to both acute (maximum) and chronic (mean) exposures. In 1996, we observed a PCE level of 2800 ppt in

Old O Field Hive JZ1 on September 2. The highest PCE value recorded during the 1997 field season, in comparison, was only 115 ppt in Hive OFS2 on July 15. The 1997 mean PCE concentration was 21 ppt compared to 207 ppt in 1996. Because the 1997 mean hive level for PCE (21 ppt) is close in magnitude to the mean ambient air level (28 ppt), much of the remaining low level exposure is probably a result of soil vapor releases. PCE was trapped at measurable levels in every ambient air sample at Old O Field.

The pattern of PCE levels was not evenly distributed throughout the season (Figure 3.6). The highest 1997 measurements were clustered in the latter half of July. Data from the weather station and the bi-directional flight activity counters support a scenario in which standing puddles of rain provided a point of contact for foraging bees to bring PCE back to the hive. A dip in flight activity in late July is visible in the Old O Field behavior data and could be attributable to a chemical stress imposed by PCE exposure.

Mean values for other contaminants measured in both years showed signs of reduction in 1997 compared to 1996 levels, though none as dramatically as PCE. The 1997 BTEX maxima and means, for example, were about one-third those of the 1996 means. TCM and DCB showed slight drops in mean values, but their maximum concentrations were as high or higher than in 1996.

BTEX contaminants were distributed more consistently across the summer field season than PCE (Figure 3.7), consistent with a soil vapor release or an ongoing release of gasoline and diesel exhaust from vehicles or equipment in the area that utilize internal combustion engines. TCM appeared throughout the field season while DCB was distributed more like the PCE pattern.

Old O Field continues to be among the sites that exhibit the highest bioavailable and ambient air loads of contaminants. It possessed the highest ambient air levels measured on a single day for PCE (81 ppt), benzene (165) and toluene (625). The highest hive level of ethylbenzene (512 ppt) was also recorded here. The highest average levels (across the entire field season) for PCE, DCB, benzene and ethylbenzene were noted at Old O field as well as the greatest mean levels in hive atmospheres for benzene and ethylbenzene.

Table 3.5

Comparison of 1997 vs 1996 Hive (and Ambient Air) Levels of Old O Field Volatiles and Semivolatiles

Values are ppt by volume

Compound	1997 Max	1996 Max	1997 Mean	1996 Mean
TCM	138	113	20	27
(air)	(82)	(37)	(25)	(24)
TCE	172	188	13	19
(air)	(10)	(15)	(6)	(8)
PCE	115	2814	21	207
(air)	(81)	(50)	(28)	(38)
DCB	28	30	5	10
(air)	(21)	(6)	(7)	(3)
Benzene	240	710	66	180
(air)	(165)	(197)	(78)	(113)
Toluene	1074	3197	274	605
(air)	(625)	(323)	(227)	(206)
Ethylbenzene	512	219	58	38
(air)	(146)	(220)	(48)	(4)
Naphthalene	10	142	5	19
(air)	(17)	(4)	(6)	(1)

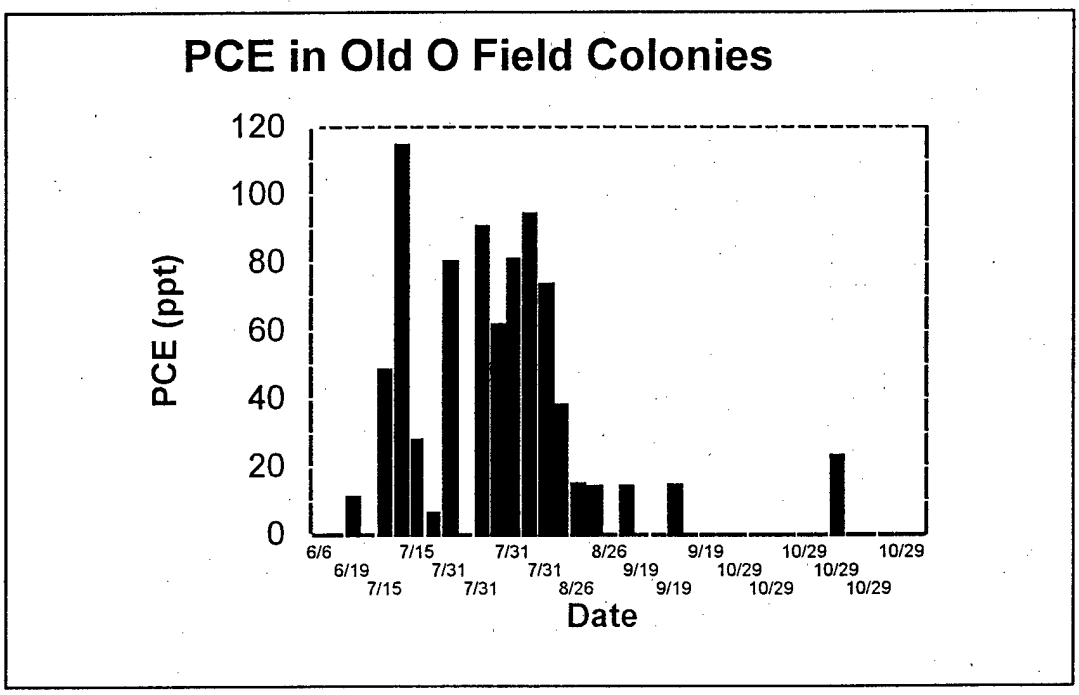


Figure 3.6. Seasonal distribution of PCE levels in Old O Field colonies. Samples per date: 2-6/6; 2-6/19; 4-7/15; 8-7/31; 4-8/26; 6-9/19 and 12-10/29

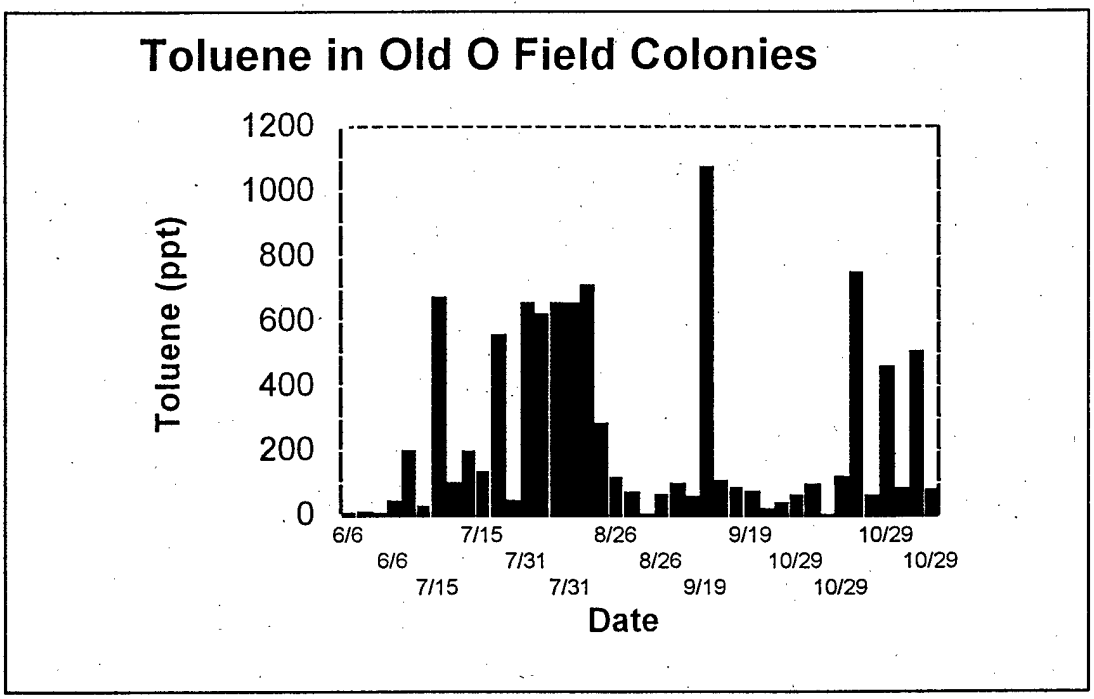


Figure 3.7. Seasonal distribution of toluene levels in Old O Field colonies. Same sample distribution as previous figure.

3.3.1.2 J Field

Contaminant levels found at J Field were comparable to those at Old O Field in most respects. The hive maximum for TCE at J Field was about 20% above that of Old O Field during the pre-capping period of 1996. Ambient air levels were even more pronounced, about double that measured at Old O Field. It did seem to drop below detection limits in our October sampling period, suggesting that reduced volatility at lower temperatures could have lowered vapor release from the soil.

Table 3.6
1997 J Field Hive (and Air) Volatiles and Semivolatiles
Values are ppt by volume

Compound	1997 Max	1997 Mean
Carbon tetrachloride (TCM)	58	10
(air)	(78)	(20)
Trichloroethylene (TCE)	224	24
(air)	(36)	(18)
Perchloroethylene (PCE)	118	23
(air)	(38)	(23)
p-Dichlorobenzene (DCB)	45	3
(air)	(23)	(6)
Benzene (Benz)	170	55
(air)	(160)	(55)
Toluene (Tolu)	1786	206
(air)	(285)	(163)
Ethylbenzene (Ethbnz)	305	36
(air)	(45)	(32)
Naphthalene (Naph)	135	18
(air)	(21)	(8)

BTEX compounds were present in both hives and ambient air at J Field. The maximum toluene concentration in a hive atmosphere sample, 1786 ppt, was much higher than in any ambient air sample, suggesting a different source media, such as an oily film on standing water. Most hives had measurable BTEX residues on any sampling day, a testimony to the ubiquitous nature of these contaminants. No exceedingly alarming levels were seen for any contaminant, just a background with a strong presence of TCE and PCE.

J Field was the site where the highest single sample concentrations were noted for TCE and naphthalene in hive atmospheres, and DCB in ambient air. Mean hive levels of TCE, PCE and napthalene and air levels of TCE were also higher than at any other APG site in 1997.

3.3.1.3 Bush River

The Bush River sites are divided into three subgroups based on their locations - colonies placed within the secured area, colonies placed in the Cluster 3 area and colonies placed outside the fence along the southern boundary of the secured area. Because colonies were widely spaced in these study sites, the location of each maximum reading is noted in the middle column of the tables below that summarize the 1997 field application results. Sampling locations are designated by a two-letter site code and a number for the specific location (e.g. BR10) to indicate the colony placement. Each hive is designated by an H plus the hive number (e.g., H2=Hive 2). "Air" is used to specify whether it was a specific hive or ambient air sample that contained the maximum.

Table 3.7 holds concentrations measured in hive atmospheres and ambient air at five locations within the secured area of Bush River (BR1 through BR5). BR1 was situated on the 22nd Street Landfill. BR2 was within the Radioactive Material Disposal Facility yard. BR3 was adjacent to Bush River in the Toxic Gas Yard Ton-Container Steamout Site. BR4 was at the 26th Street Disposal site, while BR 5 was near the Open Storage Areas.

All five sites within the secured area showed measurable levels of a suite of organic contaminants that were consistent with findings in the Southern Bush River Remedial Investigation Report (RI). BR1 exhibited the highest benzene levels among this subgroup. Hive 314 at BR2 held the highest concentrations of DCB and ethylbenzene. BR3 was associated with the subgroup high for TCE. BR4 held the subgroup maximum levels of PCE (in air). The RI report noted that PCE levels in groundwater below BR4 exceeded the maximum contaminant limit (MCL) for tap water. BR5 headed the Secured Area subgroup with readings for TCM, toluene and naphthalene (in air). The RI report stated that TCM levels in ground water below the Open Storage Areas of BR5 exceeded tap water MCL's.

Table 3.7
1997 Bush River Secured Sites
Hive (and Air) Volatiles and Semivolatiles
Values are ppt by volume

Compound	1997 Max	Hive ID	1997 Mean
Tetrachloromethane (TCM) (air)	135 (47)	BR5 H343 10/31/97	12 (6)
Trichloroethylene (TCE) (air)	50 (44)	BR3 H341 7/29/97	10 (10)
Perchloroethylene (PCE) (air)	43 (76)	BR4 air 10/8/97	13 (20)
p-Dichlorobenzene (DCB) (air)	25 (7)	BR2 H314 10/31/97	5 (3)
Benzene (Benz) (air)	214 (147)	BR1 H345 10/31/97	57 (76)
Toluene (Tolu) (air)	1042 (322)	BR5 H343 8/29/97	305 (182)
Ethylbenzene (Etbnz) (air)	131 (65)	BR2 H314 10/31/97	31 (28)
Naphthalene (Naph) (air)	26 (79)	BR5 air 8/29/97	7 (13)

Three levels measured within the Secured Area subgroup emerged as 1997 field season maxima - two as single-sample highs, the other a seasonal-mean high. First, the ambient air level of naphthalene (79 ppt) on September 29th at BR5 was higher than in any other air sample. Second, the ambient air level of TCE (44 ppt) seen at the BR3 site on October 8th was also a 1997 season high for APG. Third, the subgroup mean for toluene in hive atmospheres (305 ppt) was also the highest seen among all APG locations. It should be noted, however, that toluene is seen almost everywhere as it is a common octane-booster in unleaded fuels. The mean hive atmosphere level in our off-base, reference site at Churchville was similar in magnitude at 283 ppt during the 1996 sampling season.

The second Bush River subgroup represents three colony pair placements in and around the Cluster 3 area. BR6 was outside the fence at the southwest corner, not too distant from an open lot in which power poles were stored on racks. BR7 was inside the fenced area about 100 feet from the southeast burn. Finally, BR8 was located near the bottom of the drainage within Cluster 3 and next to the northeast burn pile containing mask buckles, mask glass and snap closures.

A summary of the maxima and mean chemistry results is contained in Table 3.8. Five of the eight contaminants had their maximum values within the subgroup registered at the burn pile site, BR8, on September 19th in the sample from Hive 334. TCM, PCE, DCB, toluene and ethylbenzene all reached their highest subgroup levels here. The PCE and DCB levels, in fact, were not only the subgroup highs, but also the highs across all sites for the entire 1997 APG sample season. A maximum of 158 ppt was recorded for PCE in that sample. This is well below the 2814 ppt episode at Old O Field that was associated with queen loss in half the colonies during the 1996 field season. On the other hand, the DCB reading of 91 ppt is the greatest concentration yet measured during the bee project.

Means for two categories of contaminants from Cluster 3 samples were also the highest observed across all APG site for the 1997 season. Eight of twenty Cluster 3 hive samples had DCB levels in excess of 10 ppt. When these are averaged with the single high of 91 ppt noted above, the mean hive atmosphere level was 10 ppt. The second contaminant that was seen at its highest mean value across all site samples was naphthalene levels in ambient air. This is not unexpected because of the nearness of a pole storage yard located close to the Cluster 3 sites. The poles were stored in piles above ground and had their bottom ends treated with creosote as a preservative. Since creosote is typically 85% polycyclic aromatic hydrocarbons, and the bulk of those napthalenes, it is not surprising that an elevated background of naphthalene was observed here.

The final Bush River subgroup is comprised of three colony pair placements outside the fencing of the secured area in the Kings Creek Chemical Disposal Area (Cluster 15) and a fourth pair in the Tapler Point Disposal Area (Cluster 18). BR9 is located at the actual Chemical Disposal Site (Site 1 in the RI report). Drummed chemical containers and disposal activities at this site are thought to date from the period between 1920-1940. BR10 and BR11 enclose a small inlet that is part of the 30th Street Landfill site (RI Site 2). Aerial photographs reveal it was active during the late 1960's or early 1970's. BR12 is in the Tapler Point Dredge Material Site, a forested location on the west side of the point and close to another inlet of the Bush River peninsula. This area received dredge material during the 1940's when channel work was performed for the Bush River Dock.

Table 3.8
1997 Bush River Cluster 3 Sites
Hive (and Air) Volatiles and Semivolatiles
Values are ppt by volume

Compound	1997 Max	Hive ID	1997 Mean
Tetrachloromethane (TCM) (air)	71 (68)	BR8 H334 8/19/97	10 (19)
Trichloroethylene (TCE) (air)	21 (16)	BR7 H329 10/8/97	4 (4)
Perchloroethylene (PCE) (air)	158 (35)	BR8 H334 8/19/97	11 (15)
p-Dichlorobenzene (DCB) (air)	91 (13)	BR8 H334 8/19/97	10 (4)
Benzene (Benz) (air)	114 (157)	BR7 air 10/8/97	39 (67)
Toluene (Tolu) (air)	1641 (1045)	BR8 H334 8/19/97	227 (284)
Ethylbenzene (Etbnz) (air)	470 (90)	BR8 H334 8/19/97	44 (34)
Naphthalene (Naph) (air)	54 (27)	BR6 H382 10/8/97	7 (15)

A summary of the subgroup maxima and means are provided in Table 3.9. No subgroup maxima were found at BR12, a result consistent with the site history presented above -- the contaminant loading to this site was less intense than at the others. Ambient air at BR9 contained the highest subgroup levels for both PCE and naphthalene. BR10 ambient air held the highest TCM level noted. Since higher readings were found in the ambient air, as opposed to inside the hives, a soil vapor release is implicated.

Table 3.9
1997 Bush River Southern Boundary Sites
Hive (and Air) Volatiles and Semivolatiles
Values are ppt by volume

Compound	1997 Max	Hive ID	1997 Mean
Tetrachloromethane (TCM) (air)	22 (68)	BR10 air 10/8/97	2 (15)
Trichloroethylene (TCE) (air)	59 (22)	BR11 H344 10/8/97	6 (4)
Perchloroethylene (PCE) (air)	45 (56)	BR9 air 10/8/97	4 (8)
p-Dichlorobenzene (DCB) (air)	13 (5)	BR9 H384 @10/31/97	4 (1)
Benzene (Benz) (air)	282 (100)	BR10 H385 10/8/97	31 (57)
Toluene (Tolu) (air)	3156 (282)	BR10 H385 7/29/97	285 (140)
Ethylbenzene (Etbnz) (air)	72 (431)	BR9 H384 @10/31/97	16 (18)
Naphthalene (Naph) (air)	39 (51)	BR9 air 8/29/97	5 (8)

Three 1997 APG-wide maxima were observed in the Southern Boundary subgroup - hive maxima for benzene and toluene and the ambient air maximum for ethylbenzene. Interestingly, the benzene and toluene maxima were recorded in the same hive at the BR10 site, number 385, but on different dates. The high benzene level of 282 ppt was recorded on October 8th while the high toluene mark of 3156 ppt was observed on July 29th. This suggests that standing water with a fuel film was probably present after precipitation events. Despite these levels being the APG-wide highs, BTEX contaminants are found in civilian settings at this magnitude all the time. Samples collected at our reference farm site near

Churchville showed 1996 maximum levels of 275 ppt and 1422 ppt for benzene and toluene in hive air, respectively. The ambient air level maximum for APG during 1997 was measured at 431 ppt on September 29th at BR9. Again, this is a BTEX contaminant is not particularly significant compared to levels encountered in civilian settings where fuel spills have occurred.

No Boundary Subgroup organic contaminant level was consistently high enough to appear on the APG-wide list of highest mean values.

3.3.1.4 West Branch Canal Creek

The West Branch Canal Creek site has been monitored by the honey bee system for three years now. It was the initial site employed for the 1995 pilot study. Contaminants noted in 1995 were scarce since we had not yet determined how much air needed to be pulled through the sorption traps to capture a measurable quantity. Our 1996 sampling did find some contaminants present, but the West Branch site had among the lowest levels observed among our APG sites last year.

The 1997 chemical results (Table 3.10) uphold this same conclusion. The maximum levels of contaminants encountered at West Branch Canal Creek levels are a fraction of the highs elsewhere except for TCM. A stand-alone nucleus colony that was placed on the ground near the electronic condos displayed a 1997 maximum hive concentration of 209 ppt on July 20th. Other values near 50 ppt were observed in three other hive samples. Taken together, the overall West Branch Canal Creek 1997 mean for hive TCM was also the highest noted (27 ppt).

As was true with the 1996 study, the contaminant levels measured at West Branch Canal Creek were generally lower than those found at our off-base Churchville farm site. The mean level of all contaminants except TCM and ethylbenzene were lower at Canal Creek than at the farm. The mean level of TCM at the farm was 18 ppt; that for ethylbenzene was nearly identical -- 20 ppt at Canal Creek versus 19 ppt at the farm.

Table 3.10

Comparison of 1997 vs 1996 Levels of
West Branch Canal Creek Volatiles and Semivolatiles
Values are ppt by volume

Compound	1997 Max	1996 Max	1997 Mean	1996 Mean
TCM (air)	209 (66)	31 (47)	27 (15)	10 (24)
TCE (air)	20 (22)	34 (12)	4 (10)	4 (4)
PCE (air)	16 (26)	44 (18)	7 (11)	11 (14)
DCB (air)	34 (2)	13 (4)	4 (1)	2 (1)
Benzene (air)	215 (112)	391 (89)	28 (42)	108 (92)
Toluene (air)	777 (212)	856 (303)	94.4 (62.5)	373 (239)
Ethylbenzene (air)	186 (19)	35 (9)	20 (6)	14 (8)
Naphthalene (air)	49 (6)	16 (4)	4 (2)	4 (2)

3.3.1.5 Edgewood Area Youth Center

Chemical residues measured at the Youth Center were separated out for this year's annual report after samples from the Youth Center consistently had among the higher levels noted in the Edgewood residential and office area. Had the Youth Center not been separated, it would be listed as the location within this site group where the maximum hive levels appeared in four

Table 3.11

Comparison of 1997 vs 1996 Levels of Youth Center Volatiles and Semivolatiles

Values are ppt by volume

Compound	1997 Max	1996 Max	1997 Mean	1996 Mean
TCM (air)	68 (21)	69 (53)	26 (8)	39 (49)
TCE (air)	50 (1)	355 (67)	13 (0.4)	96 (39)
PCE (air)	18 (22)	158 (148)	10 (10)	53 (84)
DCB (air)	6 (6)	23 (20)	2 (2)	9 (13)
Benzene (air)	77 (131)	468 (382)	32 (59)	206 (476)
Toluene (air)	233 (345)	861 (593)	140 (166)	364 (417)
Ethylbenzene (air)	47 (74)	58 (42)	20 (34)	27 (29)
Naphthalene (air)	5 (15)	26 (8)	2 (6)	8 (8)

out of the eight contaminants and three of the ambient air contaminants. Because there was no APG history to suggest why comparatively high levels were found near the Youth Center, an effort went into checking whether a culvert discharging into the adjacent ditch was the source of these contaminants. Thus, ambient air was checked at three positions on some days -- at the standardized sign height (about 2 meters above ground level), at the ground level next to the hive placement and in the mouth of the culvert. A summary of the maxima and means measured at the Youth Center site during 1997 is compiled in Table 3.11.

Despite the fact that the Youth Center has among the higher hive levels of contaminants, the levels observed in more hazardous locations at APG eclipse these levels. Samples from Old O Field, J Field and some of the Bush River Area are substantially higher. Nevertheless, we deemed it worth some effort to determine the source of the Youth Center contaminants. The culvert seemed a likely target so we conducted a round of sampling on July 25th (Table 3.12).

Table 3.12

Multilevel Air Sampling at the Edgewood Youth Center
July 25, 1997

Values are ppt by volume

	Hive 303	Hive 388	Ambient Air Sign Level	Ambient Air Ground Level	Ambient Air Culvert Mouth
TCM	68	49	18	4	26
TCE	6	5	5	4	7
PCE	18	13	22	19	20
DCB	5	4	6	6	6
Benz	58	77	131	86	143
Tolu	323	233	345	264	323
Etbz	47	41	74	51	59
Naph	1	2	15	13	12

With the exception of TCM, the culvert was not higher in contaminant levels than the ambient air measured at sign level. Were the culvert a local point source for contaminant emissions, a much larger concentration would have to be present in the restricted space of its mouth. Thus, the culvert seems an unlikely source at this point. Furthermore, the levels of TCM found in the hives are twice as high as in the air, suggesting either an accumulation in the hive from the air or a different route/media of entry, such as a contaminated pool of standing water.

3.3.1.6 Other Upper Edgewood Area Sites

Five other sampling sites in the Upper Edgewood Area were monitored with honey bees for a second year -- Lauderick Creek, East Branch Canal Creek, G Street, the National Guard Armory and Beach Point. Results of the chemical survey are summarized in Table 3.13.

Table 3.13

Comparison of 1997 vs 1996 Levels of
Other Upper Edgewood Area Volatiles and Semivolatiles
Values are ppt by volume

Compound	1997 Max	1996 Max	1997 Mean	1996 Mean
TCM (air)	56 (83)	98 (97)	13 (37)	20 (44)
TCE (air)	18 (10)	108 (24)	5 (4)	15 (4)
PCE (air)	41 (45)	214 (134)	8 (16)	37 (45)
DCB (air)	15 (21)	40 (19)	4 (4)	17 (6)
Benzene (air)	121 (123)	478 (384)	33 (49)	152 (265)
Toluene (air)	478 (362)	1847 (644)	163 (121)	442 (260)
Ethylbenzene (air)	42 (36)	58 (48)	12 (16)	19 (17)
Naphthalene (air)	5 (12)	101 (43)	2 (6)	13 (9)

Levels of TCM at these sites provided both the 1997 APG maximum for a single ambient air sample and the overall seasonal ambient air TCM mean. The maximum level of TCM of 83 ppt occurred near the National Guard Armory on August 12th. The high seasonal ambient air mean for TCM resulted from significant contributions from each of the five, individual sites -- 59 ppt near the National Guard Armory, 50 ppt at East Branch Canal Creek, 32 ppt at Beach Point, 22 ppt at G Street, and 18 ppt at Lauderick Creek.

3.3.1.7 Churchville Reference Site

As in 1996, a farm near Churchville, MD was used as an off-base reference site to provide a snapshot of regional levels of contaminants that do not originate at APG. The farm's owner, David Simmons, is a chemical engineer/hobbyist beekeeper well able to assure the security and integrity of our instrumented hives. The farm is approximately 10 miles to the northwest of the Edgewood area, generally upwind of APG and local industry. None the less, the same suite of contaminants is present at the Churchville site, often at levels comparable to those found at APG.

A comparison between 1996 and 1997 levels of volatiles and semivolatiles at Churchville is summarized in Table 3.14. The large spike in TCE that was observed in two hives during 1996 was not repeated in 1997. There was, however, a PCE maximum in 1997 about twice as high as that noted the year before. BTEX levels were greater in 1996 for hive atmospheres, but higher in ambient air during the 1997 field season.

Tables 3.15 and 3.16 compare contaminant levels measured at APG and those at the farm. In Table 3.15, the maximum hive and ambient air concentration recorded at APG is listed in the second column, hive level on top and air level in parentheses below. The third column notes the APG location where both the hive and air highs were observed. The fourth column holds the highest concentration seen at the Churchville site over our two years of field work. Finally, the last column indicates the year in which the Churchville high was observed.

Note that Churchville has been the site of the largest TCE and naphthalene levels in hive atmospheres. It also points out that the APG maxima are typically associated with sites where known contamination histories exist. The only maximum value on the list from a location other than a disposal site is the high TCM near the National Guard Armory. The remainder come from Old O Field, J Field and Bush River -- settings with high probabilities for significant contaminant availability. What is surprising is that contaminants levels at these known hazardous sites are only a few times higher than the levels seen on an ordinary farm.

Table 13.16 holds analogous comparative information for APG and Churchville except, in this case, the values listed are the means for the entire sampling season. The same general conclusions can be drawn here, too. The highest mean levels at APG are usually associated with Old O Field, J Field and Bush River sites. The differences between APG means and Churchville means are even less pronounced than the relative maxima. With respect to hive

means, only ethylbenzene is not within a factor of two, and Churchville has the higher average TCE level. All ambient air levels are within a factor of two except where Churchville has the higher ambient means for DCB, benzene and ethylbenzene. If labels were not provided with a data set, one would be hard pressed to know whether it came from one of the "dirty" sites at APG versus the Churchville farm. This is not intended to say there are no health concerns with the levels at APG, just because they also are seen at the farm. A more prudent statement

Table 3.14

Comparison of 1997 vs 1996 Levels of Churchville Volatiles and Semivolatiles

Values are ppt by volume

Compound	1997 Max	1996 Max	1997 Mean	1996 Mean
TCM (air)	66 (15)	79 (52)	16 (13)	18 (34)
TCE (air)	21 (18)	2564 (17)	5 (9)	183 (6)
PCE (air)	183 (48)	70 (36)	16 (24)	18 (26)
DCB (air)	16 (65)	23 (12)	4 (32)	5 (5)
Benzene (air)	86 (110)	275 (106)	37 (72)	67 (186)
Toluene (air)	409 (896)	1422 (328)	101 (472)	283 (204)
Ethylbenzene (air)	72 (274)	74 (22)	19 (140)	18 (17)
Naphthalene (air)	50 (13)	253 (12)	6 (7)	19 (1)

would be that farmers need to exercise good management practices with agrochemicals since they can lead to significant fluxes of organic contaminants into the hive and possibly food webs.

Table 3.15

Comparison of 1997 APG Versus Churchville Hive (and Air) Maxima for Volatiles and Semivolatiles

Values are ppt by volume

Compound	1997 APG Max	APG Site	Churchville Max	Year
TCM (air)	209 (83)	Canal Ck (Guard Armory)	79 (52)	1996 (1996)
TCE (air)	224 (44)	J Field (Bush River 3)	2574 (18)	1996 (1997)
PCE (air)	158 (81)	Cluster 3 (BR8) Old O Field	183 (48)	1997 (1997)
DCB (air)	91 (23)	Cluster 3 (BR8) (J Field)	23 (65)	1996 (1997)
Benzene (air)	282 (165)	Bush River 10 (Old O Field)	275 (110)	1996 (1997)
Toluene (air)	3156 (625)	Bush River 10 (Old O Field)	1422 (896)	1996 (1997)
Ethbenz (air)	512 (431)	Old O Field (Bush River 9)	74 (274)	1996 (1997)
Naph (air)	135 (79)	J Field (Bush River 5)	253 (13)	1996 (1997)

Table 3.16

Comparison of 1997 APG Versus Churchville Hive (and Air) Means of Volatiles and Semivolatiles

Values are ppt by volume

Compound	1997 APG Mean	APG Site	Churchville Mean	Year
TCM (air)	27 (37)	Canal Ck (Up Edgewood)	18 (34)	1996 (1996)
TCE (air)	24 (18)	J Field (J Field)	183 (9)	1996 (1997)
PCE (air)	23 (28)	J Field Old O Field	18 (26)	1996 (1996)
DCB (air)	10 (7)	Cluster 3 (Old O Field)	5 (32)	1996 (1997)
Benzene (air)	66 (78)	Old O Field (Old O Field)	67 (186)	1996 (1996)
Toluene (air)	305 (284)	Bush R. Secured (Cluster 3)	283 (472)	1996 (1997)
Ethbenz (air)	58 (48)	Old O Field (Old O Field)	19 (140)	1997 (1997)
Naph (air)	18 (15)	J Field (Cluster 3)	19 (7)	1996 (1997)

3.3.1.8 East Missoula Experiments in Contaminant Transport by Bees

Two sets of experiments were undertaken in Missoula to demonstrate a mechanism through which organic contaminants can be transported from the ecosystem to the hive. The East Missoula bee yard was used for this study so that only four colonies of bees (and not our entire research and development apiary) would be exposed to the organic contaminants we dispensed.

One mechanism we have suggested to explain the high levels of contaminants captured in hive atmosphere samples has been that bees contact the substances when they visit standing pools of water. Thus, we trained four colonies of bees to use watering stations that we scented with anise as an odor attractant. Once the bees were habituated to using these water stations, they were subsequently dosed with a small amount of PCE in one trial and naphthalene flakes in the second trial. In both instances, the contaminants showed up at our sorption tube pumping locations.

The water stations were fabricated out of 8" glass pie plates that were partially filled with washed sea sand and set at a slight angle. A separatory funnel was positioned at each station such that a slow drip delivered fresh water beneath the sand through Tygon tubing. As the anise scented water accumulated, a standing pool was established that had a shallow sand slope descending into it -- a good access route for bees.

Background runs were performed for two days prior to release of the contaminant. Then hive and ambient air samples were collected daily for six days after release. In the case of PCE, five drops of the neat fluid were added with a Pasteur pipette to the sand above the water line. With naphthalene, 0.065 grams of flakes were distributed on top of the sand near the water line. The chemical results of the study are tabulated numerically in Table 3.17 and presented graphically in Figures 3.8 and 3.9.

Table 3.17
East Missoula Contaminant Transport Results

		PCE	PCE	Naph	Naph
Type	Run	Hive	Air	Hive	Air
Background	Run 1/bg	0	0	0	0
Background	Run 2/bg	0	0	0	0
w/PCE	Run 3	256.6	170.5	0	0
w/PCE	Run 4	428	172.9	0	0
w/PCE	Run 5	151.3	232.8	0	0
w/Naph	Run 6	146.4	230.9	50.3	169.3
w/Naph	Run 7	50	72.7	8.2	9.7
w/Naph	Run 8	31.2	48.5	5.25	8.5

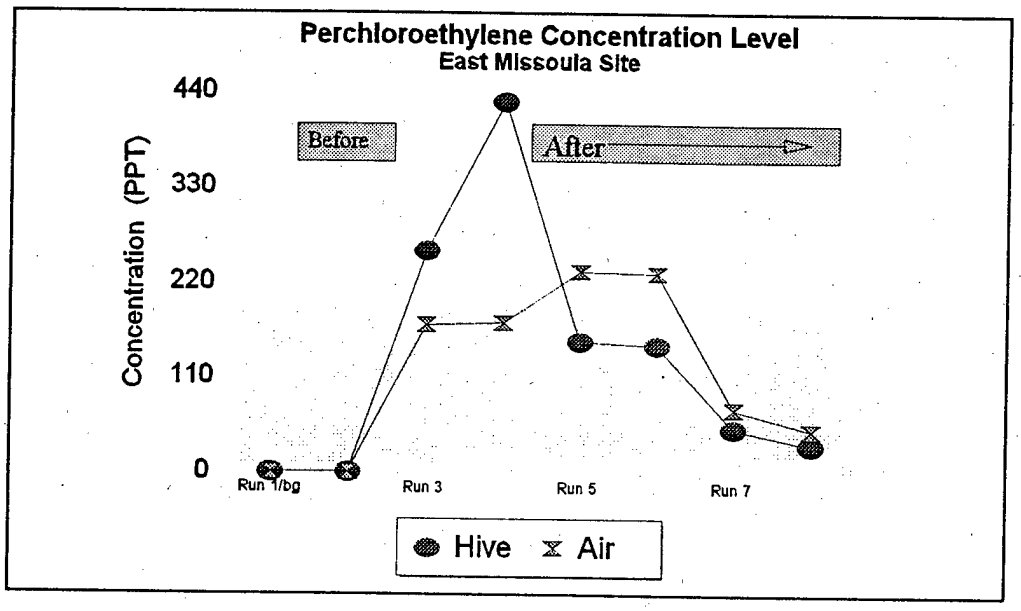


Figure 3.8. PCE transport study

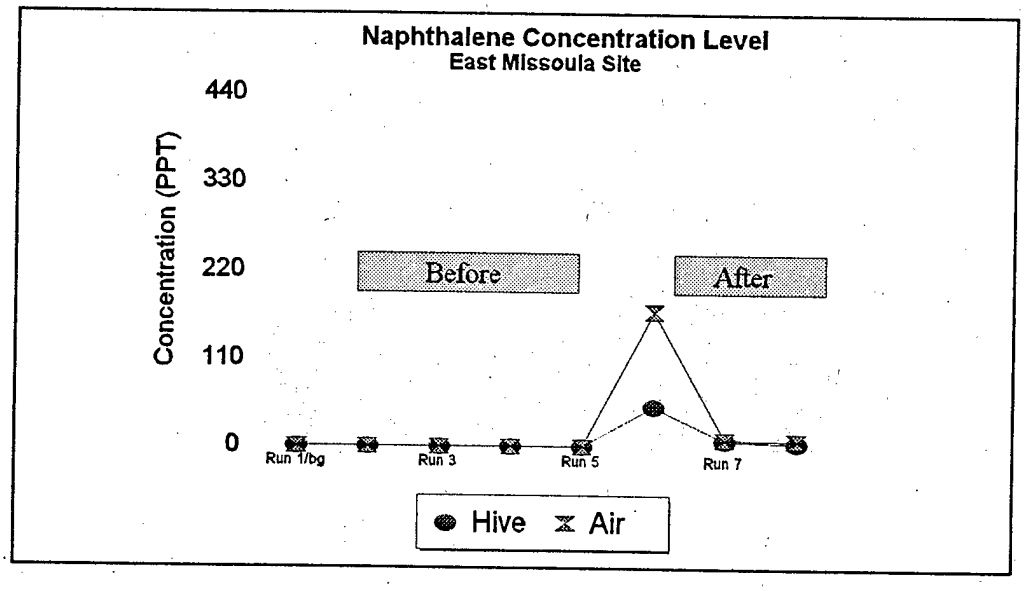


Figure 3.9. Naphthalene transport study

It is immediately apparent with the PCE experiment that a liquid organic material is more readily transportable by the bees. PCE formed an oily film in the water that the foraging bees came into contact with and transported back to the hive. It was seen during the first possible sampling period at a level well above its concentration seen in the ambient air. After three days, it appears that the amount in hive air dropped off and then paralleled that in ambient air. This would suggest that the bees were no longer contacting the liquid film, but the ambient air was diffusing into the hive with some resistance.

The solid naphthalene took a longer time to appear in our chemical sampling, testimony to its lower volatility and that it probably did not form an immediate liquid film for the bees to contact. In fact, the only napthalene we see is at a level lower than in ambient air, suggesting again that the outside air is diffusing into the hive box with some resistance. Transport of solid naphthalene was not likely since the flakes too large to be directly transported by the bees.

3.3.2 Trace Element Contaminants

Trace element analysis of bees and pollen from the 1997 season were conducted samples which had been slowly dried over a two-week period in a 45 °C oven to minimize losses of volatile elements such as arsenic and lead. They were then ground to an 80-mesh size and packaged for ICP-MS determinations at USACEHR laboratories in Fort Detrick, MD. Elements determined were Be, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Cd, Cs, Ba, Tl, Pb, Bi and U. Among this list, there was no evidence for the presence of Be, Bi, Cs, Tl, or U at any of the sites.

Because the Bush River Study Area had not been previously surveyed for trace element contaminants and since these sites are somewhat more widely spaced than colonies in other APG locations, a more detailed summary of the trace element results is provided in this report for these twelve sites. Table 3.18 holds trace element levels, reported as parts per million by weight (ppm), found in honey bee foragers at each paired colony location on three dates across the 1997 field season - early summer (June 26), mid-summer (August 25) and late summer (September 17). In addition, one round of pollen samples was analyzed; they were collected from Bush River pollen traps on August 11.

Some trace elements were found at the sub-ppm level only. Arsenic, cadmium, cobalt, chromium, gallium, selenium and vanadium all fell into this category. With arsenic and cadmium, there were only marginal traces of their presence in the pollen. Pollen levels of chromium, selenium and vanadium were about equal to forager levels. On average, gallium showed slightly higher concentrations in bees than in pollen. Chromium was just the opposite; the foragers carried about half as much as did the pollen.

Four trace elements -- Ba, Cu, Mn and Rb -- showed a definite seasonal trend in the Bush River area, the high levels being observed in the early sample round. Barium dropped off in

Table 3.18 Trace Elements in Forager Bees and Pollen from the Bush River Study Area

		6/26	8/25	9/17	Ave	Pollen
As (ppm)	BR1	0.2	0.2	0	0.1	0
	BR2	0.2	0.2	0	0.1	0
	BR3	0	0	0.2	0.1	0.1
	BR4	0.3	0.1	0.1	0.2	0
	BR5	0	0.3	0	0.1	0
	BR6	0.2	0.2	0	0.1	0
	BR7	0.2	0.2	0	0.1	0
	BR8	0.2	0	0	0.1	0
	BR9	0.2	0.2	0.1	0.2	0
	BR10	0.2	0	0	0.1	0
	BR11	0	0.4	0	0.1	0.1
	BR12	0.2	0.4	0	0.2	0.2
Ba (ppm)	BR1	9.9	5.7	6.2	7.3	2.5
	BR2	13	3.6	4.4	7	2.1
	BR3	12	3	5	6.7	1.6
	BR4	14	2.4	4.9	7.1	1.7
	BR5	5.5	5.1	8.2	6.3	1.6
	BR6	12	5.4	7.3	8.2	2.4
	BR7	17.5	2	6.5	8.7	1.9
	BR8	5.5	3.2	7	5.2	2.1
	BR9	9.1	2.2	7.2	6.2	2.2
	BR10	13	4.4	6.8	8.1	2.5
	BR11	5.7	4.7	4	4.8	2
	BR12	7.3	3.5	5.2	5.3	2.7
Cd (ppm)	BR1	0.1	0	0.2	0.1	0
	BR2	0.2	0.3	0.1	0.2	0
	BR3	0.2	0.2	0.1	0.2	0
	BR4	0.1	0.3	0.1	0.2	0
	BR5	0.1	0.2	0.2	0.2	0
	BR6	0.2	0.3	0.2	0.2	0
	BR7	0.1	0.3	0.2	0.2	0
	BR8	0	0.2	0.2	0.1	0
	BR9	0.1	0.3	0.1	0.2	0.1
	BR10	0.1	0.3	0.2	0.2	0
	BR11	0.1	0.3	0.1	0.2	0.1
	BR12	0	0.3	0.1	0.1	0.2

Table 3.18 Trace Elements in Forager Bees and Pollen from the Bush River Study Area (Cont'd.)

		6/26	8/25	9/17	Ave	Pollen
Co (ppm)	BR1	0.5	0.3	0.2	0.3	0.4
	BR2	0.6	0.4	0.1	0.4	0.3
	BR3	0.5	0.2	0.1	0.3	0.1
	BR4	0.6	0.3	0.2	0.4	0.3
	BR5	0.5	0.3	0.1	0.3	0.1
	BR6	0.7	0.5	0.3	0.5	0.4
	BR7	0.5	0.4	0.3	0.4	0.2
	BR8	0.4	0.4	0.3	0.4	0.2
	BR9	0.5	0.3	0.2	0.3	0.4
	BR10	1.0	0.3	0.2	0.5	0.3
	BR11	0.4	0.4	0.1	0.3	0.3
	BR12	0.4	0.4	0.0	0.3	0.2
Cr (ppm)	BR1	0.4	0.3	0.4	0.4	0.7
	BR2	0.3	0.3	0.3	0.3	0.5
	BR3	0.4	0.4	0.4	0.4	0.4
	BR4	0.3	0.3	0.4	0.3	0.6
	BR5	0.4	0.3	0.4	0.4	0.7
	BR6	0.3	0.4	0.4	0.4	0.5
	BR7	0.4	0.3	0.5	0.4	0.5
	BR8	0.4	0.4	0.5	0.4	0.5
	BR9	0.4	0.3	0.4	0.4	0.5
	BR10	0.2	0.3	0.4	0.3	0.6
	BR11	0.3	0.2	0.3	0.3	1.1
	BR12	0.4	0.3	0.3	0.3	0.8
Cu (ppm)	BR1	33.9	18.3	14.8	22.3	5.7
	BR2	27.0	14.5	14.2	18.6	5.1
	BR3	26.1	12.3	13.6	17.3	4.6
	BR4	30.3	15.6	13.5	19.8	8.5
	BR5	39.1	21.5	15.4	25.3	4.9
	BR6	28.4	15.1	16.1	19.9	4.5
	BR7	25.4	15.1	15.2	18.6	4.5
	BR8	23.1	15.9	16.4	18.5	5.3
	BR9	18.4	14.5	16.1	16.3	4.4
	BR10	42.8	14.4	10.5	22.6	5.9
	BR11	20.0	18.0	11.7	16.6	5.3
	BR12	27.2	17.8	12.4	19.1	9.2

Table 3.18 Trace Elements in Forager Bees and Pollen from the Bush River Study Area (Cont'd.)

		6/26	8/25	9/17	Ave	Pollen
Ga (ppm)	BR1	0.7	0.4	0.3	0.5	0.1
	BR2	0.8	0.3	0.2	0.4	0.1
	BR3	0.8	0.2	0.3	0.4	0.0
	BR4	0.8	0.2	0.3	0.4	0.1
	BR5	0.3	0.3	0.5	0.4	0.1
	BR6	0.6	0.4	0.4	0.5	0.1
	BR7	1.1	0.1	0.4	0.5	0.1
	BR8	0.4	0.2	0.4	0.3	0.1
	BR9	0.7	0.2	0.4	0.4	0.2
	BR10	0.6	0.3	0.4	0.4	0.1
	BR11	0.4	0.2	0.2	0.3	0.2
	BR12	0.4	0.3	0.3	0.3	0.2
Pb (ppm)	BR1	1.7	3.1	2.6	2.5	18.7
	BR2	3.0	5.0	2.7	3.6	20.7
	BR3	2.7	2.4	2.3	2.5	44.0
	BR4	5.2	5.8	2.3	4.4	17.2
	BR5	3.7	7.2	3.0	4.6	5.4
	BR6	3.7	6.2	2.5	4.1	11.4
	BR7	4.7	2.0	3.0	3.2	24.5
	BR8	6.1	4.8	2.7	4.5	22.6
	BR9	4.4	5.7	3.5	4.5	16.5
	BR10	18.0	1.8	2.5	7.4	13.0
	BR11	4.4	4.6	2.4	3.8	8.3
	BR12	3.4	6.7	2.7	4.3	4.2
Mn (ppm)	BR1	500.0	163.0	78.0	247.0	38.0
	BR2	584.5	244.0	47.0	291.8	40.3
	BR3	620.0	189.5	44.0	284.5	43.0
	BR4	367.0	142.0	35.0	181.3	16.0
	BR5	501.0	219.0	89.0	269.7	37.5
	BR6	534.0	250.0	76.0	286.7	24.5
	BR7	532.0	129.5	74.5	245.3	29.0
	BR8	326.5	185.0	93.5	201.7	25.8
	BR9	411.0	138.0	72.0	207.0	54.0
	BR10	889.0	237.5	154.0	426.8	24.5
	BR11	395.0	213.5	47.0	218.5	22.0
	BR12	372.0	169.5	48.0	196.5	53.0

Table 3.18 Trace Elements in Forager Bees and Pollen from the Bush River Study Area (Cont'd.)

		6/26	8/25	9/17	Ave	Pollen
Ni (ppm)	BR1	0.2	0.2	0.2	0.2	4.5
	BR2	0.6	0.8	0.3	0.6	10.6
	BR3	0.3	0.2	0.4	0.3	7.9
	BR4	0.7	0.4	0.5	0.5	6.5
	BR5	0.5	0.4	0.4	0.4	2.6
	BR6	1.6	1.6	0.3	1.2	2.7
	BR7	0.4	0.2	0.5	0.4	5.5
	BR8	0.5	0.2	0.3	0.3	9.3
	BR9	0.5	1.1	0.5	0.7	6.4
	BR10	4.5	0.2	0.3	1.7	7.4
	BR11	0.5	0.9	0.3	0.5	7.5
	BR12	0.5	2.8	0.2	1.2	0.7
Rb (ppm)	BR1	13.0	4.0	2.8	6.6	18.7
	BR2	17.4	5.3	2.3	8.3	18.4
	BR3	13.9	5.0	2.2	7.0	10.8
	BR4	13.9	6.5	5.0	8.5	23.2
	BR5	15.4	6.3	5.8	9.2	12.1
	BR6	14.4	6.5	7.8	9.6	16.2
	BR7	20.4	2.8	7.6	10.3	18.5
	BR8	13.9	3.4	7.7	8.3	15.5
	BR9	9.8	5.4	6.6	7.2	16.9
	BR10	18.9	6.8	7.2	11.0	30.6
	BR11	12.5	7.9	5.9	8.8	19.8
	BR12	16.8	7.9	6.0	10.2	6.3
Se (ppm)	BR1	0.3	0.3	0.3	0.3	0.3
	BR2	0.3	0.2	0.3	0.3	0.2
	BR3	0.3	0.3	0.3	0.3	0.2
	BR4	0.2	0.3	0.3	0.3	0.4
	BR5	0.3	0.1	0.3	0.2	0.3
	BR6	0.2	0.3	0.3	0.3	0.2
	BR7	0.3	0.4	0.4	0.3	0.3
	BR8	0.3	0.4	0.5	0.4	0.3
	BR9	0.3	0.2	0.3	0.3	0.2
	BR10	0.2	0.4	0.2	0.3	0.3
	BR11	0.3	0.3	0.3	0.3	0.3
	BR12	0.3	0.3	0.2	0.3	0.3

Table 3.18 Trace Elements in Forager Bees and Pollen from the Bush River Study Area (Cont'd.)

		6/26	8/25	9/17	Ave	Pollen
Sr (ppm)	BR1	4.1	5.0	2.8	4.0	1.5
	BR2	3.8	6.5	2.3	4.2	1.4
	BR3	3.1	5.8	2.2	3.7	1.2
	BR4	3.7	5.2	2.3	3.7	1.0
	BR5	2.8	8.0	4.0	4.9	1.8
	BR6	5.6	5.0	2.8	4.5	0.9
	BR7	6.9	2.8	2.5	4.1	1.2
	BR8	1.5	3.4	2.8	2.6	1.0
	BR9	3.8	5.4	3.1	4.1	1.8
	BR10	3.9	6.8	6.0	5.6	1.2
	BR11	2.4	7.9	2.3	4.2	0.8
	BR12	2.5	7.9	2.5	4.3	1.9
V (ppm)	BR1	0.8	0.6	0.7	0.7	1.0
	BR2	0.7	0.4	0.6	0.6	0.8
	BR3	0.7	0.8	0.6	0.7	0.7
	BR4	0.6	0.6	0.5	0.6	1.0
	BR5	0.9	0.4	0.6	0.6	1.1
	BR6	0.6	0.7	0.6	0.6	0.8
	BR7	0.7	0.7	0.9	0.7	0.8
	BR8	0.8	0.8	0.8	0.8	0.9
	BR9	0.8	0.3	0.6	0.6	0.9
	BR10	0.6	0.7	0.7	0.7	1.0
	BR11	0.7	0.5	0.7	0.6	1.3
	BR12	0.8	0.5	0.5	0.6	0.7
Zn (ppm)	BR1	69.3	69.9	41.0	60.1	31.5
	BR2	53.1	73.5	46.4	57.7	44.6
	BR3	61.2	68.9	41.4	57.2	32.9
	BR4	68.8	69.6	31.4	56.6	43.9
	BR5	61.4	77.3	71.7	70.1	26.7
	BR6	64.6	77.1	51.5	64.4	50.8
	BR7	62.8	59.0	52.3	58.0	35.9
	BR8	47.4	77.6	44.6	56.5	52.5
	BR9	48.2	58.5	58.5	55.1	23.9
	BR10	79.3	51.3	36.7	55.8	20.2
	BR11	46.5	76.5	54.7	59.2	32.6
	BR12	62.2	75.3	46.5	61.3	152.0

the mid-summer round and then rebounded during the late summer period. Bees had barium levels 2- to 3-times higher than in the pollen. No one site was consistently high. BR7 exhibited the first sample round high at 17.5 ppm. BR5 had the third round high at 8.2 ppm.

Rubidium concentrations were uniformly higher in the June sample period compared to the other two. By summer's end, levels were about half of what they were in June. The August pollen samples held rubidium levels about the same as or slightly higher than those in the June foragers. Pollen concentrations of rubidium were two- to three-fold higher than forager concentrations.

Manganese, the most prominent trace element in bees, displayed the largest seasonal drop off. The June sample round produced measurements in the 300-900 ppm range. Those from August had dropped to the 130-250 ppm range. The final round in September yielded a range of 35-150. The BR10 site exhibited the highest Mn concentrations in both the first and last sample periods. Pollen levels of manganese landed in a range of 16-54 ppm, obviously not a controlling factor in forager bee levels.

Zinc, another trace element at relatively high concentrations in bees, showed fairly stationary levels throughout the three sample periods. They were on the low side of normal ranges seen in been foraging in Western US locations (80 to 120 ppm). However, zinc levels on the Bush River sites were not significantly different from those measured in colonies at our off-base reference site in Churchville.

Copper is used by honey bees for oxygen transport the same way that humans employ iron in hemoglobin. Typical levels in healthy Western bees is around 25 ppm. Bush River foragers were slightly above this level in the June samples and low by about a third in the other two periods. As with zinc, the Churchville reference colony displayed the same levels, so no APG-specific significance is apparent.

Lead and nickel are found at elevated levels in pollen compared to forager bees. Lead levels in foragers were generally in the 2-8 ppm range. Those for pollen ranged from 4.2 ppm at BR12 to 44 ppm at BR3. Lead levels in pollen at BR1-BR4 and BR6-BR10 are sufficiently high to warrant further evaluation since their presence in pollen is a route of entry into the local food chain. The same concerns hold for nickel. While bee burdens are fairly low, pollen levels at BR1-BR4 and BR6-BR12 are sufficiently elevated to justify some ecosystem risk assessments.

Strontium was uniformly present in forager samples at the 2- to 8-ppm level. As discussed below, this is a consistent characteristic of APG bees. Pollen levels of strontium were in the 0.8 to 1.9 ppm range.

An overall summary of trace element levels in foragers is given in Table 3.19 which has 1997 site means for trace element concentrations, and Table 3.20, a comparative panel with 1996 site means. Several observations are worth noting in reviewing these results. First, Old O Field has consistently had the highest levels of barium observed among the APG sites. Second, West Branch Canal Creek foragers exhibited a higher level of nickel than foragers at other 1997 sites. Third, since arsenic levels are comparable between 1997 and 1996 foragers, any potential loss of arsenic due to the 105 °C drying temperatures used in 1996 was not significant. Lead, however, appears to demonstrate the opposite results. Duplicates (splits) of 1996 samples taken from frozen storage, dried at 45 °C, and analyzed in 1997 revealed that some lead may have been lost from the 1996 samples during processing. Also, the 1996 site

Table 3.19 Trace Level Site Mean Concentrations in 1997 Foragers (values are ppm by weight)

	O Field 1997	*Canal Cr 1997*	*Churchville 1997*	*Edgewood 1997*	*Bush River 1997*
	Ave. Conc. ± % RSD	Ave. Conc. ± % RSD	Ave. Conc. ± % RSD	Ave. Conc. ± % RSD	Ave. Conc. ± % RSD
As	0.1±8.81	0.1±7.46	BDL	BDL	0.1±10.9
Ba	**6.9±1.11**	4.2±1.56	**4.2±0.45**	3.2±0.89	4.7±1.19
Be	BDL	BDL	BDL	BDL	BDL
Bi	BDL	BDL	BDL	BDL	BDL
Cd	**0.2±3.63**	**0.2±8.05**	**0.1±2.81**	0.4±4.38	0.1±5.54
Co	0.2±9.86	0.3±6.49	0.2±3.49	0.4±8.58	0.2±6.83
Cs	BDL	BDL	BDL	BDL	BDL
Cr	0.4±5.18	0.5±6.65	0.4±2.85	0.4±7.07	0.3±7.85
Cu	12.7±2.88	15.6±3.45	15.8±2.07	15.3±2.44	13.8±3.872
Ga	**0.4±2.46**	**0.3±7.28**	0.2±2.31	0.2±8.34	0.3±6.24
Mn	166±3.01	137±3.06	95±1.63	**200±2.03**	174±3.64
Ni	0.3±4.47	**1.6±4.26**	**0.5±2.63**	**0.3±9.67**	0.5±6.47
Pb	**2.8±1.83**	**4.8±2.56**	**2.6±1.38**	**1.9±2.1**	2.7±1.34
Rb	7.35±1.31	5.39±1.63	**7.13±0.80**	7.08±1.24	6.43±1.77
Se	0.2±10.1	0.3±12.65	0.3±8.81	**0.3±10.39**	0.2±14.73
Sr	**5.42±1.61**	**3.37±1.1**	1.7±0.54	**4.24±0.76**	2.92±1.29
TL	BDL	BDL	BDL	BDL	BDL
U	BDL	BDL	BDL	BDL	BDL
V	**0.7±5.07**	**0.9±3.95**	**0.7±2.79**	0.7±5.61	0.7±6.02
Zn	44.9±2.54	56.6±3.19	47.9±2.0	46.8±1.702	41.9±3.52

Table 3.20 Trace Level Site Mean Concentrations in 1996 Foragers (values are ppm by weight)

	O field 1996	*Canal Cr 1996*	*Churchville 1996*	*Edgewood 1996*
	Ave. Conc. ± % RSD	Ave. Conc. ± % RSD	Ave. Conc. ± % RSD	Ave. Conc. ± % RSD
As	0.3±11.3	0.1±18.1	0.2±10.8	0.7±4.6
Ba	5.5±1.2	5.1±0.8	3.4±0.7	5.3±1.8
Be	BDL	BDL	BDL	BDL
Bi	BDL	BDL	BDL	BDL
Cd	0.2±7.6	BDL	BDL	0.6±4.4
Co	0.3±4.2	0.3±2.7	0.3±2.5	0.4±4.0
Cs	BDL	BDL	BDL	BDL
Cr	0.4±4.8	0.5±3.0	0.5±2.5	0.4±4.6
Cu	18.7±1.1	23.0±0.7	20.4±1.2	26.3±2.0
Ga	0.3±3.8	0.2±2.3	0.2±6.0	0.3±6.0
Mn	164.8±1.6	169.4±1.5	159±2.4	148.9±2.51
Ni	0.4±4.7	0.3±2.2	0.4±2.7	0.2±8.1
Pb	0.4±4.2	0.4±1.4	0.3±3.0	0.6±2.8
Rb	7.5±1.1	8.2±1.0	6.6±1.5	7.3±2.6
Se	0.2±29.2	0.2±18.6	0.3±52.5	0.2±18.7
Sr	4.6±1.2	2.1±0.9	1.5±0.5	3.1±1.7
TL	BDL	BDL	BDL	BDL
U	BDL	BDL	BDL	BDL
V	0.4±4.4	0.6±3.7	0.4±4.2	0.7±4.7
Zn	73.5±0.8	115±0.5	86.8±1.0	126.5±1.8

means were all in the sub-ppm range (when samples were dried at 105 °C), while 1997 means (when samples were dried at 45 °C) ranged from 1.9 to 4.8 ppm. Finally, note that Churchville means for strontium are uniformly lower than those for any of the APG sites. It is uncertain whether this outcome, however, is due to a contamination history at APG or, instead, a result of a difference in soil geology between the two locations.

3.4 Focus Areas for 1998 Field Season

Areas of chemistry to be pursued for the 1998 field season include:

- Expansion of and routine application of calibrated standards for quantification of organic contaminant levels.

  We have already been using standard EPA suites of contaminants with our new calibration device for studies outside the USACEHR project. These have proved to be quite reliable and will be applied to the analysis of the 1998 samples.

- Removal of terpene and sugar residues from the sample matrix prior to introducing a sample into the GC/MS.

  We will explore several strategies to prevent the build-up of sample varnish that has taken place in our transfer lines with each round of hive atmosphere samples. Potential techniques include use of a cold-finger to condense them out, use of a pre-trap guard column and altering the thermal desorption program to avoid their bake-off into the chromatograph.

- Development of screening software such that peaks corresponding to normal beehive compounds can be distinguished from those that represent agents of harm.

  The advent of the United Nations Chemical Weapons Treaty has spawned the development of mass spectral software that can mask background peaks in GC/MS that are not of interest. Already available are versions specific to chemicals associated with chemical weapons. This seems like a means to make data screening more efficient and reproducible by personnel with less specialized training.

- Additional experiments to establish how and if contaminants in various physical states are taken up, transported, and deposited in or on bees and in beehives.

  We need to expand upon the preliminary studies done in East Missoula to ascertain exactly how honey bees bring contaminants back to the hive. Experiments of this type should lead to an understanding of how hive atmosphere levels relate to the levels actually present in the field.

- Design and fabrication of equipment that can accurately dispense known amounts of specific contaminants in dose-response studies.

  Because we have observed evidence of changes in bee behavior in colonies exposed to relatively low levels of PCE, it will be valuable to perform dose-response studies to

"calibrate" bee behavior to known levels of PCE. Devices that deliver a metered amount of contaminant will need to be designed, built and tested.

- Conduct a boundary site survey of chemical exposures at sites both at APG-Edgewood and along transects extending northward through the communities of Harford county.

  The boundary study will provide the information needed to determine whether any of the bioavailable chemical agents that we have detected inside bee colonies and in the ambient air at APG sites are migrating off-site. Equally important will be the opportunity to determine whether off-site sources are contributing contaminants to the community or to APG. This study may also help discern the source of the somewhat elevated strontium observed at all APG sites.

- Conduct a survey of chemical exposures at J Field across the 1998 season. An early April startup is anticipated.

  Another particular emphasis will be to examine the uptake, transfer, and fate of chemicals translocated by the trees used for phyto-remediation and the potential for transfer of these chemicals into bee colonies.

SECTION 4

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 (ANN). Software programs that learn real-valued functions from examples.

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

Bioavailable. The chemical agent of interest can be collected and transported back to the hive in or on a bee or in or on the materials collected by the bee. For purposes of this report, bioavailable does not imply physiological uptake.

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

Brood nest. 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.

Coefficient of Variation (C.V.). 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.

Inductively Coupled Plasma Mass Spectrometry (ICP/MS). An instrument used to analyze for trace elements and heavy metals (i.e., inorganic chemicals).

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 honey flow.

No Observed Effect Level (NOEL). 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.

Queen right. 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.

Relative Humidity (RH) Probe. An electronic relative humidity sensor.

Relative Standard Deviation (R.S.D.) A statistical term that is equivalent to 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.

Thermal Desorption/Gas Chromatography/Mass Spectrometry (TD/GC/MS). Instrumentation for the analysis of volatile and semi-volatile 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.

SECTION 5

REFERENCES

A.B. Dindal, Cheng Yu Ma, J. Todd Skeen, R.A. Jenkins. *Novel Calibration Technique for Headspace Analysis of Semivolatile Compounds.* Journal of Chromatography (Galley Proof, accepted 5 September, 6 pages, 1997).

J.J. Bromenshenk, G.C. Smith, R.A. Seccomb, B.E. King, G. Alnasser, C.B. Henderson, and C.L. Wrobel, *New and Improved Methods for Monitoring Air Quality and the Terrestrial Environment.* 1997 Annual Report, (U.S. Army Center for Environmental Health Research, Ft. Detrick, MD, USA, 1997).

Ma Cheng-Yu, J. Todd Skeen, A. B. Dindal, C.K. Bayne, and R.A. Jenkins. *Performance Evaluation of a Thermal Desorption/Gas Chromatographic/Mass Spectrometric Method for the Characterization of Waste Tank Headspace Samples.* Environmental Science & Technology, 31(3):853-859, 1997.

E.J. Hentschel. Busy Bees: *The Bee as a Bioindicator, The Bee as a Microbiological Laboratory.* Innovation 3, Carl Zeiss, pages 28-29, 1997.

APPENDIX A

SITEVIEW© USER'S MANUAL

SITEVIEW© DATA VIEWING, ANALYSIS, PRESENTATION, AND PROCESSING SOFTWARE.

Version 6.5

*All of the following software has been developed at the University of Montana—Missoula under funding from the United States Army Center for Environmental Health Research (USACEHR).

SITEVIEW© was written to ease the numerical processing and viewing of data recorded and archived by our bi-directional **honey bee** counters. The **FILEFORM©** program first converts field data files into a database format for use by **SITEVIEW©**. Data files are stored in a BRES directory on a hard drive, removable drive, or CD-ROM. Field data files, data formatting routines, and data processing and viewing software can be distributed on a CD-ROM. The CD-ROM installs a BRES directory in the root directory of the user's computer. This directory provides for the temporary storage of decompressed data files. **SITEVIEW©** provides the user with an array of viewing and secondary data processing options for data from both honey bee and bumble bee flight activity. All of the menu selections provide choices in parentheses, with the defaults capitalized. Site codes and dates are checked by the program to verify existence of files.

Site codes for our USACEHR study sites are:

CC	West Branch Canal Creek
CV	Churchville Reference
OF	Old O-Field
FM	Fort Missoula
JF	J-Field

Date Codes use the DD/MM format:

0808	August 8
0929	September 29

The program asks for the YR (e.g., 96, 97)

MENU OPTIONS:

NOTE ABOUT SAVING DATA TO FILES:

Many of the MENU options allow data to be saved to a comma delimited text file. Pressing the RETURN when asked if you wish to save the data will not generate an additional file. Pressing RETURN (or ENTER) is the default. Only enter "Y" (yes) if you need to use the data to generate a hard-copy printout using the plotting software. Text files will be saved to the /PLOT/ directory of the corresponding site directory. The path name is displayed on the screen prior to saving.

Each SITEVIEW option is a custom software utility program written at UM. These utilities offer data processing and visualization capabilities ranging from: (1) data file formatting and storage, (2) simultaneous display of raw or smoothed data from seven colonies at each site, (3) site by site comparisons, (4) honey bee and bumblebee species comparisons, and (5) complex numerical

analysis methods that are highly sensitive to unusual flight activity behavior. The following text is taken from the main menu of the SITEVIEW program.

WELCOME TO THE BEE-COUNT DATA MANIPULATOR AND VISUALIZATION

THERE ARE SEVERAL SELECTIONS AVAILABLE FOR PROCESSING AND VIEWING DATA OBTAINED FROM SITES CONTAINING SEVEN BEE COUNTERS.

(1) PLOT TOTAL ACTIVITY FOR 7 HIVES (NOISY DATA)
(2) PLOT SMOOTHED TOTAL ACTIVITY DATA FOR 7 HIVES
(3) PLOT DIFFERENCE BETWEEN IN AND OUT ACTIVITY FOR 7 HIVES
(4) PLOT IN AND OUT DATA SIMULTANEOUSLY TO SEE OVERLAP
(5) PLOT SMOOTHED PERCENT RETURNED FOR 7 HIVES
(6) CALCULATE AND PLOT SMOOTHED DERIVATIVES OF FLIGHT ACTIVITY
(7) PLOT COMPONENTS OF THE DER*DIFF ALGORITHM USED IN OPTION 7
(8) PLOT DERIV*DIFF RATIO TO SEE PHASE SHIFT SENSITIVE ANALYSIS
(9) DISPLAY AN HOURLY OR USER SELECTED INTEGRATION PERIOD
(10) PLOT SITEWIDE DAILY ACTIVITY FOR ENTIRE SEASON
(11) SIMULATE THE FIELD DISPLAY
(12) PLOT FOUR DAYS/SITES SIMULTANEOUSLY
(15) REVERSE BACKGROUND / FOREGROUND COLOR
(19) DELETE OPENED DATA FILES FROM DISK, LEAVES COMPRESSED FILES ALONE
(20) END SITE/STUDY VIEWER MENU AND RETURN TO MAIN MENU
PLEASE ENTER A NUMBER FROM THE ABOVE LIST:

INDIVIDUAL OPTIONS

(1) PLOT TOTAL ACTIVITY FOR 7 HIVES (NOISY DATA)

This option plots the data for all of the hives for one day at one site with no smoothing. Once the date, year, and site code have been entered, another prompt allows the user to select Total (incoming plus outgoing bees), In (incoming bees) or Out (Outgoing) for the bee flight data. These options can be selected by typing **T**, **I**, or **O** respectively. The plot displayed to the screen has eight traces. The traces are numbered from bottom to top as COUNTERS 1 through 7, where 1 shows the data from the counter on hive #1, 7 displays the data from hive counter #7. The eighth trace presents the average flight activity for all seven colonies. At the bottom of the screen is an x-axis grid and time labels. Since most flight activity occurs between 06:00 and 22:00 hr, these values usually represent the beginning and end of the data collected and plotted for each day.

The plotting algorithm identifies the time corresponding to each data point before displaying it to the screen. Data is plotted by drawing a line from the current point to the next point in the array. Breaks in the sequence of data collection will cause the last point plotted before the data break to be the first point after the data break occurs.

(2) PLOT SMOOTHED TOTAL ACTIVITY FOR 7 HIVES

This option is similar to option 1, but allows the user to select smoothing parameters. Four types of smoothing are available, as shown by the screen prompt below:

4 TYPES OF OVERLAPPING BOXCAR SMOOTHING ARE AVAILABLE.
DESCRIPTIONS FOLLOW:

OPTION	*SMOOTHING TYPE*
1	*SINGLE PASS*
2	*DOUBLE PASS*
3	*SINGLE PASS, 2 POINT INNER BOXCAR*
4	*DOUBLE PASS, 2 POINT INNER BOXCAR*

DEFAULT IS 2

The first option is a simple overlapping boxcar average, while the second option repeats the overlapping boxcar average on the smoothed data, also called double smoothing. Options 3 and 4 follow the same sequence, but include a 2 point high frequency filter inside each of the user selected boxcars. Do not choose a boxcar length of less than 2 for options 1 and 2, or less than 3 for options 3 and 4. Pressing RETURN will run the default double filtering (highly recommended) mode. For most cases a boxcar length of 6 is sufficient. Choosing 6 will smooth the data over a 3 minute period for each point plotted. This applies to all subsequent choices that use any form of smoothing. Output to the screen consists of eight traces as before.

(3) PLOT DIFFERENCE BETWEEN IN AND OUT ACTIVITY FOR 7 HIVES

This option plots the difference between the bees entering and exiting a counter for each thirty second interval through the selected day. Eight traces are displayed on the screen with the lowest one representing counter 1 and the topmost trace the total difference between bees entering and bees exiting the counter for all of the hives.

The general flight pattern for a sunny day with strong flight activity should start with a long dip as the bees begin foraging. Soon this dip returns to zero as foraging bees return to the hive in appreciable numbers. As the end of the day approaches, the difference between incoming and outgoing bees reaches a maximum when foragers return in greater numbers than those that leave. Any unusual peaks or valleys should be investigated further. If you would like to view the following or previous day, enter "X" and then follow the prompts. If "X" is entered a second time the program returns to the main menu.

(4) PLOT IN AND OUT SIMULTANEOUSLY TO SEE OVERLAP

This option allows you to plot data simultaneously from bees both entering and exiting the counter throughout a single day. This option is highly recommended for data interpretation and is very useful in identifying and assigning the cause flight activity events. You can choose between

raw (raw data) and smoothed (filtered) data by entering "R" or "S". Data for bees entering the counter is plotted in green; bees exiting the counter are plotted by red. The display shows eight pairs (in/out) of traces. As in other displays, the counter data for hive #1 appears at the bottom of the screen, the average for seven hives at the top. If you would like to view the data for the following or previous day, enter "X" and then follow the prompts. If "X" is entered a second time the program returns to the main menu.

(5) PLOT SMOOTHED PERCENT RETURNED FOR 7 HIVES

This option provides smoothing parameters for the following percentage of bees returning to the hive over each 30 second interval. Again we have found that entering the number 6 usually works best for each smoothing parameter. However, you may wish to try other values. This produces a complex screen display. We suggest that you view one of these plots while reading the following description. One or two sets of traces are displayed on the screen. The default plots a single set of traces, containing 14 lines, two for each counter. Each pair of traces is laid over a horizontal, straight line that is color coded to match the traces of interest. These straight lines represent a 100% return rate for the corresponding traces. The actual percent returned is calculated as:

100 * (bees entering counter / (bees leaving counter + 1))

Adding 1 to the denominator eliminates 'divide by zero' errors encountered at run time. The resultant raw data plots are summarized for 30 second periods and tend to be data rich, "noisy", and visually difficult to interpret. The smoothing routines can greatly facilitate data visualization.

A second set of traces can be displayed at the top of the screen to show either: (1) the smoothed total flight activity for each counter (7 traces), or (2) the total percent returned for the site (2 traces, one of which is the 100% level). Before the data is plotted, the program will ask whether you want to display total activity or the total percent returned. Note that the percent returned for each counter should start out below 100% in the morning as more bees are leaving than entering the hive. Slowly the traces should rise to the 100% level during mid-day. At the end of the day, the plots may exhibit more than a 100% return, as more bees come home to the hive than go out. Any unusual drops or peaks in the percent return data during the day should be investigated.

(6) CALCULATE AND PLOT SMOOTHED DERIVATIVES OF FLIGHT ACTIVITY

This option plots derivatives based on smoothed flight activity data. A derivative can help isolate events by displaying them as distinct peaks. You can plot total activity, or data from bees entering (in data) or exiting (out data) the counter or choose to plot smoothed data with the derivatives. In the latter case, the derivative peak(s) should stand out against the smoothed data traces. Again, we suggest that you try using 6 and 6 for the boxcar lengths, 60 or 100 (or whatever makes it look best) for the derivative multiplier, and 6 for the for the derivative span. The derivative multiplier is used to adjust the scaling of the derivative data. Because some modifications of the

derivative are calculated using the average (AVE) of each half of the derivative spans (half above and half below the middle of the derivative span) an even number must be chosen for the derivative span. There are four options available for the derivative calculation as shown below:

STANDARD DERIVATIVE CALC USES THE AVE OF EACH HALF OF THE DERIVATIVE SPAN YOU CAN MODIFY THIS BY USING THE ABSOLUTE DERIVATIVE AS WELL AS USING THE EXTREME DATA POINTS TO CALC THE DERIVATIVE (POINT TO POINT DERIVATIVE):

OPTION	*MODIFICATION*
N	*NO MODIFICATION, AVE OF EACH HALF OF THE DERIVATIVE*
1	*POINT TO POINT DERIVATIVE CALCULATION*
2	*ABSOLUTE VALUE OF THE AVE OF EACH HALF OF THE DERIVATIVE*
3	*ABSOLUTE VALUE OF THE POINT TO POINT DERIVATIVE CALCULATION*

DEFAULT IS N

*NOTE: Option 3 is highly recommended for most purposes. The point to point derivative calculation is in the form of:

MULT * (SM(D) - SM(D - DERIVSPN)) / (DERIVSPN * H1)

where SM is the array holding smoothed flight activity data, D is the current data point in the array, DERIVSPN is the user entered derivative span, and MULT is the user entered derivative multiplier. H1 is the average of the upper half of the derivative span, which is used here to aid presentation by eliminating large peaks due to low flight activity at the beginning and end of the day. The H1 correction is automatically removed in options 7 and 8 (see below) in order to maintain true derivative peak height consistency.

Smoothing can be used to filter out short term events and enhance long term events. A large (>10) boxcar, enhances long term events, while you can filter long-term and enhance short term events by using a small (<10) boxcar. A large derivative span can hide short term events, and conversely, small derivative span may be insensitive to long term events. Choosing a derivative span that is greater than the chosen boxcar smoothing lengths increases sensitivity. For example, if you are attempting to detect changes in flight activity during a single day, and the event you are trying to detect lasts for about five minutes, choose a boxcar length of 6 to smooth over 3 minute intervals.

A simple method of determining how quickly an event occurred is to vary the derivative span. Rapid, short duration changes in flight activity will result in a peak if a small enough derivative span is used. Long term changes in flight activity will not be detected by short derivative spans. Note the rise and fall in activity at the beginning and end of the day. A short derivative span will not see this as a change in activity. Successively longer derivative spans will increase as the length of the event is approached, then eventually begin to decrease as the duration of the event is overtaken. Thus, when the result of changing the derivative span is decreasing peak heights, the duration of the event has been passed, but can be approximated by dividing the derivative span by 2.

(7) PLOT COMPONENTS OF THE DER*DIFF ALGORITHM USED IN OPTION 8

The previous options have all displayed on-screen either the raw data for flight activity data or some combination of smoothed flight activity data and secondary data processing. It was obvious from the raw data that some type of smoothing was necessary. Once smoothing was performed, patterns of flight activity could be studied. For example, it is easy to see that flight activity usually changed slowly throughout a warm, sunny day. Sudden deviations from this pattern usually indicated a response to weather, beekeeping activities, housekeeping by the bees, or other externally induced events. However, retrospective evaluations of real-time data is time consuming. More importantly, it fails to fully realize our objective of being able to conduct not only real-time acquisition, but also real-time reporting and analysis of colony behavioral data. Our goal is to not only detect these events, but to assign the observed responses to potential causes in real-time. **SITEVIEW©** is a testing ground for algorithms that can be implemented to detect these events and discern their causes.

One of these algorithms is DER*DIFF for calculating phase shift sensitive analysis of parameters. The process is straightforward. Data from the counters is smoothed according to user selected boxcar lengths using methods from option 2. This smoothed data is then collected into arrays called SMI and SMO. SMI contains smoothed data for bees entering the counter and SMO contains data for bees leaving the counter. Next, the difference between the SMI and SMO is calculated. Derivatives are taken of the smoothed data following the method used in option 6, but excluding the H1 activity correction factor. We recommend a derivative multiplier of 10 to align the y-axis scaling of all of the traces. These derivatives look back from the current smoothed data point to a point that is as far back in the array as the user selected derivative span (DERIVSPN). The derivatives are collected into arrays called DVI and DVO to yield the derivative of in data and out data, and the difference between DVI and DVO is calculated. The absolute difference in the percent returns are calculated as shown below:

$$\mathbf{ABS((SMI(x) / (SMO(x) + .1)) - (SMI(y) / (SMO(y) + .1)))}$$

where x is the number of the current data point in the array and y is calculated as the number of the current data point minus the user selected derivative span. The addition of .1 in each denominator prevents 'divide by zero' errors while exerting little influence on the calculated result. Artificially induced phase changes are eliminated because the derivative and the difference in percent return are calculated by looking back in the array by the same user selected derivative span.

The are two options for calculating the components of the DER*DIFF algorithm. A screen prompt will allow selection of the desired calculation method. Because of the number of traces being displayed, we recommended using the A option.

*YOU CAN PLOT THE INDIVIDUAL ELEMENTS OF THE DER*DIFF ALGORITHM TO VIEW THE PROCESS BY WHICH THEY COMBINE. THE FIRST PART IS THE DIFFERENCE*

BETWEEN THE SMOOTHED IN AND OUT DATA, AND THE SECOND PART IS THE DIFFERENCE BETWEEN THE SMOOTHED DERIVATIVE OF THE IN AND OUT DATA. YOU CAN SELECT AN OPTION BELOW TO PLOT THE ELEMENTS:

OPTION	*DISPLAYS*
N	*PLOT THE DIFF OF THE SMOOTHED DATA AND THE DERIVATIVE*
A	*PLOT THE ABSOLUTE VALUE OF THE DIFFERENCES*

DEFAULT IS N

The y-axis is scaled according to the minimum and maximum values of the site-wide total data in the analysis. This is not done for individual counters because of the high level of variability displayed among the counters. If you would like to display data for all of the counters simultaneously, it is recommended that you manually adjust the y-axis and multiply the maximum value by 2 to 3. If you find a day where the phase shift analysis is not providing a reasonable y-axis autoscale, write it down and report it to us. The smallest y-axis span available is currently 65, resulting from y-axis values of -40 and 25. The largest y-axis span produced via autoscaling is 101000, with y-values of -1000 and 100000.

After you have entered the appropriate parameters, you will be prompted to display results from all seven counters or from the total of all seven counters. Although the option to display the components of the DER*DIFF calculation for all seven counters is available, it is not recommended because a large number of overlapping traces will be drawn on the screen. If you are looking for something that happened at one of the counters you can enter "Y" (yes), but don't be surprised by a very cluttered screen. We recommend entering "N" (no) to display only the analysis and the combined activity for the site. This will produce a single line for each component of the phase shift analysis and two lines representing in and out activity for the site throughout the day. If the peaks do go off scale, you can adjust the y-axis or, in extreme cases, select a smaller derivative multiplier.

The colored numbers displayed at the top left corner of the screen identify the individual counter traces. Directly below the colored numbers are the labels for each of the traces normally on the display. SMT is the difference between the smoothed in and out data, DRV is the difference between the derivatives of the smoothed in and out data, PRC is the percent returned bees for the smoothed data, DRPRC is the derivative of the percent returned of the smoothed data, IN is the smoothed in data, and OUT is the smoothed out data. The bottom line of numbers is the maximum smoothed activity per sampling period, the y-axis maximum, the y-axis minimum and the y-axis span. The in and out activity are plotted so that the bottom of the screen is zero on the y-axis, while the phase-shift analysis automatically adjusts.

(8) PLOT DERIV*DIFF RATIO TO SEE PHASE SHIFT SENSITIVE ANALYSIS

This is an ultra-sensitive phase shift analysis that can detect smoke events and possibly, what we believe may be acute chemical events (although they often can only be reported as anomalous events). A smoke event falls under the characterization of an acute chemical event. Events such as this can be used to provide sensitive flight activity-based analysis for comparison with weather and

colony temperature to initiate chemical sampling. Currently, we are conducting dose-response trials to build a library of colony responses to external events. Until a database of real-time chemical data can be produced, many of the changes revealed by phase shift analysis must be listed as anomalous events.

To achieve the highest sensitivity and best false alarm rejection, the difference between the smoothed in and out activity, the difference between the derivatives of the in and out activity and the derivative of the percent returned are employed. Each of these methods provides a detectable peak or valley when a flight activity event occurs. They also have individual limitations such as: (1) the derivative being overly sensitive to change in flight activity during periods of low activity, and (2) the percent return being relatively insensitive in the morning and overly sensitive in the afternoon. The derivative and percent returned are multiplied together to minimize the impact of early and late day differences in bees entering and leaving the counter while enhancing the differences in mid-day events.

Setting this up to run is very simple. You must first select the date and site of interest. Next you will be prompted to plot smoothed flight data. We recommend entering "Y" because it will help correlate flight activity patterns with DER*DIFF calculated peaks throughout the day. Next, you will be prompted for smoothing and derivative parameters. Because this analysis compares differences between in and out activity, it can be overly sensitive (noisy) to boxcar lengths of less than 6. We recommend using a boxcar length of 6 to 14, a derivative multiplier of 100 and a derivative span of 8 to 14 (even numbers only).

Enter the appropriate derivative method (N or 1 for maximum sensitivity and 2 or 3 for noise and error rejection) and select a phase sensitive analysis method from the list below:

*THE STANDARD DER*DIFF CALCULATION MULTIPLIES THE DIFFERENCE OF THE SMOOTHED IN AND OUT DATA BY THE DIFFERENCE OF THEIR RESPECTIVE DERIVATIVES.*

YOU CAN SELECT AN OPTION BELOW TO MODIFY THE CALCULATION:

OPTION	*MODIFICATION*
N	*NONE*
S	*ABSOLUTE VALUE OF THE DIFFERENCE BETWEEN SMOOTHED IN AND OUT DATA*
D	*ABSOLUTE VALUE OF THE DIFFERENCE BETWEEN DERIV OF IN AND OUT DATA*
B	*ABSOLUTE VALUE OF DIFF FOR BOTH SMOOTHED AND DERIVATIVE DATA*

DEFAULT IS N

Choosing option B produces all positive peaks and a display that is easy to interpret. Hence, this is the preferred method.

The y-axis is scaled according to the minimum and maximum values of the site-wide total data in the analysis. This is not done for individual counters because of the high level of variability displayed among the counters. If you would like to display data for all of the counters simultaneously, it is recommended that you manually adjust the y-axis and multiply the maximum value by 2 to 3. If you find a day where the phase shift analysis is not providing a reasonable y-axis autoscale, write it down and report it to us. The smallest y-axis span available is currently 500, resulting from y-axis values of -250 and 250. The largest y-axis span produced via autoscaling is 104000, with y-values of -4000 and 100000.

After you have entered the appropriate parameters, you will be prompted to display results from all seven hives or from just the total of all seven hives. If you are looking for something that happened at one of the hives you can enter "Y", but don't be surprised by a very cluttered screen display. We recommend entering "N" to display only the analysis and the combined activity for the site. This will give one line that represents the phase shift analysis and two lines representing in and out activity for the site throughout the day. If the peaks do go off scale, you can fix this by adjusting the y-axis or, in extreme cases, selecting a smaller derivative multiplier.

The colored numbers displayed in the top left corner of the screen identify the individual traces. Directly below the colored numbers are two lines of numbers, the first line being the number of samples collected that day. The bottom line of numbers is the maximum smoothed activity per sampling period, the y-axis maximum, the y-axis minimum and the y-axis span. The in and out activity are plotted so that the bottom of the screen is zero on the y-axis, while the phase-shift analysis automatically adjusts. If the x-axis tick marks are not visible, it is because the y-axis maximum is too large. Try lowering the y-axis maximum value if the tick marks are necessary for identification of event sequence.

If all colonies are foraging normally, the analysis will produce a flat line. If a rain event occurs, peaks will be present as the bees recognize the onset of rain. A good example of a rain event is 7/19/97 at Fort Missoula. If an anomalous event occurs, the resulting phase shift (bees inside the hive stop leaving while bees outside the hive continue to return) will be detected as a sharp peak. Some excellent examples of this are 7/28/97 and 8/5/97 at Fort Missoula (methanol and Pine Sol respectively), 8/8/96 at Canal Creek (smoke event), and 6/22/97 at Old O-field (unassigned event). On 8/5/97 at Fort Missoula the second set of triplet shaped peaks looks like a possible cloud cover event or light rain, while the sharp, tall peak is the peak of interest.

An interesting example of the results of changing smoothing and derivative parameters is what happened on 7/21/97 at Fort Missoula. This was the "stand in front of the hive" experiment. Sharp bursts of bees entering hives occurred after the beekeeper moves from the front of the hive. The peaks don't show up in the total activity, but instead separate out when all hives are displayed. Try to eliminate the peaks at the end of the day without eliminating the peaks of interest by changing the length of the smoothing boxcar and/or the derivative span. Another example is the combination of the weather event and possible chemical event on 8/5/97. Shortening the smoothing boxcar and derivative span will enhance the short lived weather event, while lengthening these same parameters will favor the anomalous events (possible chemical event).

(9) PLOT HOURLY AND USER ENTERED SUMS FOR HONEY BEE AND/OR BUMBLE BEE DATA

All of the Maryland sites have honey bee flight data processed for hourly or 2, 5, 10, and 15 minute sums, while only Fort Missoula has bumble bee data processed for hourly or 2, 5, 10, and 15 minute sums. You are not given the option to mix sites. When entering the user selected sum, you must use a 2 digit number, thus, for 2 minute sums the appropriate entry will be "02". The same is true for 5 minute sums, while 10 and 15 minute sums are entered as 10 or 15. Not all sites have data for 365 days of the year. **There are missing days in the bumble bee data.** This program does not detect days missing from the bumble bee experiments, and will crash out if a missing day is selected. The bumble bees were brought online in Missoula on July 31, 1997 and taken off-line about September 11. This option was written to demonstrate the utility of integrating data at different intervals and the comparison of flight activity of *Apis mellifera* and *Bombus occidentalis* under identical environmental conditions.

Selecting option 9 will provide another list of options that can be used to display either integrated honey bee or bumble bee activity. The options in this list are described below:

(1) PLOT HOURLY SUM OF HONEY BEE FLIGHT DATA

This option plots the hourly sum of honey bee flight activity for total, in, or out data. The traces are set up as before, with the trace for counter 1 being at the bottom of the screen and the total (in, out, or total) activity for the seven counters at the top.

(2) PLOT HOURLY SUM OF BUMBLE BEE FLIGHT DATA

This option allows you to plot the hourly sum of bumble bee flight data for Fort Missoula. Even though it asks for a site code, only Fort Missoula had bumble bees. The bottom trace is colony number 1, and the top trace is the total activity for all three colonies.

(3) PLOT USER ENTERED SUM OF HONEY BEE FLIGHT ACTIVITY DATA

This option allows the user to enter a number corresponding to the number of minutes in the integration period. The traces displayed follow the pattern of option 1. The intervals available, as described above, are 2, 5, 10 and 15 minutes, and must be entered as a two digit sequence. The two minute interval has a high frequency component that is the result of the integration algorithm relying on the actual sampling time instead of the number of samples.

(4) PLOT USER ENTERED SUM OF BUMBLE BEE FLIGHT ACTIVITY DATA

This option follows that of option 3, but is used with the bumble bee flight activity data. The traces displayed are similar to that of option 2. Again, bumble bees have only been monitored at Fort Missoula during the 1997 season.

(5) PLOT HONEY BEE AND BUMBLE BEE HOURLY SUM FLIGHT DATA TOGETHER

This option allows direct comparison of the hourly sums of flight activity for the honey bees and the bumble bees. The top trace is the honey bee total activity divided by a number that makes it fit on the screen with the bumble bee data. Again, you can't mix sites.

(6) PLOT HONEY BEE AND BUMBLE BEE USER ENTERED SUM FLIGHT DATA TOGETHER

This option allows direct comparison of the user entered sums (2, 5, 10 and 15 minute intervals) of flight activity for the honey bees and the bumble bees. The top trace is the honey bee total activity divided by some number that makes it fit on the screen with the bumble bee data.

(7) SUM AND PLOT A USER SELECTED AMOUNT OF 30 SECOND SAMPLING INTERVALS

This option sums a user selected number of consecutive sampling periods. Since each field sampling period is 30 seconds in duration, summing 10 sampling periods will be roughly the equivalent of a five minute sampling period. This is another demonstration of the utility of 30 second sampling intervals. This option was added to eliminate the high frequency component of the 2, 5 and 10 minute sampling intervals.

(10) PLOT SITE-WIDE DAILY ACTIVITY FOR THE ENTIRE SEASON

This option allows you to plot site-wide activity for an entire flight season. All of the data plotted with the following options are available in a comma delimited form in the SUM directory for the site of interest. The menu is descriptive. Just enter the number of the option you want to show. In some cases, two scaling options are available. The first option plots all traces on the same y-axis, while the second option offsets the traces so that individual counters can be easily identified. The first display option is the default. The first five options are self-explanatory, as the plot of the daily activity totals over the entire sampling season. Option six plots the daily net loss (bees out - bees in). See below for a detailed description and interpretation of the significance of the available options:

(1) DAILY SUM OF TOTAL ACTIVITY
(2) DAILY SUM OF BEE IN DATA
(3) DAILY SUM OF BEE OUT DATA
(4) DAILY SUM OF IN-OUT DATA
(5) DAILY SUM OF OUT-IN DATA

Each of the above options reads daily sum data from the *.SUM file. The eight traces displayed on the screen represent the 7 counters at the site and the site-wide total for each

option. An option is presented to display the traces in a spread out or condensed form. The default condensed format creates a common y-axis for all of the counters. The spread format creates a unique y-axis for each counter, with counter 1 being the lowest on the screen and the total being the highest.

(6) DAILY NET LOSS

This option displays the net loss, also displayed in option 5 as the daily sum of out-in data. Net loss is calculated as bee leaving the counter minus bees entering the counter. Positive values represent a net loss and negative values a net gain of bees for the day.

(7) DAILY ADJUSTED NET LOSS

This option corrects the net loss for the total activity of the hive by dividing the net loss by the total flight activity of the counter. This is useful for detecting any unusual behavior resulting from either a colony or a counter malfunction. Adjusted net loss typically varies between -3 and +4, any outliers should first be investigated for weather events, power outages, or counter failure. If none of these factors are involved, then the number of bees returning at the end of the day was less than expected.

(8) DAILY PERCENT RETURN DATA

This option will plot the percent return data for a single site throughout the season. It is also useful for identifying unusual colony or counter behavior. Daily percent returns typically range from 94% to 100% due to natural attrition, any outliers should be investigated.

(9) DAILY NORMALIZED PERCENT RETURN DATA

This option corrects the percent return for the normalized (against the total activity of the site) activity of each hive. This can help identify strong and weak colonies and how well their return rate is functioning. It could be used to help identify hives being robbed at the end of the season. An unusually high normalized percent return (NPC) may indicate a strong colony robbing from other colonies.

(10) DAILY C.V. AMONG CHANNELS OF EACH COUNTER IN
(11) DAILY C.V. AMONG CHANNELS OF EACH COUNTER OUT
(12) DAILY C.V. AMONG CHANNELS OF EACH COUNTER TOTAL

Options 10-12 display the daily coefficient of variation (C.V.) among channels of each counter at the selected site. Option 10 displays the C.V. for bees entering the counter, option 11 displays the C.V. for bees exiting the counter and option 12 displays the C.V. for bees both entering and exiting the counter. This plot has 7 traces, each representing the C.V. of the channels for a single counter on the site. This can help identify which counters have

alignment problems or defective channels. If alignment is perfect, one would expect the C.V. for that counter to have a low value on a high activity day. If the alignment is not optimal, or if some of the emitters are not operating, the C.V. will be high because most channels will have normal activity while others will be 0 for the day. Occasionally, counters may have a higher C.V. for bees entering than exiting the counter. If this is the case, it may indicate more serious alignment problems.

(13) DAILY C.V. AMONG COUNTERS CHANNELS AT A SITE IN
(14) DAILY C.V. AMONG COUNTERS CHANNELS AT A SITE OUT
(15) DAILY C.V. AMONG COUNTERS CHANNELS AT A SITE TOTAL

Options 13-15 display the C.V. among counter channels at a site. This plot has 14 traces, with each trace representing a single counter channel among all of the counters at a site. For example, the C.V. of channel 1 from all the counters is calculated, same for channel 2... This provides a method of determining which channels are out of alignment or malfunctioning. As with the previous set of options, a high C.V. value indicates a defective channel.

Combining options 10-12 and 13-15 enables the detection of malfunctioning counters and helps identify the problem therein. Once a malfunctioning counter has been identified, further diagnosis with another program, **CHANVIEW©** (about to be upgraded) can be performed. Alignment or replacement recommendations can be based on the final analysis.

(16) DAILY MAX OF DER*DIFF ALGORITHM 6 POINT BOXCAR
(17) DAILY MAX OF DER*DIFF ALGORITHM 10 POINT BOXCAR
(18) DAILY MAX OF DER*DIFF ALGORITHM 14 POINT BOXCAR

Options 16-18 display the results of applying the DER*DIFF algorithm (see option 8 for information regarding the algorithm) to the flight activity for each hive at a site. These options plot the maximum values of the DER*DIFF algorithm obtained by scanning the entire day using a predetermined boxcar length. The length of the boxcar dictates the length and severity of the change in flight activity detected by the DER*DIFF algorithm. Peaks composed of all seven hives indicate an event that affected the entire site equally. Peaks composed of one or two hives may indicate an event that provoked a change in activity for those hives.

(11) SIMULATE THE FIELD DISPLAY

This option allows demonstration and testing of the field display using previously collected data. This is a fully functional field display. All dates and sites are included and trapped. If data doesn't exist for the date and site that you choose, you will be forced to re-enter a new date. The user can choose between a format that displays the total flight activity for all days including the current day, and a format that plots both in and out flight data for the current day and for the previous

five days. A new version that will include the daily summary indicator alarms is being implemented in the field software.

(12) PLOT FOUR DAYS/SITES SIMULTANEOUSLY (For One of More Sites)

This option allows plotting of up to four days of raw or smoothed site-wide flight activity data. The days selected could be from the same site or from different sites. The user only needs to know the site and year for which data exist to produce a date selection table. A date from this table must be chosen and verified. It is possible to select one day from four sites if there are four sites collecting data during that day. It is also possible to select any combination of days and sites. The purpose of this option is to compare activity at different sites. An excellent example of this is the comparison between Fort Missoula and the Maryland sites. Fort Missoula typically has a long steady forage period, while the Maryland bees forage with more intensity over a shorter period during the day. Another comparison can be made about the location of forage materials. When foraging begins, there is a lag time difference between the outgoing bees and the incoming bees. This difference should be smaller if forage materials are available within a short distance of the hives.

(15) REVERSE BACKGROUND / FOREGROUND COLOR

If you dislike the default black background, you can choose any color you would like. You just need to know the codes. Here's a hint, black is 0 and white is 4144959. The others are in between. We strongly suggest using the defaults displayed when you are asked to provide the color numbers. Even the lightest colors I've tried are too dark and/or conflict with other colors chosen for the data traces. **Pressing ENTER at both prompts will change the background from black to white and the text from white to black**. You may have to activate this option twice to get the background color to change to reverse. To return to the black background/white lettering, simply enter this option again and press ENTER for the two prompts. Eventually this software will include an improved, user selectable color scheme for identification of all the channels.

(19) DELETE OPENED DATA FILES FROM DISK, LEAVES COMPRESSED FILES ALONE

This option will clean up the decompressed data files in the TEMP and PLOT directories. It will detect whether the CD-ROM is being used to determine which directories should have extraneous data files deleted. Only decompressed data files and plot files will be deleted.

(20) END SITE/STUDY VIEWER MENU AND EXIT PROGRAM

This ends the session and exits the program.

APPENDIX B

PROPOGATION OF ERROR ANALYSIS FOR REPORTED CONCENTRATIONS

Appendix B
Propagation of Error Analysis for Reported Concentrations

Propagation of error analysis was performed on the measurements and calculation stream that lead to reported contaminant concentrations. This places an upper bound on the uncertainty associated with reported levels.

The three values utilized in computing reported concentrations are shown in Eqn. B-1

$$\text{conc. (nanograms/liter)} = (a \pm \sigma_a) / (m \pm \sigma_m) / (V \pm \sigma_V) \qquad \text{B-1}$$

where a is the absolute ion abundance for the contaminant of concern, m is the slope of the calibration curve's best-fit regression line; V is the volume of air that drawn through the sorbent trap, and the σ's are the absolute uncertainties for each of the variables.

The uncertainty in the absolute ion abundance, σ_a, has been estimated from the data used to construct each contaminant's abundance vs. nanograms calibration curve according to the formula of Eqn B-2 {1}.

$$\sigma_a \approx \sigma_a = [\sum(d_i^2) / (n-2)]^{1/2} \qquad \text{B-2}$$

The d_i's represent the vertical deviations between each data point and the best fit straight line. The number of observations used in the regression is n.

The uncertainty in the regression line slope is computed from the standard formula for a least-squares linear regression:

$$\sigma_m = [\, n \sigma_a^2 / \{ n \sum(c_i^2) - (\sum c_i)^2 \} \,]^{1/2} \qquad \text{B-3}$$

where the c_i's are the concentrations of the standard solutions measured for the calibration curve.

The uncertainty of the volume measurement, σ_V, has been estimated from pump factor plots such as that shown in Figure 3.4 (page 3-14).

Overall uncertainties in the computed concentration are dependent on the magnitude of the absolute ion abundance. Since the error in the ion abundance, σ_a, is constant, its relative impact is reduced as the magnitude of the abundance, a, increases. Two values have been computed and

tabulated in Table 3.4 -- one for a concentration from a strong "hit" at 1000 ng, a second for a trace level observation at 100 ng. Computational errors for a 1000 ng sample size cluster around 10%. Those for a 100 ng sample have the potential for greater error, in the range of 17% to 141%. Because of these results, trace level observations, i.e., those less than 10 ppt, are only reliable to one significant figure. Values greater than 100 ppt are probably good to two significant digits.

1. Harris, Daniel C., 1995. **Quantitative Chemical Analysis. Fourth Edition**. W.H. Freeman, New York, NY, 837p

Contam-inant	Regression Slope	r^2	% error 1000 ng	% error 100ng	A ipl * A = ppt	B ipl * B = ng/m3
PCE	35.0±0.3	1.00	9	17	4.20	28.6
TCM	41.9±1.0	0.99	10	49	3.79	23.9
TCE	39.1±1.5	0.98	11	65	4.74	25.6
Benz	124 ± 8	0.91	13	141	2.50	8.06
Tolu	121 ± 1	1.00	9	19	2.19	8.26
Etbz	104 ± 2	1.00	10	28	2.21	9.62
DCB	68.3 ± 1.0	1.00	9	28	2.43	14.6
Naph	189 ± 4	0.99	10	38	1.01	5.29