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Identification of Plant Sources for Wildflower Honey Production, Final Report RIF-2008-10

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Identification of Plant Sources for Wildflower Honey Production

Final Report RIF-2008-10

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Introduction

Recent trends in the food industry have created a demand for high quality, locally produced products. Wildflower honey produced in a specific region will have unique flavour, aroma, and colour characteristics due to the variety of plant sources involved in its production. Knowledge of the botanical origin of honey is useful for making decisions on which plants to maintain in a certain area, and for product marketing purposes.

The major objectives of this project were to identify the plant sources used for the production of wildflower honey on a local beekeeper's property, and to determine how the quality and general characteristics of the honey changed throughout the summer with respect to the seasonal horticultural shift.

Secondary objectives based on the project proposal requirements included collecting background information on the local honey industry to identify areas for future applied research, and material that could be used for course content and curriculum development. The project also provided an employment opportunity for a co-op student in the recently established biotechnology applied degree program.

Field Sampling

Field trips to the test site were conducted every two to three weeks throughout the project and reports were written detailing the findings for each visit. Figure 1A (Appendix 1) is an overview of the test site, and is marked with the sampling locations. Test sites 1/3 and 2 were permanent sampling sites that were fully monitored during each visit. Test sites 1 and 3 were adjacent to each other. Location 1 was monitored during the first visit, but the test plot was moved to location 3 for subsequent visits so that it would not interfere with the activities of a local farmer. The area of each of these sites was five square metres. Air temperature, light conditions, bee activity (number of visits per minute), vegetation, and general weather conditions were monitored. Data on wind speed, humidity, and UV index on the day of each sampling trip was obtained from the nearest Environment Canada monitoring station to track the climate conditions over the summer. Sites 4, 5, 6, 7, and 8 were specific locations where plant material was collected based on observed bee activity or when known honey plants were in bloom. Soil samples were also taken from several sites to determine the level of nutrients in the area.

Table 1A (Appendix 1) summarizes the data collected during the field trips. As expected, there was a marked change in the bee activity and types of plants in bloom throughout the season. Bee activity was difficult to monitor for several reasons. The bees are less inclined to go on foraging trips at temperature below 15°C, or during cloudy or windy conditions¹. Also, bees can collect nectar up to 4 km away from their hives². Since the furthest sampling site was only 1 km away, it wasn't possible to monitor all of the plant sources involved. Site 7 showed the most bee activity of all of the test sites, and there were significant counts recorded around individual honey plants such as cucumbers, zucchini plants, pumpkins, and Rose of Sharon bushes. The plant sources listed in this table will be discussed in detail in the next section.

Table 1B (Appendix 1) summarizes the soil testing results for potassium (K), nitrogen (N), phosphorous (P), acidity (pH) and moisture. Potassium was adequate or in excess, however, the test sites were generally poor in nitrogen or phosphorous. The acidity was close to a neutral pH of 7 for all sites. Although these are not ideal growing conditions for cultivated plants, various types of wildflowers, trees and bushes thrive in these conditions. The moisture results ranged from 9-12%, with the exception of site 2, which was at almost 25%. This site is near the garden and house of the property owners, and is routinely watered, whereas the other sites only receive moisture through precipitation.

Plant Source Identification

A literature search conducted at the beginning of the project found various lists of local honey plants.^{3,4,5} Determining the plant sources in specific geographical regions where honey is synthesized reveals honey's identity, how it could be promoted and price justification. For example, Clovermead Apiaries sell their summer blossom (mixed wildflower source), blueberry, clover, golden rod, basswood and buckwheat honey at different prices. In this study, more than forty plant samples were collected and tested. The data collected was analyzed to determine whether or not there was a correlation between the volatiles emitted by the plants and those present in the honey. Table 2A (Appendix 2) summarizes the plants identified. Several field guides proved useful to confirm the identity of the plants involved.^{6,7,8}

Melissopalynology is the study of honey and the pollen related to its botanical origin. It is a very labour intensive and time consuming technique, which is best done by plant experts. Other techniques used for determining the origin of honey are performed by testing the actual honey itself instead of the

plant material. The honey is tested to determine its amino acid profile, inorganic content, sensory characteristics, and volatile profile. For our project, some pollen analysis was performed using light microscopy, however, the majority of our analysis was done by volatile profiling using headspace solid phase microextraction with gas chromatography/mass spectrometry (SPME/GC-MS) on both honey and the plants suspected of being nectar sources.

The SPME sampling device consists of a coated fibre contained inside a syringe assembly. When the plunger is pushed down, the fibre is exposed to a plant sample. Any volatile chemicals given off by the plant are trapped in the fibre coating. The device is then placed inside a heated injection port of a gas chromatograph/mass spectrometer (GC/MS) instrument, where the trapped chemicals are released and analyzed. The GC/MS instrument is capable of determining the identity and concentration of the trapped chemicals.

During field sampling, plant samples were collected and put into glass vials. The vials were immediately sealed with a cap containing a hole blocked by a Teflon liner. The samples were kept refrigerated until analysis. During testing, the vials were heated to 40°C in a water bath for one hour so that the plant material could release its volatiles. The SPME device was then pushed through the Teflon liner of the bottle cap and the fibre was exposed to the sample. After 30 minutes, the SPME device was removed and put into the GC/MS instrument for analysis.

Table 2B is a summary of the dominant compounds identified in each of the plant samples tested and their concentrations. Although most plants emitted a variety of volatiles, a few plant samples had definitive marker compounds. For example, 96% of the volatile material from white sweet clover is due to a single chemical, 2H-1-benzopyran-2-one. Many of the plants tested gave off benzaldehyde and benzene acetaldehyde in various amounts (Table 2C). These two compounds were also detected in the honey samples, which will be discussed in detail. Flowers within the same biological family give off similar compounds. For example, pumpkin and zucchini emit some of the same volatiles, as did red and white clover. Different anatomical parts of the same plant also had similar profiles. Both cherry blossoms and leaves gave off significant amounts of benzaldehyde.

Many volatiles released by the plants did not appear in the honey. The dominant compound in this category was (E)-2-hexenal, which was present in sixteen of the plants. This chemical is a known bee attractant and is given off by plants pollinated by honey bees.⁹ 1-Octene-3-ol, is another known compound within this category.¹⁰ It was present in significant quantities in the red clover sample, accounting for 66% of the volatile material. This compound was also dominant in bull thistle, mullein, field mint, violet, and rough-fruited cinquefoil. Benzaldehyde and benzene acetaldehyde are also in this category, as is limonene, 2-phenylethanol, and methyl salicylate.¹¹ Project test plants emitting

limonene were golden rod and Queen Anne's Lace. False Solomon's Seal and lamb's quarters showed evidence of 2-phenyl alcohol emission in their SPME results, and methyl salicylate was found in the burdock and field mint samples.

Besides scent, bees are also attracted to plants based on colour, in particular blue, purple, violet, yellow and white.¹² Many of the plants collected in our field study fell into these colour categories. Examples of blue flowers were chickory and bluet. Purple and violet flowers found at the site consisted of violets, burdock, cow vetch, bull thistle, and red clover. Yellow flowered plants included rough-fruited cinquefoil, evening primrose, mullein, dandelion, greater celandine, and wild mustard. Examples of white-coloured flowering plants tested included Queen Anne's Lace, white and sweet clover, aster, deutzia and Rose of Sharon.

Honey Analysis

Three batches of honey were collected. Each was tested for its volatile profile by headspace solid phase microextraction to identify any compounds that may account for its aroma, taste, or flavour characteristics, as well as its botanical origin. Some chemical tests routinely used in honey analysis were also performed, including colour intensity, sugar content (fructose:glucose ratio), acidity, and moisture. Amino acid profiles were obtained to determine if there was a significant difference in composition. These profiles have also been used to provide information on the botanical origin of honey.¹³ Infrared spectra were also obtained. These spectra can indicate any significant differences in the chemical structure between batches, and if any unusual impurities are present.

The procedure used for the solid phase microextraction (SPME) analysis on the honey samples was similar to that for the plant sample testing. For honey analysis, 70g of sample was used. The sample was heated and stirred for one hour before being sampled by the SPME device and then analyzed by the GC/MS. Three samples were analyzed from each batch of honey.

Table 3A summarizes the compounds identified in the honey samples tested during the project, along with their concentrations. All of the compounds detected had been previously reported in the literature for SPME analysis of honey.^{14,15} All batches contained 3-methyl-butanoic acid, 2-methyl-butanoic acid, 3-methyl-pentanoic acid, benzaldehyde, and benzene acetaldehyde. There were, however, significant variations in concentrations between the batches. The third batch, extracted on August 6th, had the greatest variety of compounds and contained significant amounts of 4-methylpentanenitrile, which was not found in the other two batches. The second batch, extracted on

July 26th, had highest concentration of benzaldehyde, but did not have the same variety of volatiles. This is not surprising, since this batch had the lightest colour of the three. It did, however, contain 3-phenyl-2-propenal, which was not present in any other batch. This chemical is the main flavour component of cinnamon and its common name is cinnamaldehyde. The first batch of the season was extracted on July 12th. The extractable compounds were intermediate in amount and variety. It was the only one of the three that contained hexane, a short-chain alkane.

3-Methylbutanoic acid, a known flavour compound responsible for a cheesy, sweaty-like flavour, has been known to be found in wildflower honey.¹⁶ It was detected in all our batches, along with 2-methylbutanoic acid and 3-methylpentanoic acid. None of the acid compounds were found in the plant samples tested. They were most likely formed in the oxidative atmosphere of the honey comb, where some aldehydes are oxidized to organic acids.¹⁷

Benzaldehyde and benzene acetaldehyde are flavour compounds that were identified in many of the plant samples. This may be the result of direct transfer from the bees to the final honey product without significant degradation by bee metabolism or the ripening process that occurs in the honey comb. Benzaldehyde, depending on its concentration, can impart bitter almond¹⁸, cherry¹⁹, and burnt sugar or caramel-like flavours.²⁰ It was found in significant quantities in both the cherry blossoms and cherry leaves tested. The bees would have used the nectar from the cherry blossoms as a food source, which would account for its presence in the regurgitated honey. Benzaldehyde emitted from cherry leaves may be trapped by the moisture that collects on leaf surfaces after a rainfall. Foraging bees collect water from leaves and bring it back to their hives to nourish the worker bees. It is possible that benzaldehyde may also have been transported back to the hives through this process.

Benzene acetaldehyde is a known flavour compound that is responsible for a flowery odour.²¹ The first batch had the greatest amount, which may be due to the significant amounts of fruit trees and wildflowers in bloom during the period of its production.

Other types of honey tested included President's Choice Wildflower Honey, (a commercial supermarket brand), and samples from Clovermead Apiaries, Munro Honey and Muskoka Honey. Only limited quantities were available from these other sources so smaller vials and amounts of honey were used. The ratio of the amount of honey to the amount of vapour phase near the top of the vial was consistent with the larger vials. Extensive analysis was not possible due to time constraints and limited sample sizes. The results did, however, indicate some general trends. Buckwheat honey from two apiaries was the only honey tested that contained 2-methyl butanal, making this a potential marker compound. The basswood honey had significantly higher amounts of benzaldehyde, with values greater than 50% volatile content. All honeys tested showed traces of furfural, a compound formed

when sugars breakdown due to heat or acid. This compound, however, was not found in our test site honey. This may be due to the age of the honey from other the sources. The summer blossom honey from Clovermead apiaries also contained cinnamaldehyde.

Pollen analysis was also conducted on the project site honey samples. Table 3B lists the types of pollen found in the honey that could be matched to the pollen obtained from the plant samples. The pollen types correspond well with the plants in bloom around the time that each batch was collected according to Table 1A.

Table 3C lists the chemical tests results. The first test was a visual inspection of the product. According to Canadian federal government regulations, Canada No. 1 white grade honey must be free from foreign material that would be retained on a U.S. National Bureau of Standards standard 80-mesh screen (0.0055").²² No foreign particles were visible in the honey tested, and all samples had a pleasing colour. No off-odours were detected in any of the batches.

Colour intensity is typically measured using a Pfund meter. This specialized piece of equipment is not available in our laboratory, so an ultra-violet/visible (UV/VIS) spectrophotometer was used to provide an indication of relative intensity between samples. A wavelength of 560 nm was used based on standards published by the U.S. Department of Agriculture.²³ This wavelength is typically used to measure caramel-like colours. The third batch of honey had the highest absorbance reading, which would correspond to its dark amber colour. The second batch was had the lightest colour and also the lowest absorbance reading.

The moisture content of the honey was measured by a commercial beekeeper using a refractometer. The first two batches meet the Canadian regulations for No. 1 grade honey, which is moisture content not exceeding 17.8%. The third batch, a No. 2 grade honey, is slightly above this limit. The beekeeper noted that this result may not be accurate as the honey sample had been left out on a warm day just before the test, and was at a higher temperature than the test equipment at the time of analysis.

The main sugars in honey are fructose and glucose. Fructose is the sweeter of the two sugars and is less likely to crystallize out over time. All batches meet the Canadian regulations for blossom honey which has a minimum requirement of 65% (total of fructose and glucose content), however, the ratio between the two types of sugar varied between batches. All batches had values within the 0.76-1.86 range typically found in honey²⁴ but the first had a much lower value than the others. The pH of honey can be used as a relative measure of its acidity. All had similar values ranging from 3.4 to 3.6, which is within the typical 3.4-6.1 range listed by the U.S. National Honey Board.²⁵

Amino acid profiles were obtained for each of the batches. The results are presented in Table 3B. All had similar concentrations for the more acidic amino acid histidine. The amino acids with basic

functional groups such as methionine and cysteine were either not detected or present in low amounts, with the exception of lysine, which was present in all three batches. Proline, the major amino acid component in honey, was found in the highest concentration in batch 1. Batch 3 contained a very low level of this amino acid.

Figure 3A shows a comparison between the infrared spectra of all three batches. All batches have a very similar overall chemical composition. No significant impurities or unexpected anomalies were found. All profiles have major bands that are typically found in honey samples²⁶ such as a broad alcohol band at 3300cm^{-1} , a strong carbonyl band at 1640cm^{-1} , and a series of broad closely spaced bands from $1500\text{-}800\text{cm}^{-1}$, due to the various functional groups present in the sugars.

Sensory evaluation (organoleptic analysis) was performed on the honey. In this series of tests, a product is examined using the five senses or organoleptic attributes: colour, odour, taste, texture and noise. This type of analysis is used in many areas to establish a product profile and to determine its consumer perception. It can also be used in quality control testing. In industry, the organoleptic profile is established using experts, controlled techniques, and statistical analysis. Untrained panelists are also used for consumer taste testing. For honey, sensory analysis can be used to distinguish between products originating from different plants, as well as any defects, such as fermentation by-products, impurities, and off-odours or flavours.^{27,28} Parameters tested include the appearance of the honey (colour intensity and colour tone), scent (intensity of scent and description of the odour), and taste (sweetness, acidity, bitterness, intensity of aroma, aftertaste and other mouth perceptions). Physical characteristics typically tested include grittiness and spreadability. According to the Canadian Honey Council, when judges evaluate liquid honey for competitions, the parameters they look for are: density (moisture content), freedom from crystals, freedom from foreign material, freedom from air bubbles (in suspension or froth), brightness, flavor and aroma, colour, product uniformity, and fill, appearance and uniformity of the containers used.²⁹

In this study, a sensory evaluation test was performed on the three batches in order to determine which one had the most desirable characteristics for product marketing purposes. The data was analyzed to determine any correlation between the preferred batch and the lab test results. An untrained panel was set up using twenty volunteers from the college. Appearance, taste, odour grittiness, runniness, and spreadability were all tested. The sensory evaluation form used for the test is included in Appendix 3 - Table 3D. Table 3E summarizes the results. The batches were identified as A, B, and C. These corresponded to test site batches extracted on July 12 (A), July 24 (B), and August 6 (C). For the aroma/odour category, the dominant parameter was the "floral" classification, with Batch A having the highest score. This corresponds with the findings from the SPME analysis. This

sample contained the highest amount of benzene acetaldehyde, which is responsible for a flowery odour and taste. Sample C, however, had the most intense aroma for all the other categories, whereas Sample B generally demonstrated only very weak aromatic traits. Sample C also had the most dominant taste characteristics in the majority of the categories tested, except for sweetness, floral, and fruity/citrus categories, where A proved to be dominant. This also parallels the SPME results, since Sample C had the greatest variety of volatile compounds.

Industry Overview

An important aspect of biotechnology is generating economic activity from a biological process. In Canada, about 80% of honey is produced by commercial companies, and 20% by hobbyists³⁰. Seven hundred million pounds is produced annually, with two-thirds generated from Alberta, Manitoba and Saskatchewan, and one-third from the remainder of the country. Approximately one half of this honey is exported, mostly to the United States. Worldwide, Canada is the 11th top producer of honey³¹.

Recent food marketing trends have targeted local products including honey. London- Middlesex has produced and distributed a local food guide, in which honey and spreads such as maple syrup are listed as a major category. Our project site gave us an insight into honey production on a small, hobby-scale level. This is an important segment of the industry since approximately 80% of the 100,000 beekeepers in Canada operate on this level.³² Registration is required through the Ontario Beekeeper Act³³. The honey produced at these sites is usually sold locally and not exported.

Local beekeepers can add to their knowledge through industry organizations such as the Ontario Bee Association. Small scale producers have the option of being able to harvest several batches of honey, with each batch having its own distinctive flavour. Their various products can be marketed as spring, summer, and autumn wildflower honey. Beekind, a honey producer based in California, markets seasonal wildflower honey with advertising information describing the characteristics of each type. Spring wildflower honey is described as having a fruity, floral flavour; summer honey as having herbal overtones, and autumn honey as having a dark colour and rich flavour.³⁴ These descriptions also correspond to the batches of honey produced at our test site. Multiple harvests are too labour intensive to be practical for large scale producers.

The project team toured a local company, Clovermead Apiaries, in Alymer, Ontario. Due to price pressures in the global market place, it is difficult to realize profits through honey sales alone. Therefore, Clovermead has expanded into agritourism, and a retail facility which sells other bee-related products such as propolis, royal jelly, medicinal creams and ointments. The soaps they sell are made in

partnership with another local company, From the Meadow, which produces products from local plant sources. Clovermead also operates one of the largest bee pollen ventures in North America³⁵ through their company API Nutrition.

The project team also toured Munro Honey, the largest commercial honey producer in Ontario. We were shown the processes and equipment used for the large scale production of honey as well as their warehouse facilities . Munro exports honey to the United States, and operates a meadery that sells award-winning meads and honey wines to an international market. The meadery gave us the opportunity to observe an industrial scale biotechnological fermentation process.

Areas for Future Research & Curriculum Development

During the course of the project, several areas of viable future research became apparent. A literature search showed that natural products made from honey or other bee-related products are a thriving and growing industry. Our small survey of the local industry identified retailers of products such as creams, ointments, soaps, candles and health supplements. According to the Food and Agriculture Organization of the United Nations, bee-related products, in particular beeswax, may be used in batik fabric production, waterproofing agents for wood and leather, manufacture of electronic components and CDs, modelling and casting procedures in industry and art, polishes for shoes, furniture and floors, and specialized industrial lubricants.³⁶ Although honey has been used as a home remedy for centuries, it is worth noting that apitherapy also has a place in modern medicine. The Multiple Sclerosis Association of America and the National Multiple Sclerosis Society are participating in clinical trials involving the use of bee venom as a form of treatment.³⁷ ApiNate® is a bandage/wound dressing product marketed by a Japanese company.³⁸

The foundation of future honey-related projects may lie within these products and/or processes. The wildflower component of the project could also provide background for future research projects involving insect/plant interactions, food analysis, herbal potions. The biological and ecological aspects of the project hold great potential for expansion at a later date. Data collection over multiple seasons would be informative. Currently these results sit in isolation but many avenues of exploration remain. This study has established a number of parameters for statistical analysis which would provide useful ecological and ethological data that could be compared to the soil, weather, barometric pressure and light intensity conditions existing throughout the honey season.

Several aspects from the project will be used as course content in the applied biotechnology degree program. The analysis method used for testing the sugar content of honey is being incorporated into the laboratory portion of the Analytical Chemistry II course, and the feasibility of performing amino acid profiles for the Instrumentation I course is currently under review. Information gathered during the Munro Honey tour will be available to the instructors of the bioprocess and manufacturing engineering courses.

The co-op student funded by the project will be using material collected over the summer for her Communications/Thesis course. The project also provided the student with the opportunity to develop useful employment related skills such as the operation of laboratory test equipment, literature search and report writing, and field work.

Conclusion

From the data gathered in the field studies and laboratory tests, we were able to identify the major honey plants present on the beekeeper's property. The dominant ones that made their way to the final product in significant amounts were red clover, white clover, sweet white clover, dandelion, cherry, and maple. Traces of a variety of wildflowers and other plants were also found. These included Queen Anne's Lace, False Solomon's Seal, basswood, deutzia, violet, wild mustard, celandine, cucumber, buckwheat, evening primrose, and aster.

The beekeeper can now use the botanical origin information for making decisions on bee habitat preservation and hive placement. He can also use the results from the sensory evaluation testing for marketing purposes. Findings from the industry overview section of our report also identified a range of bee-related products that can be sold to consumers.

The volatile analysis results from the solid phase microextraction (SPME) sampling of the wildflowers and honey samples resulted in some interesting scientific correlations. Many of the plants tested require bees for pollination and give off volatile compounds to attract them. Several of these compounds were detected by the SPME device and were identified with the gas chromatography/mass spectrometer (GC/MS) instrument in the lab, providing some valuable insight into plant/insect interactions.

The SPME analysis of honey samples detected several known aroma and flavour compounds such as benzaldehyde and benzene acetaldehyde. The latter is known to impart a floral aroma, which was also confirmed in the sensory evaluation test. The first batch of the season had the highest amount of

this compound based on the SPME analysis results, and was also rated the highest in this category by the taste testing panel.

The chemical test results indicated that the quality of all batches tested met various regulatory requirements for sale in Ontario, however, there were definite differences between the batches. Colour intensity was the most obvious. The SPME results showed a variety of compounds present in the batches which explained their different flavour characteristics. Sugar analysis results also provided useful information about the product. For example, the first batch of the season had the lowest fructose:glucose ratio and can therefore be expected to crystallize out sooner than the other batches. Although this does not affect honey quality, it is information that should be passed on to consumers who may not be aware that unpasteurized honey can granulate over time. When this occurs, the honey can then be used as a thick spread, or liquefied by gentle heating.

The possibility of using the information collected over the summer for publication or poster/seminar presentations is currently under discussion. Some of the lab procedures used will be incorporated into the program this fall and the data generated also provides the basis for future research in the areas of honey production, plant-insect interaction, and wildflower volatile extractables. This project provided the opportunity to promote the college and the applied biotechnology degree program to the local agricultural community through the demonstration of our applied research capabilities.

Appendix 1 - Field Sampling

Table 1A - Field Sampling Data

Date	Test Plot	Time	General Weather Condition	Temp. °C	Light Reading (Lux)	UV Index	Pressure (kPa)	Tendency	Dew Point	Humidity (%RH)	Wind Direction	Wind Speed (km/h)	Bee Activity (average # of visits /minute)	Vegetation	
														Dominant	Trace
27-May-08	1	12:45 PM	sunny	15.2	73,800	9	101.6	Rising	4.3	80%	NNW	22	1.2	wild mustard*, grass, red clover (not in bloom)	dandelion
	2	2:10 PM	sunny	15.0	81,200	9	101.6	Rising	4.3	80%	NNW	22	1.1	violets*, creeping charlie, field cress	yellow clover, blue, tulip
	hives		sunny		81,200	9	101.6	Rising	4.3	80%	NNW	22	1.1	grass, maple pollen	
9-Jun-08	2	12:35 PM	sunny	32.9	59,000	10	100.8	Falling	22.3	79%	SSW	37	0	creeping charlie, wood sorrel, yellow clover	smart weed, white clover
	2B	12:51 PM	sunny	32.9	59,000	10	100.8	Falling	22.3	79%	SSW	37	4.8	white clover*	
	2C	12:53 PM	sunny	32.9	59,000	10	100.8	Falling	22.3	79%	SSW	37	4.6	white clover*	dandelion, wild raspberry, chokecherry, American Elm, yellow wood sorrel, Norway maple
	3	11:05 AM	sunny	28.0	65200	10	100.8	Falling	22.3	79%	SSW	37	0	grasses, red clover, lamb's quarters, wild mustard	dame's rocket, celandine white clover, False Solomon's seal, cherry blossoms
	4		sunny			10	100.8	Falling	22.3	79%	SSW	37	0		
	5	1:37 PM	sunny	32.9	59000	10	100.8	Falling	22.3	79%	SSW	37	7.8	deutzia*	
	6	1:38 PM	sunny	32.9	59000	10	100.8	Falling	22.3	79%	SSW	37	0.4	Eastern white pine*	

Notes

UV index, Pressure, Tendency, Dewpoint, Humidity, Wind Direction, Wind Speed values taken from daily reports posted by the nearest Environment Canada weather station

*bee activity observed around this plant

plant names in bold face = known honey plant

Date	Test Plot	Time	General Weather Condition	Temp. °C	Light Reading (Lux)	UV Index	Pressure (kPa)	Tendency	Dewpoint	Humidity (%RH)	Wind Direction	Wind Speed (km/h)	Bee Activity (average # of visits /minute)	Vegetation	
														Dominant	Trace
30-Jun-08	2	2:04 PM	mostly cloudy	20.1	15800	7	100.7	Rising	13.9	86%	NW	13	0	grasses, red clover	wild raspberry, chokecherry, American Elm, yellow wood sorrel, Norway maple
	3	12:42 PM	mostly cloudy	19.5	11,000	7	100.7	Rising	13.9	86%	NW	13	0	grasses, creeping charlie, violets (not in bloom), yellow clover	white clover, bladder campion
	4		mostly cloudy		11,000	7	100.7	Rising	13.9	86%	NW	13	0	feabane, cow vetch, rough fruited cinque-foil, lamb's quarters	field mint, Herb Robert
	6	12:46 PM	mostly cloudy	19.5	11,000	7	100.7	Rising	13.9	86%	NW	13	76	cherry trees* (not in bloom, site of bee swarming activity)	
	7		mostly cloudy		11,000	7	100.7	Rising	13.9	86%	NW	13	0	grasses	viper's bugloss, yarrow
14-Jul-08	2	1:13 PM	variable cloudiness / sunny periods	27.0	20,000-86,000	8	100.9	Rising	13.0	62%	W	26	0.4	yellow clover, white clover	common mallow, wood sorrel
	3	2:22 PM	variable cloudiness / sunny periods Some clouds/ sunny periods	25.0	14,600-85,000	8	100.9	Rising	13.0	62%	W	26	0	red clover (not in bloom)	raspberry canes, Queen Anne's Lace, burdock, red clover
	4	2:05 PM	clouds/ sunny periods	27.0	18,500-96,600	8	100.9	Rising	13.0	62%	W	26	0.4	grasses, Queen Anne's Lace	jewelweed, sweet clover, aster

Notes:
 UV index, Pressure, Tendency, Dewpoint, Humidity, Wind Direction, Wind Speed values taken from daily reports posted by the nearest Environment Canada weather station.
 *bee activity observed around this plant
 plant names in bold face = known honey plant

Date	Test Plot	Time	General Weather Condition	Temp. °C	Light Readin g (Lux)	UV Index	Pressure (kPa)	Tendency	Dewpoint	Humidity (%RH)	Wind Direction	Wind Speed (km/h)	Bee Activity (average # of visits /minute)	Vegetation	
														Dominant	Trace
28-Jul-08	2	10:20 AM	mostly sunny, with some clouds	24.0	59,300	9	101	Rising	17.6	64%	W	15	0.2	grasses, violets (not in bloom), white clover, yellow clover	Heal-all
	3	10:38 AM	mostly sunny, with some clouds	27.0	64,500	9	101	Rising	17.6	64%	W	15	0	red clover, Queen Anne's lace, burdock	
	7	11:07 AM	mostly sunny, with some clouds	26.0	60,500	9	101	Rising	17.6	64%	W	15	1.8	chickory*, Queen Anne's Lace*, white sweet clover*	mullein, golden rod
	8D	10:22 AM	mostly sunny, some clouds	24.0	59,300	9	101	Rising	17.6	64%	W	15	11.8	zucchini*	
	8E	10:16 AM	mostly sunny, with some clouds	24.0	59,300	9	101	Rising	17.6	64%	W	15	11.8	cucumber*	
11-Aug-08	2	10:55 AM	sunny with clouds	25.0	46,200-60,300	8	101.4	Rising	14.4	88%	NW	9	0.4	grasses, violets (not in bloom)	white clover, smartweed
	3	10:33 AM	sunny	24.9	42,000	8	101.4	Rising	14.4	88%	NW	9	1.2	Queen Anne's lace*, burdock, red clover, golden rod	asters, dandelion, goat's beard
	5	11:39 AM	sunny	21.0	46,200-60,300	8	101.4	Rising	14.4	88%	NW	9	74.5	Rose of Sharon (white)*	
	7	11:33 AM	sunny	22.0	54,700	8	101.4	Rising	14.4	88%	NW	9	3	Grasses, Queen Anne's Lace*, viper's bugloss, chickory, golden rod, sweet clover*	asters, yarrow, bull thistle
	8D	10:55 AM	sunny	21.0	46,200-60,300	8	101.4	Rising	14.4	88%	NW	9	11.2	zucchini*	
	8E	11:02 AM	sunny	21.0	46,200-60,300	8	101.4	Rising	14.4	88%	NW	9	2.2	cucumber*	
	8F	11:02 AM	sunny	21.0	46,200-60,300	8	101.4	Rising	14.4	88%	NW	9	4.2	pumpkin*	

Notes

UV index, Pressure, Tendency, Dewpoint, Humidity, Wind Direction, Wind Speed values taken from daily reports posted by the nearest Environment Canada weather station

*bee activity observed around this plant

plant names in bold face = known honey plant

Figure 1A - Test Site Overview

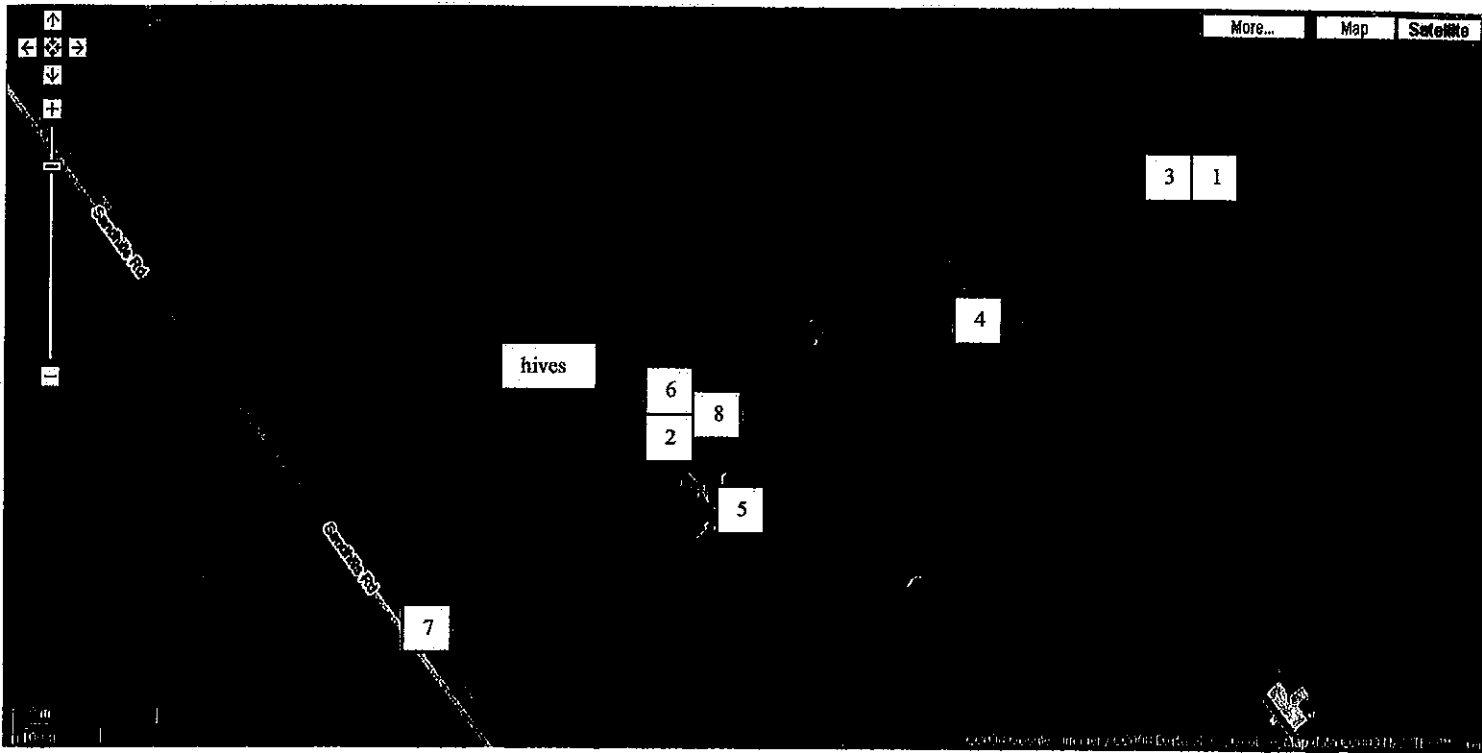


Table 1B - Soil Testing Results

Parameter	Site 1/3	Site 2	Site 4	Site 7
Potassium mg/g	1.8 adequate	4.9 surplus	2.5 sufficient	2.5 sufficient
Nitrogen mg/g	<0.03 depleted	0.03 depleted to deficient	0.03 depleted to deficient	<0.03 depleted
Phosphorous mg/g	<0.3 depleted	0.3 deficient	0.5 deficient to adequate	0.3 deficient
Acidity (pH)	6.5 slightly acidic	7.5 alkaline	6.5 slightly acidic	7.5 alkaline
%Moisture	12.1	25.4	12.7	9.5

Notes:

- 1) %moisture obtained by comparing the weights of the original sample to the air dried sample after 24 hours
- 2) results for potassium, nitrogen, phosphorous and pH obtained using test kits provided by: Luster Leaf Products, Inc, 2220 Techcourt Woodstock IL, 60098
- 3) test kit calibration documented in project laboratory notebook 1, pages 38, 42, 43, 49, 50, 54
- 4) descriptions for acidity, potassium, nitrogen, and phosphorous levels are based on the information provided in the soil kit manual and instructions

Appendix 2 - Plant Source Identification

Table 2A - Field Site Plants

(Known honey plants are shaded in grey)

Common Plant Name	Scientific Name
American Elm	<i>Ulmus americana</i>
Basswood	<i>Tilia americana</i>
Birdsfoot-Trefoil	<i>Lotus corniculatus L.</i>
Bladder Campion	<i>Silene vulgaris</i>
Bluet (Bedstraw)	<i>Galium triflorum</i>
Bull Thistle	<i>Cirsium vulgare</i>
Cherry	<i>Prunus sp.</i>
Chicory	<i>Cichorium intybus L.</i>
Chokecherry	<i>Prunus virginiana</i>
Common Burdock	<i>Artium minus</i>
Common Mallow	<i>Malva neglecta</i>
Common Mullein	<i>Verbascum thapsus</i>
Common Yarrow	<i>Achillea millefolium</i>
Cow Vetch	<i>Vicia cracca</i>
Creeping Charlie (Ground Ivy)	<i>Glechoma hederacea</i>
Cucumber	<i>Cucumis sativus L.</i>
Dame's Rocket	<i>Hesperis matronalis</i>
Dandelion	<i>Taraxacum officinale</i>
Deutzia Scabra	<i>Deutzia Scabra</i>
Eastern White Pine	<i>Pinus strobus</i>
False Solomon's Seal	<i>Maiathemum racemosum</i>
Field Cress	<i>Lepidium campestre</i>
Field mint	<i>Mentha arvensis</i>
Fleabane	<i>Conyza canadensis</i>
Golden Rod	<i>Solidago L.</i>
Greater Celandine	<i>Chelidonium majus</i>
Heal-all	<i>Prunella vulgaris L.</i>
Herb Robert	<i>Geranium robertianum</i>
Jewelweed	<i>Impatiens capensis</i>
Lamb's Quarters	<i>Chenopodium album</i>
Norway Maple	<i>Acer platanorde</i>
Queen's Anne Lace	<i>Daucus carota</i>
Pumpkin	<i>Cucurbita L.</i>
Raspberry	<i>Rubus sp.</i>
Red Clover	<i>Trifolium pratens</i>
Rough Fruited Cinquefoil	<i>Potentilla recta</i>
Rose of Sharon	<i>Hibiscus syriacus</i>
Smartweed	<i>Polygonum lapathifolium</i>
Sugar Maple	<i>Acer sacchaum</i>
Viper's Bugloss	<i>Echium vulgare</i>
White Clover	<i>Trifolium repens</i>
White Heath Aster	<i>Aster ericoides</i>
White Sweet Clover	<i>Melilotus alba</i>

Wild Mustard	<i>Sinapsis arvensis</i>
Woolly Blue Violet	<i>Viola sororia</i>
Yellow Clover	<i>Trifolium aureum</i>
Yellow Goat's Beard	<i>Trifolium pratense</i>
Yellow Wood Sorrel	<i>Oxalis stricta</i>
Zucchini	<i>Cucurbita pepo L.</i>

Table 2B – Summary of Dominant Volatile Compounds Emitted by Plant Samples during SPME Analysis

Plant Common Name	Compound	%
Basswood Tree Leaves	(Z)-3-hexen-1-ol	38.4
	(E)-2-hexenal	34.3
	(E,E)-2,4-hexadienal	7.6
Birdsfoot Trefoil	(E)-2-hexenal	23.9
	(E,Z)-2,6-nonadienal	12.5
	(E)-2-nonenal	12.2
Bladder Campion	1,3-dimethyl benzene	21.3
	methyl ester benzoic acid	14.2
	methyl ester octanoic acid	6.0
Bull Thistle	cedrene	28.5
	1-hexanol	21.6
	1-octen-3-ol	12.0
Cherry Tree Blossoms	benzaldehyde	85.6
	methyl benzoate	2.3
	(E)-2-hexenal	2.1
Cherry Tree Leaves	benzaldehyde	41.3
	(E)-2-hexenal	27.7
	hexanal	5.1
Chickory	(+/-)-2,6,6-trimethyl-bicyclo[3,1,1]hept-2-ene	26.3
	3-methyl-1-pentanol	21.7
	3-hexen-1-ol	10.3
Common Burdock	methyl salicylate	38.2
	1-hexanol	20.9
	(E)-2-hexenal	14.0
Common Mullein	1-octen-1-ol	55.6
	3-octanol	22.2
	3-octanone	7.1

Common St. John's Wort	caryophyllene	16.8
	IR-alpha-pinene	11.0
	beta-phellandrene	9.4
Common Yarrow	beta-phellandrene	41.4
	[3aS-(3a.alpha.,3b.beta, 4.beta., 7.alpha., 7aS*)]-octahydro-7-methyl-3-methylene-4-(1-methylethyl)-1H-cyclopenta[1,3]cyclopropa[1,2]benzene	28.6
	caryophyllene	14.2
Cow Vetch	1-octen-3-ol	48.2
	caryophyllene	30.7
	(E)-2-hexenal	3.2
Cucumber	(E,Z)-2,6-nonadienal	24.2
	(E)-2-hexenal	22.5
	(Z)-6-nonenal	16.3
Dame's Rocket	3,7-dimethyl-1,3,7-octatriene	65.8
	(E)-3,7-dimethyl-1,3,6-octatriene	10.3
	eucalyptol	7.3
Dandelion	(E)-2-hexenal	49.9
	1-hexanol	10.3
	hexanal	5.4
	(E)-3-hexen-1-ol	4.7
	(Z)-3-hexen-1-ol	4.3
Deutzia	6,6-dimethyl-bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde	37.5
	6,6-dimethyl-bicyclo[3.1.1]heptane-2-carboxaldehyde	21.4
	(1R)-6,6 dimethyl bicyclo[3.1.1]heptan-2-one	14.4
Eastern White Pine	(1S)-6,6-dimethyl-2-methylene bicyclo[3,1,1] heptane	40.4
	IR-alpha-pinene	24.3
	[3aS-(3a.alpha.,3b.beta, 4.beta., 7.alpha., 7aS*)]-octahydro-7-methyl-3-methylene-4-(1-methylethyl)-1H-cyclopenta[1,3]cyclopropa[1,2]benzene	10.2
Evening Primrose	3,7-dimethyl-1,6-octadien-3-ol	23.1
	(Z)-3-hexen-1-ol	13.3
	1-hexanol	13.3
False Solomon's Seal	3,7-dimethyl-1,6-octadien-3-ol	16.0
	phenyl ethyl alcohol	14.0
	[1S-(1.alpha., 3a.alpha., 3b.beta., 6a.beta, 6b.alpha)]-decahydro-3a-,ethyl-6-methylene-1(1-methylethyl)-cyclobuta[1,2:3,4]dicyclopentene	12.4

Field Cress	(Z)-3-hexen-1-ol	65.9
	1-octen-3-ol	9.2
	1R, 3Z, 9s-4,11,11-trimethyl-8-methylenebicyclo[7.2.0]undec-3-ene	4.8
Field Mint	1-octen-1-ol	35.7
	methyl salicylate	17.2
	caryophyllene	15.8
	alpha-caryophyllene	11.1
Fleabane	[4aR-(4a.alpha., 7.alpha., 8a.beta.)]-decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-Naphthalene	62.6
	E-2,6-dimethyl-1,3,5,7-octatetraene	5.6
	(Z)-3,7-dimethyl-1,3,6-octatriene	5.2
Golden Rod	limonene	50.8
	IR-alpha-pinene	18.0
	[3aS-(3a.alpha.,3b.beta., 4.beta., 7.alpha., 7aS*)]-octahydro-7-methyl-3-methylene-4-(1-methylethyl)-1H-cyclopenta[1,3]cyclopropa[1,2]benzene	9.0
Greater Celandine	1-hexanol	15.8
	1,1-dimethyl-3-methylene-cyclohexane	12.0
	(E)-2-hexenal	10.0
Herb Robert	caryophyllene	26.2
	(E)-2-hexenal	21.3
	[3aS-(3a.alpha.,3b.beta., 4.beta., 7.alpha., 7aS*)]-octahydro-7-methyl-3-methylene-4-(1-methylethyl)-1H-cyclopenta[1,3]cyclopropa[1,2]benzene	10.8
Jewelweed	(E)-2-hexenal	53.4
	(E)-2-hexen-1-ol	8.3
	(Z)-3-hexen-1-ol	6.7
Lamb's Quarters	(Z)-3-hexen-1-ol	40.7
	1-hexanol	12.2
	phenyl ethyl alcohol	8.4
Pumpkin	1,4-dimethoxy-benzene	76.7
	1-octen-1-ol	8.9
	2-ethenyl-1,1-dimethyl-3-methylene-cyclohexane	3.1
Queen Anne's Lace	(1S-endo)-1,7,7-trimethyl-bicyclo[2,2,1]-heptan-2-ol acetate	33.8
	limonene	16.0
	beta-phellandrene	15.2

Red Clover	1-octen-3-ol	65.9
	decane	8.5
	1-hexanol	3.9
Rose of Sharon	1-decyne	82.2
	Z-1,6-undecadiene	7.3
	5-ethylcyclopent-1-enecarboxaldehyde	2.3
	1-hexanol	2.1
Rough-fruited Cinquefoil	1-octen-3-ol	48.2
	caryophyllene	30.7
	(E)-2-hexenal	3.2
Sugar Maple Pollen	(1S)-6,6-dimethyl-2-methylene bicyclo [3,1,1] heptane	15.9
	(1S)-6,6-dimethyl-2-methylene bicyclo [3,1,1] heptane	12.6
	3-methyl-1-pentanol	10.5
Viper's Bugloss	(E)-2-hexenal	46.7
	1-hexanol	21.3
	(E)-2-hexen-1-ol	16.1
White Clover	3-octanone	53.8
	1-hexanol	11.6
	3-octanol	8.7
White Heath Aster	[3aS-(3a.alpha.,3b.beta., 4.beta., 7.alpha., 7aS*)]-octahydro-7-methyl-3-methylene-4- (1-methylethyl)-1H-	
	cyclopenta[1,3]cyclopropa[1,2]benzene	53.0
	(1S)-6,6-dimethyl-2-methylene bicyclo [3,1,1] heptane	13.5
	3,7-dimethyl-1,3,7-octatriene	11.6
White Sweet Clover	2H-1-benzopyran-2-one	96.0
Wild Mustard	(Z)-3-hexen-1-ol	29.2
	(E)-2-hexenal	12.8
	(Z)-2-hexen-1-ol	7.8
Woolly Blue Violet	1-octen-3-ol	60.3
	3,7-dimethyl-1,6-octadien-3-ol	16.6
	(E)-2-hexenal	7.0
Yellow Clover	3-octanone	56.0
	3-octanol	15.0
	1-hexanol	9.9
Yellow Goat's Beard	(E)-2-hexenal	23.3
	1-hexanol	22.6
	(E)-3-hexen-1-ol	13.1

Zucchini	1,4-dimethoxy-benzene	58.9
	1-octen-3-ol	21.4
	3-octanone	5.7

Table 2C - Benzaldehyde and Benzene Acetaldehyde Content of Plant Samples (Vapour Phase)

Compound	Benzaldehyde %	Benzene Acetaldehyde %
Basswood leaves	0.58	n.d.
Bull Thistle	2.52	n.d.
Cherry Blossoms	85.55	n.d.
Cherry leaves	41.33	1.14
Chickory	n.d.	2.23
Common Burdock	n.d.	2.25
Cow Vetch	6.62	n.d.
Cucumber	5.35	n.d.
Dame's Rocket	n.d.	0.11
Dandelion	3.84	4.61
Evening primrose	n.d.	3.42
Field Cress	n.d.	1.46
Greater Celandine	2.13	2.45
Jewelweed	1.51	n.d.
Lamb's quarters	2.30	n.d.
Maple Pollen	n.d.	1.33
Pumpkin	0.87	n.d.
Rough-fruited Cinquefoil	1.83	n.d.
Viper's bugloss	1.74	n.d.
Wild Mustard	2.23	3.16
Woolly Violet	0.64	n.d.
Yellow Goat's Beard	4.74	6.62

n.d.=not detected

Appendix 3 - Honey Analysis

Table 3A Honey Volatile Analysis by SPME

Compound	Test site honey (12/07/08)			Test Site honey (26/07/08)			Test Site Honey (06/08/08)		
	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
Hexane	1.8	2.1	1.9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2-Methyl propanenitrile	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.8	1.4	2.0
2-Methyl butanenitrile	2.3	1.8	1.6	n.d.	n.d.	n.d.	4.7	4.0	4.2
3-Methyl-butanenitrile	3.1	2.9	2.7	n.d.	n.d.	n.d.	6.2	5.3	6.4
2-Methylpropanoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.2	1.0	1.5
Toluene	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.0	1.1	0.9
4-Methylpentanenitrile	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	25.0	20.9	19.7
3-Methyl-butanoic acid	6.6	3.6	7.3	2.1	2.9	3.6	4.3	4.2	4.7
2-Methyl-butanoic acid	2.6	8.0	5.5	5.0	4.5	5.8	4.0	6.6	5.9
3-Methyl-pentanoic acid	10.9	10.3	11.4	5.0	4.3	6.9	7.2	7.8	7.8
Benzaldehyde	19.2	17.1	14.9	28.8	29.3	37.0	15.0	14.0	13.1
Benzene acetaldehyde	5.6	4.8	5.1	2.1	2.2	3.4	3.7	3.8	4.1
Benzyl nitrile	2.5	2.3	2.2	n.d.	n.d.	n.d.	5.1	5.6	6.0
3-Phenyl-2-propenal	n.d.	n.d.	n.d.	2.3	2.2	2.1	n.d.	n.d.	n.d.

results are in % mass of headspace extract

n.d.=not detected

Table 3B - Honey Quality Test Results

Parameter	Batch 1 July 12/08	Batch 2 July 26/08	Batch 3 August 6/08
Visual Appearance	-free from bubbles or particulate matter -golden colour -no off-odours detected	-free from bubbles or particulate matter -light golden colour -no off-odours detected	-free from bubbles or particulate matter -amber colour -no off odours detected
Colour Intensity (Absorbance at 560 nm)	0.083	0.051	0.134
Moisture Content (%)	16.5%	17.0%	18.0%
Sugar Content (Fructose:Glucose Ratio)	Fructose: 33.4% Glucose: 42.2% Ratio: 0.79	Fructose: 39.0% Glucose: 33.3% Ratio: 1.17	Fructose: 35.8% Glucose: 32.9% Ratio: 1.09
pH	3.56	3.41	3.60

Honey Quality Parameters Table 3B continued

Parameter	Batch 1 July 12/08	Batch 2 July 26/08	Batch 3 August 6/08
Amino Acid Profile			
Expressed as mg/g			
Histidine	0.005	0.016	0.012
Glycine	0.003	0.002	0.002
Arginine	n.d.	0.014	0.009
Alanine	0.005	0.003	0.007
Proline	0.107	0.071	0.014
Tyrosine	0.018	0.013	0.034
Valine	0.028	0.030	0.011
Methionine	0.001	0.002	n.d.
Cysteine	n.d.	n.d.	n.d.
Lysine	0.024	0.020	0.036
n.d.=not detected			

Methods Used for Chemical Testing

Moisture – refractometer

Absorbance – 1 cm plastic cells, Shimadzu UV/VIS 1700 instrument, distilled water blank

pH - 3g of sample dissolved in 30mL of water, result read with a calibrated pH meter, samples prepared in triplicate, average result reported in the above table

Glucose and Fructose Sugar Analysis

Iodometric titrations based on modified versions sections 954.11 (Separation of Sugars in Honey, 1990), and 959.11 (Glucose in Sugars and syrups, 1990) of AOAC Method Manual, 15th edition

Amino Acid Profile

Diethyl ethoxymethylene malonate derivatization followed by high performance liquid chromatography (HPLC) analysis, procedure used filed in project data binder#2, based on several references for derivatization techniques of amino acids in honey^{39,40,41}

Figure 3A - Infrared Spectrum Comparison

Conditions used:

Thermo Nicolet Avatar 360 Fourier Transform infrared spectrometer
attenuated total reflectance sampling device used in % reflectance mode
direct analysis of honey samples
32 scans, resolution = 4
scan range 4000-650cm-1
autogain
ZnSe crystal

See attachment

Figure 3A InfraRed Spectrum Comparison (Attachment to page 26)

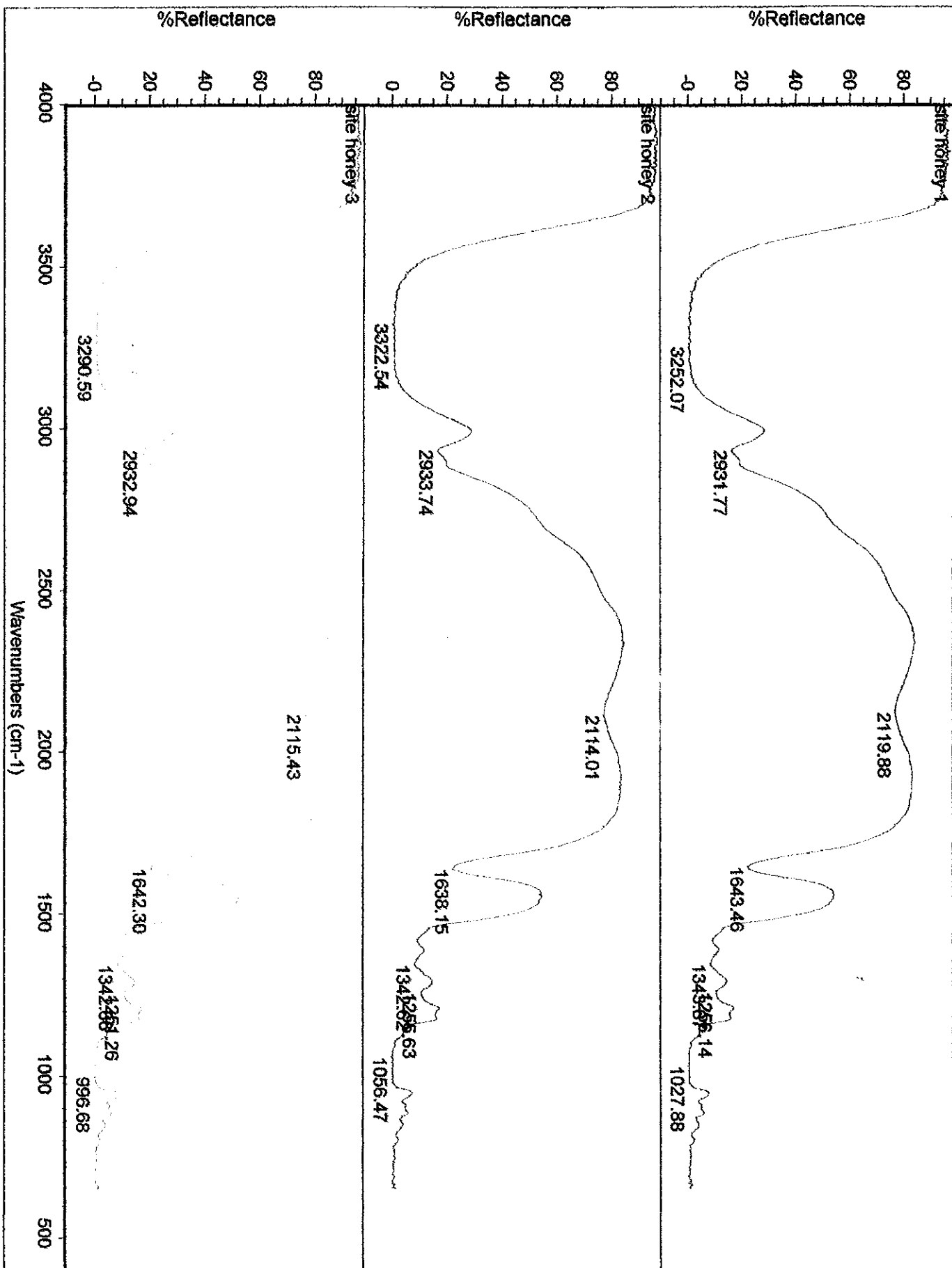


Table 3C - Pollen Grains Detected in Honey Samples

Batch 1-July 12/08	Batch 2-July 26/08	Batch 3 - August 6/08
Cherry	Celandine	Red Clover
Maple	Dandelion	Dandelion
Red Clover	Red Clover	Cucumber
Dandelion	White Clover	Buckwheat
False Solomon's Seal	White Sweet Clover	Evening Primrose
Deutzia Scabra	Cherry	White Clover
Basswood		Aster
Violet		
Wild Mustard		

Procedures Used:

Plant Samples

A few drops of ether were added to plant material on a microscope slide. After the solvent evaporated, excess plant material was removed with tweezers. The slides were then fixed with a mounting medium, and viewed with a light microscope using 40x magnification on the ocular lens and 10x magnification on the eye piece.⁴²

Honey Samples

Honey was weighed into plastic centrifuge tubes. Warm water was added, and the sample was centrifuged. The supernatant was decanted, and the pellet was resuspended in warm water. It was centrifuged again, and transferred to a microscope slide. The slide was heated, and then fixed with a mounting medium. The sample was viewed under a light microscope using 40x magnification on the ocular lens and 10x magnification on the eye piece.⁴³

Table 3D - Sensory Evaluation Test Form

Sensory Evaluation of Honey Samples

Instructions:

- 1) Taste one sample at a time and complete the survey questions before moving on to the next one.
- 2) Wait at least one minute, and drink a sip of water between samples.
- 3) Assign a rating for each characteristic using the following scale:
0 = absent 1 = weak 2 = medium 3 = strong
- 4) After testing all of the samples, complete the overall rating section at the bottom of the form.

Characteristic	Sample A	Sample B	Sample C
Appearance			
Foam/Air bubbles			
Colour Intensity			
Presence of sugar crystals or granules			
Aroma/Odour			
Floral			
Woody (e.g. cedar, pine)			
Vegetable/Herbal			
Caramel/Toffee			
Fruity/Citrus			
Spicy (e.g. cloves, nutmeg, cinnamon)			
Taste			
Acidity (sour)			
Salty			
Bitter			
Sweet			
Presence of an After Taste			
Floral			
Woody (e.g. cedar, pine)			
Vegetable/Herbal			
Caramel/Toffee			
Fruity/Citrus			
Spicy (e.g. cloves, nutmeg, cinnamon)			
Other			
Runniness			
Spreadability			
Solubility (how fast it dissolves in your mouth)			
Grittiness			

Overall Ranking	Sample A	Sample B	Sample C
Rate each honey in order of overall preference (1st, 2nd, 3rd)			

Table 3E - Summary of Sensory Evaluation Test Results

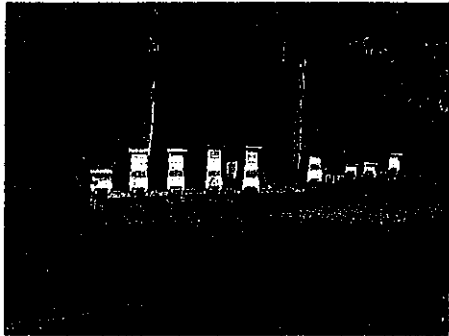
	A	B	C	# of Responses
Appearance				
Foam/Air bubbles	22	17	15	20
Colour Intensity	35	17	55	19
Presence of sugar crystals or granules	1	0	1	20
Aroma/Odour				
Floral	23	16	21	19
Woody (e.g. cedar, pine)	10	4	16	20
Vegetable/Herbal	9	4	10	20
Caramel/Toffee	12	6	16	20
Fruity/Citrus	9	6	14	20
Spicy (e.g. cloves, nutmeg, cinnamon)	13	8	16	20
Taste				
Acidity (sour)	3	2	9	20
Salty	5	4	11	20
Bitter	4	1	11	20
Sweet	41	39	33	19
Presence of an After Taste	17	17	31	20
Floral	18	13	11	20
Woody (e.g. cedar, pine)	9	7	16	20
Vegetable/Herbal	4	5	11	20
Caramel/Toffee	12	8	17	20
Fruity/Citrus	13	6	5	20
Spicy (e.g. cloves, nutmeg, cinnamon)	6	6	15	20
Other				
Runniness	35	38	47	20
Spreadability	44	42	41	19
Solubility (how fast it dissolves in your mouth)	45	45	39	19
Grittiness	1	0	2	20

Overall Rating	A	B	C	# of Responses
Overall Rating (total score - first = 1, second = 2, third = 3)	38	38	38	19
Number of "first" or "#1" ratings	4	7	8	19

Note: The values in each column indicate the total score obtained by adding together the individual ratings from the members of the test panel. A low score indicates a weak presence of a specific characteristic. A high score indicates a strong presence.

Appendix 4 – Project Photographs

Test Site



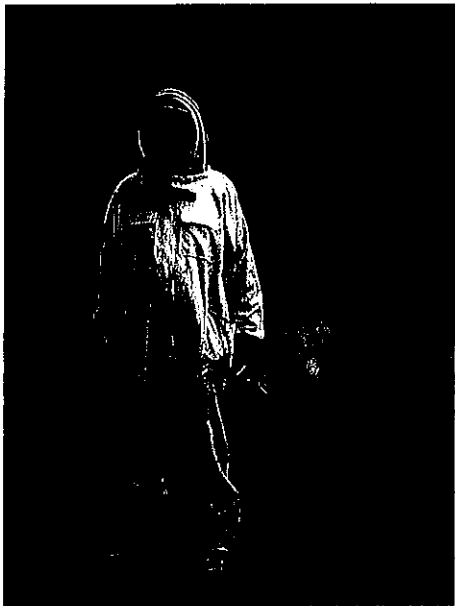
Bee Hives at the Project Test Site



"Super" Screen used for honey collection



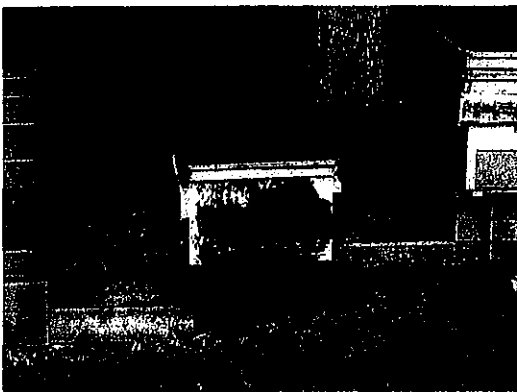
Centrifuge Used to Extract Honey



Beekeeper with Smoker



Bee Activity at the Top of the Hive

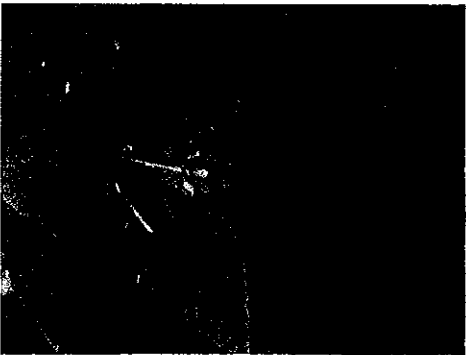


Bee Colony Captured after a Recent Swarm

Examples of Test Site Plants



Deutzia Scabra, Test Plot 5



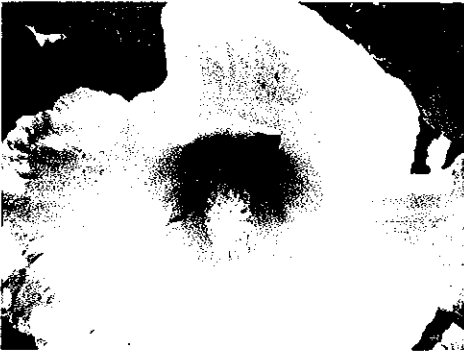
Jewelweed, Test Plot 4



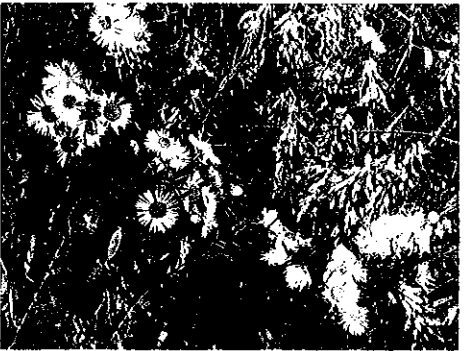
Honey Bee Collecting Nectar from a Violet,
Test Plot 2



Chickory and Queen Anne's Lace, Test Plot 7

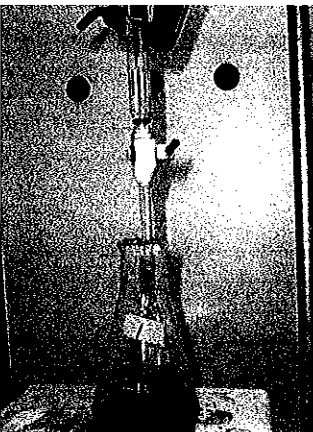


Bee collecting Pollen and Nectar from a Bloom on a Rose of Sharon Bush, Test Plot 5

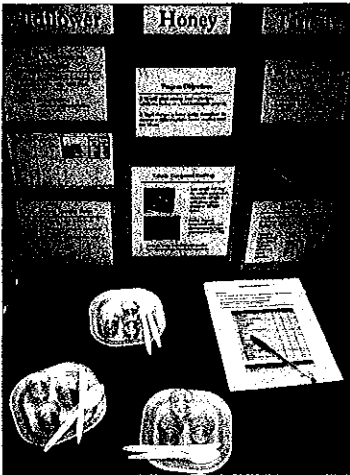


Fleabane and Cow Vetch, Test Plot 4

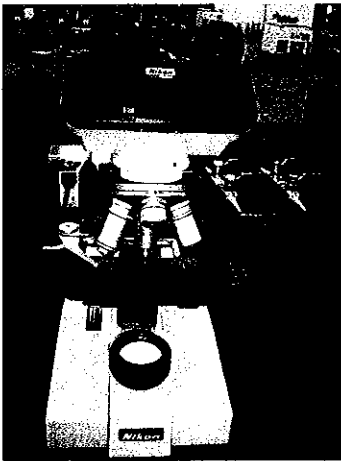
Lab Analysis



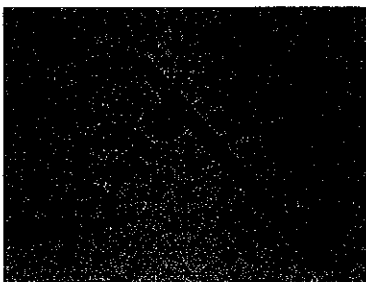
Sugar Analysis



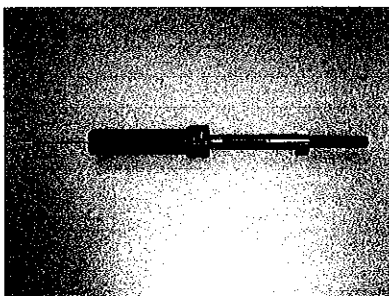
Sensory Evaluation



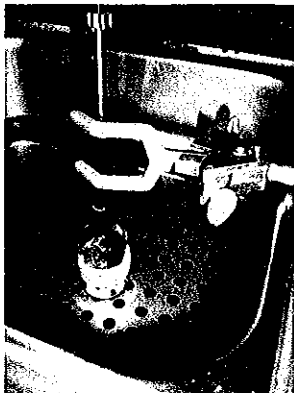
Pollen Analysis



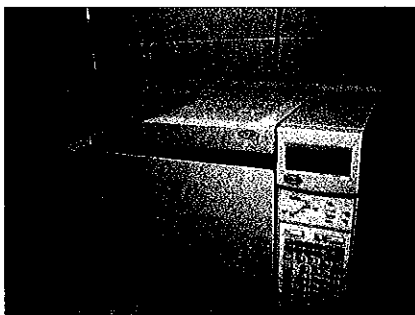
Microscope Slide of Pollen in a Honey Sample



SPME Sampling Device



Extraction of Volatiles from a Plant Sample



Transfer of Trapped Chemicals from the SPME Device
to the GC/MS Instrument

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