Final Report

Project: NS0308CO – Nova Scotia Beekeepers' Association – Nutritional value and pesticide content of pollen collected by commercial honey bees *Apis mellifera* in the Maritime Provinces and its implication for honey bee health

Schedule A: 1, 2, and 4

1. **A full report addressing each of the preceding objectives and deliverables.**

Nutrition testing was performed by AAA Laboratories Inc.; they determined percentage of protein and levels of essential amino acids in honey bee-collected pollen. Of the four sites included, pollen from cranberries had significantly higher percentages of protein than blueberry and post-crop sites (Fig. 1). There was no statistically significant difference among apple, blueberry, and post-crop pollen.

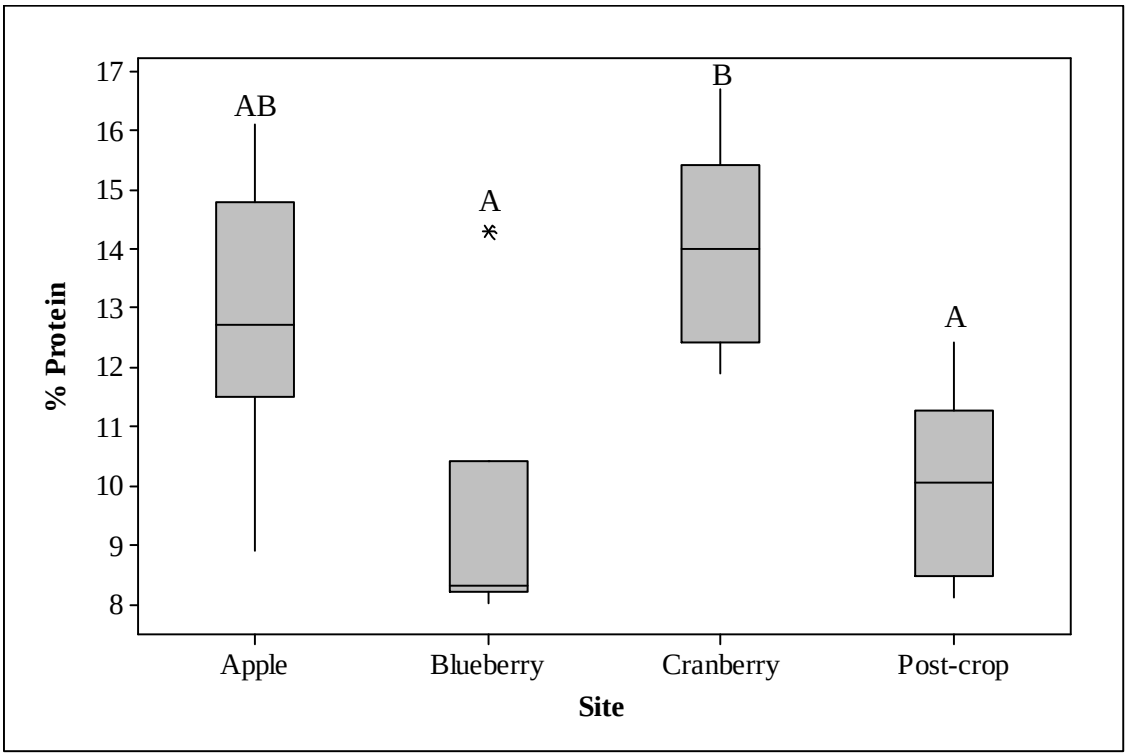

Figure 1. Percent protein from the four types of sites: apple, blueberry, cranberry, and post-crop. Site types not sharing the same letter are statistically different (One-way ANOVA; P =0.001).

Levels of essential amino acids (threonine, valine, methionine, leucine, iso-leucine, phenylalanine, lysine, histidine, and arginine) were compared with levels required by honey bees

according to de Groot (1953). The only amino acids that met these requirements were leucine, lysine, histidine, and arginine (Table 1). However, the other five amino acids were not very far off. These resultss suggest that increased use of pollen supplementation during pollination may be warranted. However, essential amino acids may have been obtained in non-crop forage that was not tested.

Table 1. Percent protein and amino acid proportions from all sites (n=29) and amino acid proportions de Groot (1953) concluded were necessary.

Nutrient	Mean	Median	St. Dev.	Min.	Max.	de Groot proportions
% protein	11.50	11.50	2.62	8.00	16.70	N/A
THR	0.10	0.10	0.00	0.09	0.11	0.11
VAL	0.12	0.12	0.00	0.11	0.13	0.15
MET	0.05	0.05	0.01	0.03	0.07	0.06
LEU	0.17	0.17	0.00	0.15	0.17	0.17
ILE	0.10	0.10	0.00	0.09	0.11	0.15
PHE	0.10	0.10	0.00	0.10	0.10	0.09
LYS	0.18	0.18	0.01	0.15	0.20	0.11
HIS	0.07	0.07	0.01	0.05	0.09	0.06
ARG	0.11	0.11	0.02	0.09	0.20	0.11

Species accumulation curves based on pollen showed that honey bees' diets were most diverse in cranberries, followed by post-crop sites, blueberries, and finally apples (Fig. 2).

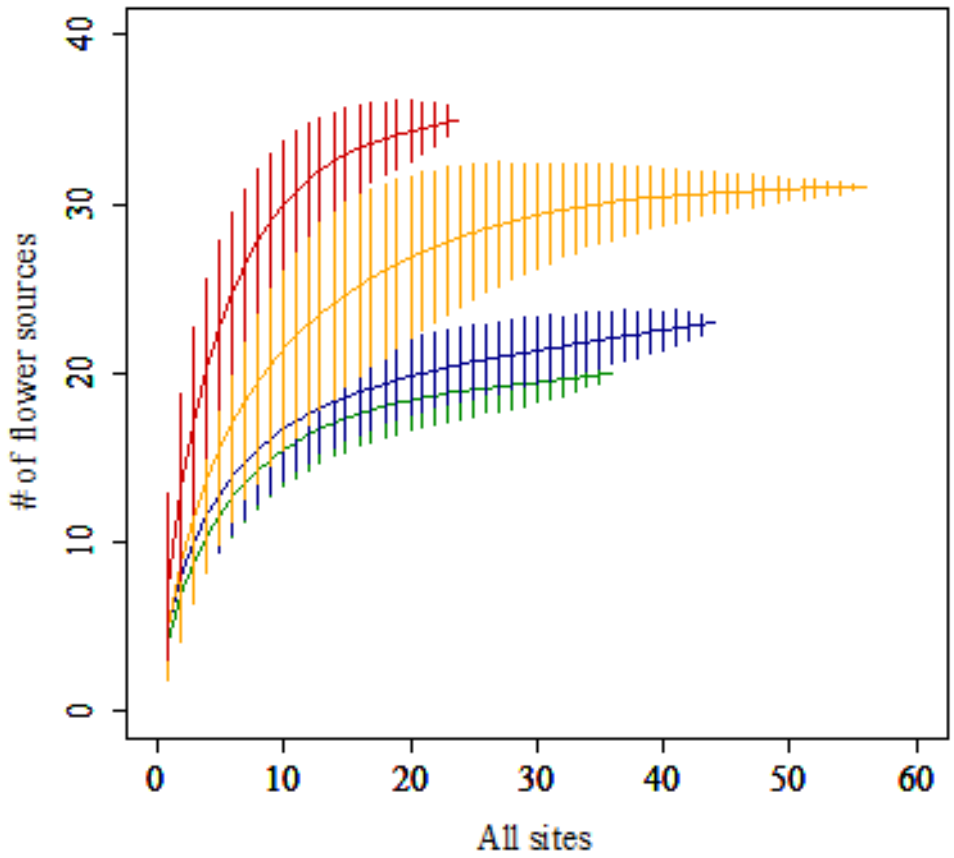

Figure 2. Species accumulation curves of pollen sources at apple (green; n=9), blueberry (blue; n=11), cranberry (red; n=14), and post-crop (yellow; n=14) sites. Vertical lines represent 95% confidence intervals.

Colonies in apple sites collected significantly greater proportions of crop pollen than they did in cranberry and blueberry sites (Fig. 3). Additionally, cranberry colonies had statistically significantly greater proportions of crop pollen than those in blueberries. In fact, there was no blueberry pollen collected by honey bees in colonies located in blueberries. This result does not mean honey bees do not pollinate blueberries, but means they did not collect blueberry pollen for their diets.

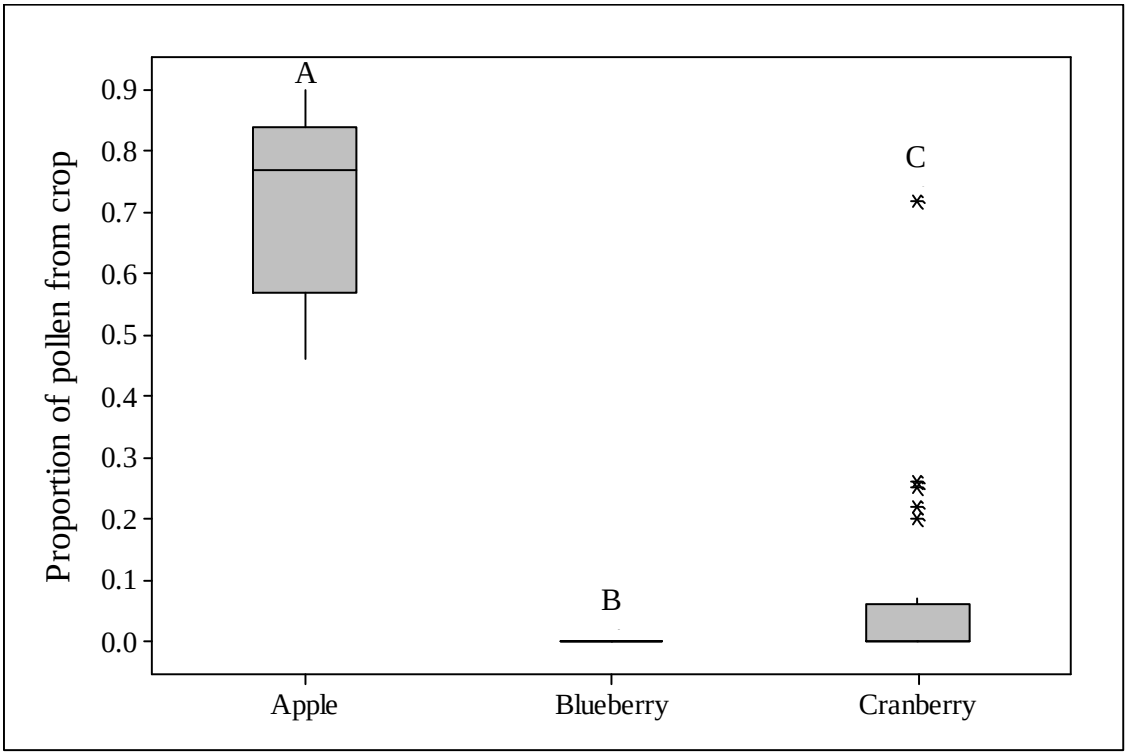

Figure 3. Proportion of crop pollen collected at the three crop specific sites: apple (n=27), blueberry (n=29), and cranberry (n=31). Site types not sharing the same letter are statistically different (One-way ANOVA; P <0.001)

Pollen was tested for 174 residues. Of the 86 samples tested, 83.7% contained pesticide residues (Table 2). There were 39 different residues detected, with a mean of 3.1 detections, and up to 11 in one sample. Residues with the highest number of detections were captan and tetrahydrophthalimide (THPI , a breakdown product of captan). Captan is a commonly used fungicide that has low to moderate toxicity to honey bees.

Levels of residues were compared to acute oral lethal doses that would kill 50% of honey bees (LD_{50}) values where available (Table 2); some residues had a range of LD_{50} values, so both the lowest and highest values were considered. LD_{50} values were converted to lethal concentrations that would will 50% of honey bees (LC_{50}) for comparison in parts per billion (ppb). No residues were at amounts equal to or exceeding LD_{50} values. However, there is a possibility of sub-lethal effects at lower than deadly concentrations.

Table 2. Summary of pesticide residue results from all sites (n=86). Detections and LC50s reported as ppb, LD50s as µg/bee. Residue types: B=Breakdown product, I=Insectide, N=Nematicide, H=Herbicide, F=Fungicide, A=Acaricide, IS=Insecticide Synergist.

								Acute oral LD50		Converted LC50	
Residue	**Type**	**# Detections**	**Median**	**Mean**	**St. Dev.**	**Min.**	**Max.**	**Low**	**High**	**Low**	**High**
1-Naphthol	B (Carbaryl)	4	182.0	167.2	43.9	103.0	202.0	no data	no data	no data	no data
Acephate	I	1	70.7	70.7	N/A	70.7	70.7	1.0	—	38461.5	—
Acetamiprid	I	16	5.0	9.9	9.2	2.4	27.4	14.5	—	558846.2	—
Aldicarb sulfone	I/N	2	3.7	3.7	2.3	2.1	5.3	0.3	—	10961.5	—
Atrazine	H	2	7.9	7.9	0.3	7.7	8.1	97.0	—	3730769.2	—
Azinphos methyl	I	7	177.0	176.5	111.5	34.8	381.0	3.4	—	129230.8	—
Boscalid	F	7	552.0	447.2	301.8	16.3	820.0	166.0	—	6384615.4	—
Captan	F	35	1570.0	2842.7	3117.2	62.0	12000.0	10.0	—	384615.4	—
Carbaryl	I	4	1325.0	1286.8	285.1	927.0	1570.0	0.2	1.27	8846.2	48846.2
Carbendazim (MBC)	F	4	113.0	119.5	45.4	71.9	180.0	50.0	—	1923076.9	—
Chlorothalonil	F	14	1435.0	7520.4	14017.1	465.0	50500.0	181.3	—	6972692.3	—
Chlorpyrifos	I	11	6.2	11.9	16.6	3.6	61.2	0.1	—	2269.2	—
Coumaphos	I/A	18	16.2	33.7	51.9	2.1	211.0	2.0	15.00	76923.1	576923.1
Coumaphos oxon	B (Coumaphos)	2	13.4	13.4	3.4	11.0	15.8	no data	no data	no data	no data
Cyhalothrin total	I	1	29.9	29.9	N/A	29.9	29.9	0.6	—	21923.1	—
Cypermethrin	I	3	25.7	20.9	14.3	4.8	32.2	0.2	—	6615.4	—
Diazinon	I	9	4.6	11.2	12.6	2.2	35.9	0.2	—	9615.4	—
Dicofol	I/A	1	2.8	2.8	N/A	2.8	2.8	12.2	—	469230.8	—
Endosulfan I	I	1	65.2	65.2	N/A	65.2	65.2	4.9	—	188461.5	—
Endosulfan II	I	1	144.0	144.0	N/A	144.0	144.0	no data	no data	no data	no data
Fluvalinate	I/A	9	35.8	34.1	13.8	17.9	58.7	0.2	1.20	7692.3	46153.8
Imidacloprid	I	25	3.2	6.0	7.2	1.0	30.4	0.0	0.50	150.0	19230.8
Imidacloprid olefin	B (Imidacloprid)	5	87.9	108.2	54.8	52.4	197.0	no data	no data	no data	no data
Linuron	H	2	140.2	140.2	162.3	25.5	255.0	120.9	—	4648461.5	—
Methamidophos	I/A	2	14.4	14.4	5.7	10.4	18.4	1.4	—	52692.3	—
Methoxyfenozide	I	13	64.6	438.4	765.3	17.9	2520.0	100.0	—	3846153.8	—

Myclobutanil	F	1	85.7	85.7	N/A	85.7	85.7	362.0	—	13923076.9	—
Phosalone	I/A	5	205.0	345.2	304.7	82.4	710.0	4.4	—	169230.8	—
Phosmet	I	5	404.0	822.6	866.1	15.1	2030.0	0.5	1.00	19230.8	38461.5
Piperonyl butoxide	IS	3	60.2	48.7	21.6	23.8	62.0	11.0	—	423076.9	—
Pyraclostrobin	F	6	101.5	85.6	56.5	11.8	141.0	100.0	—	3846153.9	—
Pyrimethanil	F	2	3.6	3.6	0.6	3.1	4.0	100.0	—	3846153.9	—
Tebufenozide	I	3	1650.0	1876.7	491.0	1540.0	2440.0	234.0	—	9000000.0	—
Thiacloprid	I	1	1.4	1.4	N/A	1.4	1.4	17.3	37.8	666153.9	1455000.0
Thiamethoxam	I/F	3	5.6	6.5	2.0	5.2	8.8	0.0	0.1	192.3	3384.6
THPI	B (Captan)	25	2750.0	3250.0	3418.0	484.0	17500.0	no data	no data	no data	no data
Thymol	F	9	78.3	72.3	13.9	46.6	85.5	no data	no data	no data	no data
Trifloxystrobin	F	4	95.2	114.6	103.4	17.1	251.0	200.0	—	7692307.7	—
Vinclozolin	F	3	2.5	2.9	1.0	2.1	4.0	100.0	—	3846153.9	—

All pollen from apples sites (21 of 21) contained pesticide residues, with a mean number of 5.57 detections per sample. Blueberry sites had 18 of 22 pollen samples containing residues, with a mean rate of 4.09 residues per sample. Cranberry sites had 18 of 21 samples containing residues, with a mean detection of 2.05 residues per sample. Only 15 of 22 samples from post-crop sites contained pesticide residues, with a mean of 0.86 residues per pollen sample. There was a significant difference in the number of residue detections between apple and post-crop sites (Fig. 4).

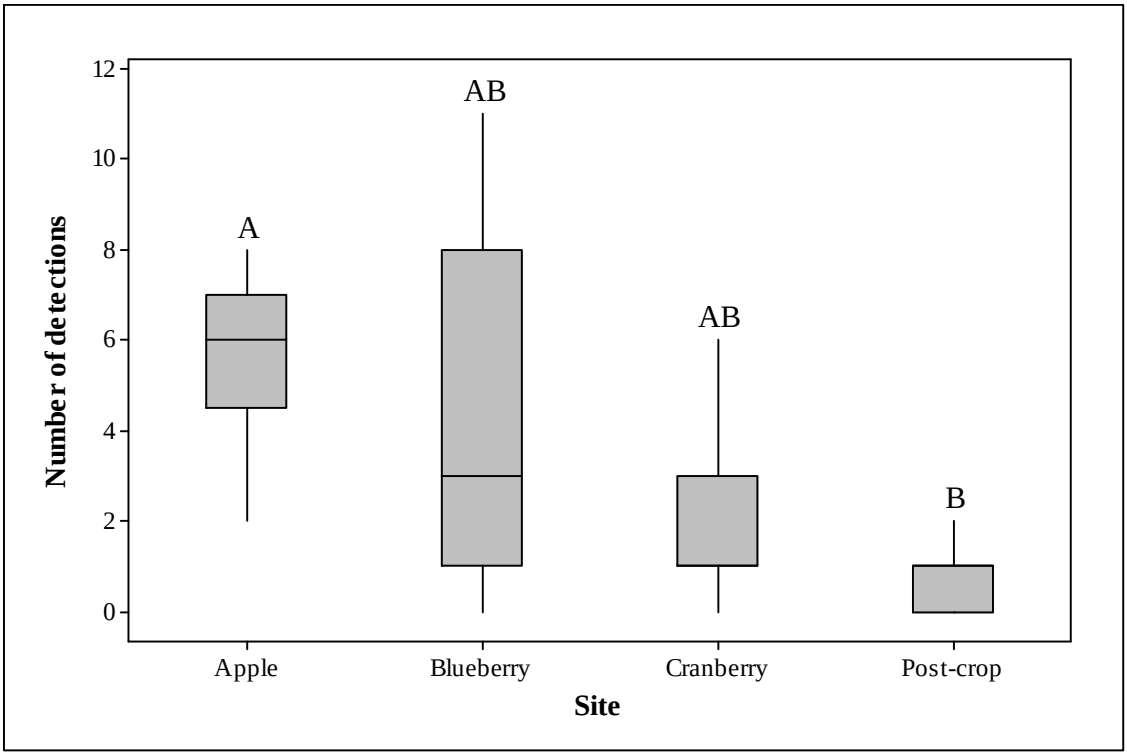

Figure 4. Number of pesticide residue detections from the four sites, apple (n=21), blueberry (n=22), cranberry (n=21), and post-crop (n=22). Site types not sharing the same letter are statistically different (One-way ANOVA; $P<0.001$).

1. An executive summary of the entire project which addresses the following points:

    a. **An assessment by the project leader of the degree to which the project fulfilled the primary objective(s) and deliverables.**

All of the major targets of this project were achieved. First, with the participation of beekeepers, we were able to collect pollen from honeybee colonies placed in each of three crops: apples, blueberries, and cranberries. Pollen was also collected from honeybee colonies situated in non-crop areas. As one measure of whether nutritional needs were being met by the three crops, we

evaluated the proportion of pollen that honeybees collected from non-crop plants. Based on our data, apples appeared to provide sufficient nutrition, whereas cranberries and particularly blueberries provided virtually no pollen to colonies. These results were to some extent mirrored in total protein analyses of pollen; honey bees were more likely to obtain non-crop pollen when they were situated in blueberries.

Second, screening of pollen for pesticides was completed, and 39 different compounds were identified. We did not find that levels were likely to be particularly hazardous, but the presence of so many compounds in so many samples suggests that regular monitoring is warranted.

In addition to meeting our proposed objectives, we also quantified two important parasites (*Varroa destructor* mites and *Nosema* spp. fungi) in all of the participating colonies.

Among the deliverables already produced are presentations to the Nova Scotia Beeekeepers' Association and the Acadian Entomological Society (won first prize) and the detailed data within this report.

b. **Actual short-term outcomes:** These are the direct or indirect effects or consequences resulting from the project; not activities that have been successfully completed, but what happened as a result of doing the activities

Honey bees' preferences for pollen from non-crop sources (i.e., no blueberry pollen was collected) raises questions about their efficacy in pollinating. This should be discussed with the beekeeper members of the Maritime associations, and further researched.

Pesticide residues should be further monitored to determine possible sub-lethal effects, and regulations associated with them should be evaluated.

c. **Project results:** At the end of the project; it is expected that these will fall under one of two headings, address one, as appropriate to the project.
   - *Improved knowledge of potential innovative products, processes or technologies;*

   or

   - Improved knowledge of solutions or strategies that have been analyzed or tested to address issues and opportunities

This project is the first to determine the nutrition and pesticide contamination provided by crops for their honey bee pollinators in the three Maritime Provinces. As such, it has provided baseline information on the quality of pollen collected by honey bees on crop sites, as well as knowledge of pesticide residues honey bees bring into their colonies via pollen. With this broad information, it could be possible to identify ways that researchers and beekeepers can work towards better colony health. This project could help create a more targeted approach to pollen supplementation and honey bee forage planting around crops.

d. **Who are the primary targets/beneficiaries of your project?** Include as many groups as are applicable; if "Other" is selected, please enter details.

Aboriginals
Agricultural producers
Consumers
Educators
Farm Families
Processors
Research Community
Rural Canadians
Women
Youth
Other _____

Other beneficiaries are beekeepers, the primary group targeted by this project.

e. **Information shared with target groups/project stakeholders:** Describe how you shared information about the project.

Information about this project has been presented at several venues, both at conferences and AGMs of the NSBA. Information was also published in the October 2011 NSBA newsletter. Conferences included the Acadian Entomological Society AGM, the Joint Annual meeting of the Entomological Society of Canada and the Acadian Entomological Society, the Acadia University Research Summit, and the North American Pollinator Protection Campaign meeting. Presentations at NSBA AGMs were made in 2011, 2012, and will also be made at the meeting in 2013.

f. **Is project information available to the general public/Canadians?** How? Is it available through a web site, press release or other type of media?

Project information could be published on the CAAP website. Additionally, the completed thesis by project team member Megan Colwell will be published on the Acadia University Vaughn Memorial Library's online database.

g. **Post project performance story:** The purpose is to describe what the project accomplished; to be included in this summary is a brief introduction to the project, its activities, results, lessons learned and next steps; this information may be used for publication on the CAAP web-site; the following items should be addressed in the story:

- **What issue/challenge/problem/opportunity did this project address?**

Apiculture is a huge industry, with many agricultural businesses dependent on pollination services; apiculture has an estimated worth of $1 billion a year in Canada alone (Agriculture and Agri-Food Canada, 2003). However, reports of declining populations and diseases signal alerts regarding the importance of enhancing our understanding of honey bee hive health. Proper nutrition enhances as well as maintains good health and hive sustainability (Brodschneider and Crailsheim, 2010). This project will help agricultural sectors of the Maritime Provinces by providing data on the quality of nutrition honey bees obtain when they are used for commercial pollination services.

Honey bees collect nectar from flowering plants that they convert to honey within the hive, providing sugars that are important for honey bee diets (Haydak, 1970). Sugars supply energy, but pollen is a honey bee's chief source of protein (amino acids), lipids, minerals, and vitamins, all of which are necessary for brood-rearing and normal development (Cook et al., 2003; Singh and Singh, 1996). If inadequate nutrition is obtained from pollen for a particular season, crop, or location, effective pollen supplementation strategies may be needed. Identifying occasions when honey bees are experiencing malnutrition, and therefore weakened hive health, could prevent some overwintering losses.

The nutritional content of pollen is highly variable among plant species and among geographic regions (Brodschneider and Crailsheim, 2010). Thus, to achieve optimal nutrition from pollen, honey bees may have to forage on a variety of species to obtain all their essential nutrients (Singh and Singh, 1996). By determining the extent to which honey bees collect pollen from non-crop plants, one could identify crops requiring nutritional supplementation, ultimately improving pollination practices.

Additional serious problems for apiculture occur because of common use of pesticides in agricultural practices; honey bees are sometimes more susceptible to insecticides than are target pests (CAPA, 1999). Knowing what pesticides are in pollen is valuable for identifying possible hazardous contaminations of honey bee forage.

- **Include a very brief overview of purpose and activities:** Summarize very briefly the activities funded under this agreement, highlighting key achievements and results.

Pollen was collected using pollen traps from various hive operations throughout the pollination season of 2011 in Nova Scotia (19 hives), New Brunswick (12 hives), and Prince Edward Island (9 hives; Fig. 5). Samples were collected from 5 operations in Nova Scotia, 4 in New Brunswick, and 3 in Prince Edward Island. At least three colonies per bee yard were used in the

study. Pollen collection was done during pollination of apples, blueberries, cranberries, and at post-commercial crop areas. Pollen traps operated for 24 hours, and pollen that was retrieved was stored separately for each trap, location, and date. Concurrent with pollen collection, a survey of flowering plants was performed around colonies to identify possible sources of forage. Pollen samples from crop and non-crop plants were taken to assist in pollen identification.

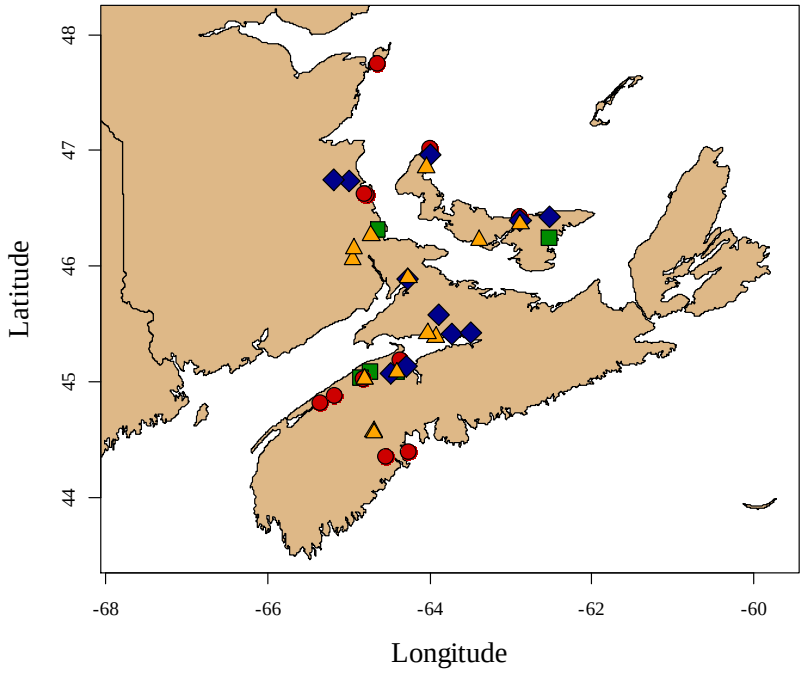

Figure 5. Locations of collection sites in the Maritimes: green squares are apple sites, blue diamonds are blueberry sites, red circles are cranberry sites, and yellow triangles are post-crop sites.

Pollen samples were tested for nutritional value (percent protein content and essential amino acids), pesticide residues (174 different residues), and floral source (crop or non-crop plants). Only four of the nine essential amino acids were at the required level as per the literature. However, the other five amino acids were not very different from required levels. There was a total of 39 different pesticide residues detected from Maritime pollen. None were at LD_{50} thresholds, although there is a possibility of sub-lethal effects and synergisms. Most of the pollen that honey bees collected from apple orchards was apple pollen, whereas pollen from cranberry bogs was only ~6% cranberry pollen. There was no blueberry pollen that honey bees collected from blueberries.

- **Why are the project and its results significant for the target group and/or stakeholders?** Include an estimate or statement of the number of persons reached; the number of persons attending meetings, etc.

Results of this study will benefit beekeepers of the Maritime Provinces, by documenting honey bees' preferences for crop versus non-crop pollens. Whereas some crops (i.e., apples) that

beekeepers rent their colonies to for pollination are used readily as pollen sources, other crops (i.e., blueberries) may not be adequate as pollen sources. This could be especially important in cases of large crop areas, where non-crop forage with possibly high nutrient value is difficult to reach or unattainable. Pollen supplementation or planting margins of crops with flowers with more nutritious pollen may help mitigate nutrient deficiencies.

Information will be shared via presentations at the AGMs of the Nova Scotia Beekeepers' Association, as well as reports to the beekeeping associations of New Brunswick and Prince Edward Island. There are approximately 230 members of the NSBA, and there is a significant number of members in beekeeping associations in the other two provinces. Additionally, these beekeepers rent out their colonies for pollination services, which means this project has the potential to have an impact on growers in the Maritimes.

- **What has been learned?** Identify the lessons learned from this project.

We have shown that honey bees scarcely use pollen from blueberries and cranberries. From the pesticide data, we have learned there is no acute threat from pesticide contamination of pollen in Maritime colonies, although there is a possibility of sub-lethal effects.

- **What are the next steps?**
  - Is the solution or strategy likely to be further implemented?

  or

  - Is the innovative product, process or technology likely to be adopted by the sector?

  If you answered yes to the preceding, describe what you expect will be the next activities. If you answered no, explain why not. Not all projects will have next steps but often there are activities planned for after the end of the project that are directly or indirectly a result of the project.

Before any kind of pollen supplementation schedule can be fully developed, there must be further research into the assessment of the nutritional requirements of honey bee colonies. Discussions with beekeepers at AGMs could help discover the next steps to take in implementing such a schedule.

Additionally, there should be investigation into possible sub-lethal effects of detected pesticide residues in Maritime colonies. There may be a lack of published information for some of the detected residues. This would warrant in lab and in field studies of sub-lethality of different compounds.

4. Acknowledgment of the contribution of Agriculture and Agri-Food Canada and Agri-Futures Nova Scotia. The letterhead logo should be used where appropriate. An electronic version of the logo will be provided on request. Suggested wording for acknowledgment is as follows: "Funding for this project has been provided in part through Industry Councils from Nova Scotia, New Brunswick and Prince Edward Island which deliver the Canadian Agricultural Adaptation Program (CAAP) on behalf of Agriculture and Agri-Food Canada." Please refer to the attached Schedule "E", CAAP Common Look & Graphic Standards User Guide, for additional information.

Acknowledgments:

Funding for this project has been provided in part through Industry Councils from Nova Scotia, New Brunswick and Prince Edward Island which deliver the Canadian Agricultural Adaptation Program (CAAP) on behalf of Agriculture and Agri-Food Canada. Additional funding and support was provided by the Maritime beekeeping associations, NSBA, NBBA, and PEIBKA, as well as Acadia University and University faculty members. Roger Simonds lab at the USDA in North Carolina, and Jay Gambee's in Oregon, performed chemical analyses of pollen. Valerie Fournier's lab at Université de Laval provided valuable expertise on pollen identification.

References

Agriculture and Agri-Food Canada. 2003. 2002-2003 Canadian honey situation and trends. http://www4.agr.gc.ca/resources/prod/doc/horticulture/honey_e.pdf. Accessed 10 July 2010.

Brodschneider, R., and Crailsheim, K. 2010. Nutrition and health in honey bees. Apidologie 41:3 278-294.

CAPA. 1999. A guide to: managing bees for crop pollination. 36th Apimondia Congress, Vancouver, BC, Canada.

Cook, S.M., Awmack, C.S., Murray, D.A., and Williams, I.H. 2003. Are honey bees' foraging preferences affected by pollen amino acid composition? Ecol. Entomol. 28: 622-627.

de Groot, A.P. 1953. Protein and amino acid requirements of the honeybee (*Apis mellifica* L.). Ph.D. Thesis. Utrecht University, the Netherlands.

Haydak, M.H. 1970. Honey bee nutrition. Annu. Rev. Entomol. 15: 143-156.

Singh, R.P., and Singh, P.N. 1996. Amino acid and lipid spectra of larvae of honey bee (*Apis cerana* Fabr) feeding on mustard pollen. Apidologie 27: 21-28.