

**Re-Supply of Fumagilin-B to Alberta
and
Strategic Plan for Development of
Fumagillin Alternatives**

Prepared for:

**Alberta Beekeepers Commission
and
Grande Prairie Regional Innovation Network**

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Project Final Report:

Re-Supply of Fumagilin-B to Alberta

Strategic Plan for Development of Fumagillin Alternatives

Objective

The initial objective of the project was to coordinate the activities of various industry stakeholders for reestablishing the supply of Fumagilin-B into Alberta in time for fall winterization of beehives by Alberta beekeepers. During the course of the project, the scope was expanded to also include the development of a Strategic Plan for adopting alternative treatments for nosema over the medium to long term.

The issues to be addressed for resupply of fumagillin and/or Fumagilin-B¹ were:

- a) timelines and economics of supply of the concentrate (fumagillin) vs. final product (Fumagilin-B)
- b) the approval process and requirements of the product by Health Canada
- c) the identification of a Canada-based pharmaceutical operation that can assume the business of importing, distributing, selling, and if necessary, compounding the final Fumagilin-B product.

Background

Fumagillin has been the primary recommended antifungal treatment for nosema infections in beehives. A supply disruption, beginning in 2018, prompted an investigation into the supply chain for Fumagilin-B, the final product sold in Alberta. It was determined that the active ingredient, fumagillin dicyclohexylammonium salt, has been sole sourced but nonetheless remains available for importation from Themis Medicare in India. The supply disruption was related to Alberta-based Medivet's decision to cease its importing and packaging operations. Therefore, a new Canadian veterinary products company is required to manage the fumagillin business in Canada. In addition, potential future regulatory challenges related to antibiotic use, along with other longer term risks, raise interest in the potential for alternative approaches to controlling nosema infections.

Work Plan

A. Fumagilin-B Resupply

1. In collaboration with industry stakeholders and government sponsored researchers, hold discussions with Health Canada to determine the specific requirements of approval of a revised supply chain:

¹ Fumagillin is the common name for the active fungicide used to treat nosema fungi. Fumagilin-B is the commercial name for the final dosage product that is purchased and applied by beekeepers.

- focus on an unchanged product manufacturing source
 - determine how the existing Drug Identification Number (DIN) can be used to expedite approval
 - discuss “ownership” of the product with CEVA and Medivet
 - engage a new veterinary drug distributor/packager as needed.
2. Discuss specifics of supply options with Themis, the active ingredient producer, including:
 - preferred packaging and business model from Themis’s perspective
 - cost implications, critical volumes, etc.
 3. Present business case to prospective Canadian veterinary product packager/distributor:
 - establish terms for a formal commitment to the business
 - determine commitments required from Themis and Alberta beekeepers
 - project timelines for implementation.

B. Strategic Plan for Fumagillin Alternatives

1. Review current work underway at various research organizations, including but not limited to work underway in Poland, at Alberta Agriculture and Food, University of Alberta (iGEM project for developing nosema-fighting bee gut bacteria as a feed additive), Agriculture and Agri-Food Canada, and University of Saskatchewan.
2. Organize a ½ day workshop involving industry experts and stakeholders in order to identify potential objectives, timelines, and target results for a hive health program that could phase out the use of fumagillin.
3. Identify a medium term (3 – 5 years) funding mechanism for a first phase of a proposed beehive health development program
4. Present Strategic Plan to ABC board.

Results

A. Fumagillin Resupply

Summary

- Discussions were held with key staff at Health Canada and the Canadian Animal Health Institute who provided insights into how best to get product available to Canada, both during the immediate and medium term.
- The contacts at these organizations are:
 - Mary-Jane Ireland, Health Canada, 613 954 1873, mary-jane.ireland@hc-sc.gc.ca
 - Manisha Mehrotra, Health Canada, 613 941 8775, manisha.mehrotra@hc-sc.gc.ca
 - Jean Szkotnicki, Canadian Animal Health Institute, 519 763 7777, jszk@cahi-icsa.ca

- The recommendation for the immediate term was to solicit the involvement of a Canadian veterinary drug manufacturer who could initiate the importation and sale of Fumagilin-B under the Emergency Drug Release Program (EDRP). This is a program that can make critical veterinary drugs available under the supervision of an authorised veterinarian. (<https://www.canada.ca/en/health-canada/services/drugs-health-products/veterinary-drugs/emergency-drug-release-veterinary-drugs.html>)
- Alberta Veterinary Laboratories (Dr. Merle Olson) was recommended by the Canadian Animal Health Institute as the best veterinary drug manufacture to contact, based on previous experience in dealing with the EDRP for other products that had been “orphaned” by their manufacturers.
- Both AVL (represented by Dave Ireland, 519 741 4344, dave.ireland@wightman.ca) and Themis Medicare (Ms. Sapna Mehta-Kumar, sapna@themismedicare.com) were contacted regarding a potential EDRP. It was agreed that AVL would work directly with Themis to establish terms for importation of either fumagillin (the active ingredient) or fumagilin-B (the final dosage). This discussion is ongoing.
- During the course of investigating veterinary drug manufacturers, other potential Canadian companies with the capacity to import an active and produce a final dosage product were identified:
 - Medisca, based in Montreal
 - Stafford Pharmacy, Calgary

These companies would be able to take on the business if the active ingredient was readily available and approved by Health Canada.

The information gathered was shared with the Canadian Honey Council, which is currently taking the lead in developing a Canadian business for resupply of Fumagilin-B.

B. Strategic Plan for Fumagillin Alternatives

Summary of Findings

The sudden disruption of fumagillin availability in the Canadian market raised numerous questions about the vulnerability of the industry to risks of losses to nosema.² These questions include:

- are alternative treatments being used successfully to control nosema infections?
- what new technologies should be advanced?
- how can promising alternative treatments be brought to the market?
- are there other bee health risk factors to be addressed that would also reduce hive vulnerability to nosema infections?

² It is generally accepted that nosema infections in Alberta are related to *nosema ceranae* as opposed to *nosema apis*. For simplicity, this document is using the term nosema to refer to these sporulating fungal infections.

Based on the potential for ongoing or future uncertainty of fumagillin availability, it was agreed that there would be value to answer these questions, document the current state of knowledge around nosema control in bees, and develop a strategy to mitigate the risks of future losses of beehives due to nosema infections.

The approach taken was to review recent literature, and consult with industry experts and apiculturists. This was accomplished primarily through consultations with the presenters and attendees of the ABC AGM in November 2018. Follow-up interviews led to additional work underway in other jurisdictions, and taken all together, a comprehensive view of the industry was obtained.

Key findings are provided below.

- Fumagillin is by far the most effective and virtually the only treatment used by Alberta beekeepers for nosema control. The 2017-18 Winter Loss survey reported that 100% of Alberta beekeepers responding to the survey used Fumagilin-B as a fall treatment. Nosema infections were not a significant cause of colony losses.
- Even though it is widely accepted that controlling nosema is critical for minimising losses during all seasons, there are reports in the literature where measurement of spore counts did not correlate with colony strength. In fact, two reports suggested that the healthiest colonies in their respective studies also had the highest spore counts, but these studies were conducted in different regions (Switzerland and California).
- The cold climate of Alberta and Saskatchewan is a factor that very likely requires differentiated best practices for hive health as compared to practices in eastern Canada, US, and in Europe. For example, the use of thymol products for controlling varroa mites has been reported to be significantly less effective in northern Europe as compared to southern Europe, since it acts by volatilization, which is much lower in lower temperatures.
- Alberta's relatively large honey producing industry and related large bee colonies (i.e., intensive apiculture) inherently leads to higher risks from disease and infestations, that in turn requires ongoing development and diligent application of best practices for maintaining healthy hives.
- The primary issues being addressed with respect to the use of antibiotics in apiculture is the prophylactic use, which is a practice that can result in microbial resistance. One area of focus has been use of antibiotics for prevention of American foulbrood (AFB). However, since the body of knowledge surrounding this pest is much larger than it had been, beekeepers are now able to effectively treat for AFB on an as needed basis.
- Use of probiotics to enhance the intestinal health of bees has been shown to be safe and is generally considered a promising route for controlling nosema. However, only those probiotics native to bee

intestines should be considered, as gut microbes from other animal species have been shown to be harmful to bees.

- One industry expert strongly supports addressing nosema indirectly by controlling varroa mites and viruses, since healthy bees are believed to be able to tolerate nosema ceranae. However, as noted above, Alberta and other parts of Canada have environmental risks that are not accounted for in other regions and likely necessitate unique hive management practices.
- Poor queens was cited as a second leading cause of overwintering losses in Canada (after weather affects), and therefore, genetics improvement is considered a valid approach towards better resilience against nosema. It is common in Alberta to import queens from Hawaii, California, and Chile, and it stands to reason that the genetics of imported queens may not be best adapted to Alberta. Local production of queens is underway in Alberta with the intention that queens with some level of desirable traits can be selected for commercial use.

Discussion

Preventing losses from fungal infections caused by nosema ceranae remains a serious concern of Alberta beekeepers. This is clearly evidenced by the extensive use of fumagillin in Alberta and by all accounts, Fumagilin-B is considered a highly reliable treatment to the point where most over-wintering losses of bees is attributed to weather, poor queens, and poor feeding (starvation). Further, in the area of chemical or other drug related pest management, varroa mite control was cited as the leading cause of losses as opposed to nosemosis. The success in preventing nosema related losses may be contributing to a lack of development of fumagillin alternatives for Alberta's market.

Trends in chemical and food safety in North America tend to follow the lead of Europe, where the use of fumagillin for the treatment of beehives is not allowed. Also, there are a number of fundamental concerns related to the use of fumagillin.

- a) Treatment with fumagillin does not prevent reoccurrence of the disease as only the vegetative forms of the parasite are killed. Therefore reapplication of fumagillin is generally required 2 -3 times each year.
- b) There is the possibility that the efficacy of fumagillin could decline due to ongoing use, as is the case with many antimicrobial treatments. However, the product has been used for more than 40 years and remains effective.
- c) Risks of antibiotic residues in honey are a general concern for the honey industry and it is possible that regulating bodies could review the current levels of acceptability (25 µg per kg honey for fumagillin). It should be noted that at this time, fumagillin, is not a concern since it has a relatively short half-life and does not persist in biological systems, it does not currently have restrictions like other antibiotics that could lead to microbial resistance, and it has been used for decades with no known adverse effects related to food safety to humans.

The state of apiculture research in Canada is well positioned for increasing the overall body of knowledge around beekeeping in areas of disease control, nutritional practices, genetics, and genomics.

The Bee Health Roundtable currently has eight working groups looking at the following issues:

1. Varroa Mite Control
2. Control of Pesticide Exposure
3. Minor Use
4. Research
5. Communications
6. Habitat and Surroundings
7. Nutrition
8. Best Management Practices (BMPs)

While research project funding can tend to be *ad hoc* in order to address unexpected problems, successful collaborations with provincial and federal organizations have resulted in a broad range of research activities. For example, the following projects are underway at AAFC's National Bee Diagnostic Centre at the Grande Prairie Research Centre in Beaverlodge, AB.

- Evaluating risk factors associated with colony survival (virus transmission)
- Using genomics and proteomics tools for developing healthier and stronger bee genetics
- Pollination and canola yields
- Studying emerging threats to pollinators
- Honey bee stock assessment (genetics)
- American foulbrood surveillance and detection
- Assessing sperm viability on queen performance
- Bee health and blueberry pollination
- National surveys

While nosema control is not a specific focus of this research, the efforts to strengthen bee genetics and other aspects of general hive health and management are likely to have potential for reducing the losses due to nosema.

Conclusions

There are a number of approaches recommended that, taken together, can act as insurance against future shortages and other factors that could restrict the use of fumagillin. A discussion of each recommendation is provided below and a summary of key issues related to new nosema control product technologies is shown in Table 1.

1. Focus on hive health as a whole

Recognizing that best practices in hive management will have a direct impact on overall bee health, it is recommended that a heightened level of attention be paid to all aspects of beekeeping as a whole, i.e.,

pesticide control and nutrition. This is based on some expert opinions that otherwise health hives can tolerate and survive with nosema infections.

One area in particular is varroa mite control which has been correlated to the presence of a range of viruses and it may be that strong varroa mite control will also contribute to reduced losses due to nosema. In Alberta, 92% of survey respondents used alcohol wash to monitor mite infestation and the same percentage utilized accepted chemical treatments (amitraz, oxalic acid, formic acid) for varroa mite control which does suggest that this pest is in well in check.

Nutritional studies are also ongoing, and are anticipated to provide additional and valuable colony feeding guidance that will help reduce starvation losses during long Alberta winters and healthy colonies for the following spring.

2. Document results from alternative Treatments

While a range of treatments for nosemosis prevention have been researched, results using commercially available products, other than fumagillin, have been marginal. Nonetheless, with Fumagilin-B in short supply during 2018, it is believed that a number of alternative methods may have been applied in the fall of 2018. It is recommended that all annual surveying in 2019 include requests for details of treatments intended to control nosema, along with reports of winter losses that may attributed to nosema.

As examples,

- Nosevit is a product containing natural phenolic extracts and is promoted as providing general bee gut health in terms of pH control, intestinal coating, intestinal elasticity, and amino peptide probiotics. Published research supports Nosevit as having some level of efficacy against nosema, but results in Alberta are less clear.
- Thymovar (containing thymol as the active ingredient) is an option for varroa mite control, but some studies suggest that it also has some impact on reducing nosema infection. The efficacy of thymol products in Alberta could be minimal for overwintering treatments, since this product acts via its vaporization, which will be much lower in cold temperatures.
- Anecdotal, essential oils (specifics are not known) may have been applied to hives with the intent that these oils have probiotic properties and may help maintain bee intestinal health and help reduce nosema spore counts.

3. Further investigate the use of bee probiotics

Research reported recently by Dr. Aneta Ptaszynska provided a good overview of the opportunities presented through the use of bee probiotics. Previous research using a range of commercially available probiotics did not provide good results vs. nosema infections and in fact, caused increases in bee mortality. Subsequent research using microbes isolated from bee intestines provided much more promising results, and these findings are in the process of being published with related intellectual property to be protected via patents.

At this stage, while data is not considered fully comprehensive, the approach is considered generally sound since it is based on intestinal health for bees rather than use of antibiotics. Further, there are numerous bacterial probiotics approved for use as animal feed, and therefore the expectation is that approval of a new and related probiotic for bees could proceed following an established procedure as a Veterinary Health Product (VHP), which is reserved for products that do not have specific therapeutic claims. The approval procedure would require the submission of safety data by the industry (no DIN required).

Once the research on bee gut probiotic use has been published, it is recommended that ABC review the state of the research and assess the potential for progressing the technology towards commercial viability in Alberta.

4. Further investigate the use of natural antimicrobial ingredients

Use of natural plant extracts that exhibit biological activity is an interesting approach for beekeeping since it is in line with the “natural” and/or “organic” nature of the honey producing industry. Dr. Aneta Ptaszynska reported on her evaluation of a range of plant extracts that are common to the natural health product (NHP) industry. Included were extracts from medicinal plants such as ginseng, rhodiola, ginkgo biloba, and others, with the intention that the adaptogenic function of the extracts would improve bee immunity by elevating the bee’s phenolic oxidase activity.

The most promising results were obtained using eleutherosides extracted from Siberian ginseng and this treatment is currently in the process of being published with related intellectual property to be protected via patents. Further work is required to confirm the efficacy of eleutherosides in field operations.

It should be noted that one of the extracts evaluated was rhodiola rosea, which is produced in Alberta. Rhodiola root extract contains active ingredients (adaptogens) known as salidroside and rosavin. While the results obtained for rhodiola were reported verbally to be less effective than extract of Siberian ginseng, the potential for a developing the regional economy using Alberta rhodiola on Alberta bees warrants further investigation.

As another example of using an NHP, a recent study at Washington State University reported that mushroom mycelia extract can substantially reduce the incidence of deformed wing virus and Lake Sanai virus.³ These findings were not able to conclude if the extract worked specifically against these viruses, or if bee immune systems were boosted, but if it is the latter, it would be interesting to determine any impact on nosema.

Use of natural plant extracts can be approved by Health Canada as VHPs.

5. Support improved genetics (selecting queens)

³ <https://www.sciencedaily.com/releases/2018/10/181004100044.htm>

Honey bee genetic research is likely to open up a wide range of opportunities to improve general health, pest resistance, and over winter survival rates in Alberta. Honey bees are not native to Alberta and therefore the importation of queen bees is the standard practice for the beekeeping industry. Currently queen bees are imported from regions such as California, Hawaii, Chile, and New Zealand.

The approach is to conduct genomic and proteomic analysis of bees selected from colonies with desirable traits, such as honey production, hygiene, disease resistance, etc. Hygiene is particularly interesting since bees with a stronger propensity to remove sick or deformed larvae and young bees from the hive will reduce the spreading of viruses. Genomic or proteomic markers specific to resistance to nosema have not yet been observed, but further research is expected to lead to more robust colonies.

6. Develop alternative veterinary drugs for treating nosema infections

Porphyrins have been identified as a potential treatment against nosema infections based on their bactericidal and antifungal activity. Porphyrins were shown to inactivate nosema microsporidia, were well tolerated by healthy bees, and reduced the mortality rates of nosema infected bees.

Based on overall trends towards antibiotic-free food production however, an alternative antibiotic to fumagillin is not considered a leading approach for managing nosema over the long term, since it would raise identical issues that go against principles of natural/organic antibiotic-free food production. In addition, the size of the bee industry is unlikely to be sufficient to provide a compelling business case for the development of a new drug for apiculture only.

7. Novel Biotechnology Solutions

The University of Alberta 2018 iGEM project presents an interesting and novel approach to managing nosema infections using synthetic biology. The concept is that a new and custom designed intestinal flora could be introduced into bees that would produce protoporphyrin IX as part of its normal metabolism, and in this way, a nosema fungal infection would be controlled “naturally” without having to introduce chemical or other treatments externally. Put another way, the bees would have a built-in nosema-fighting immune system.

The project found that bees could tolerate the approach and also confirmed that nosema is reduced by the presence of porphyrins. The view of this type of technology by the industry remains to be determined, as there are numerous major hurdles to overcome that include establishing a technical lead for the research (this was an undergrad project), funding for what is essentially a novel drug development and delivery system, as well as negotiating the regulatory environment from the perspective of introducing new genetically engineered organisms into food production.

Table 1. Summary of New Treatment Options for Controlling Nosema

Approach	Status	Main Steps	Challenges, Comments	Timeline / Cost
Bee Probiotics	Use of two probiotic strains has been filed for patenting in Europe (PL423363)	<ol style="list-style-type: none"> 1. confirm preferred microbial strains for isolation 2. develop methods for growing up and isolating the active ingredients 3. complete field testing 4. Regulatory approval 	<ul style="list-style-type: none"> – need develop a cost effective process for growing up commercial quantities of probiotic – the industry will be skeptical of a new product claiming performance equivalent to fumagillin 	<p>3 - 5 years</p> <p>>\$1 million</p>
Natural Veterinary Health Products	Use of Siberian Ginseng (eleuthero root) has been filed for patenting in Europe (PL4415155)	<ol style="list-style-type: none"> 1. confirm ingredient production process 2. complete field and safety testing 3. Regulatory approval 	<ul style="list-style-type: none"> – Availability of extract needs to be confirmed – Rhodiola should be given another look based on Alberta production – product efficacy will require stringent proof of safety and performance 	<p>2 - 3 years</p> <p><\$1 million</p>
Improved Genetics	Selection of domestic queens based on genomic markets is an emerging practice – 12 traits have been identified (honey production, disease resistance, behaviour, etc)	<ol style="list-style-type: none"> 1. Prioritize targeted traits 2. Determine how to roll out to industry 3. Use “omics” to detect causes of colony stress 	<ul style="list-style-type: none"> – longer timeline required – ensure coordination with AAFC research – no current markers known for nosema resistance 	<p>5 - 10 years</p> <p>>\$5 million</p>
New Chemical or Drugs	Promising results obtained for porphyrins, while marginal results were observed in many others (either low spore reduction, high bee mortality, or both)	<ol style="list-style-type: none"> 1. Need to confirm that efficacy will match that of fumagillin 2. establish product development partnership with veterinary products manufacturer 	<ul style="list-style-type: none"> – cost of regulatory approval for a new drug/chemical – ongoing issue of residual antibiotics in honey products – ongoing issue of microbial resistance build-up 	<p>7 - 10 years</p> <p>>\$5 million</p>
Novel Biotechnology	Concept for protoporphyrin IX [“nine”] (PPIX) expression by a probiotic (<i>E coli</i>) has been demonstrated.	<ol style="list-style-type: none"> 1. Introduce PPIX expression genetics into natural bee intestinal flora 2. Optimise the PPIX expression rate 3. Develop process for growing up the engineered probiotic 4. Complete field testing 5. Seek regulatory approval 	<ul style="list-style-type: none"> – Need a lead scientist to move technology forward – establish the feasibility of maintaining healthy strains of the engineered probiotic – regulatory issues related to use of a probiotic designed through synthetic biology are unknown 	<p>7 - 10 years</p> <p>>\$10 million</p>

Other Recommendations

1. Ensure that research priorities of AAFC are aligned with short term needs of ABC. This will require a defined focus by the ABC board, the executive director, and provincial apiculturists in terms of allocating discussion and reporting time on meeting agendas, or establishing a working group of the executive director and several producers and apiculturists to focus on near term research needs.
2. Strengthen a network of life science industry resources in Alberta that could collaboratively address the apicultural needs of ABC. Areas of interest could include genetics/genomics research, crop research, natural health and other bioactive product development work that may be taking place at universities, industry associations and government research organizations. Through collaboration with other organizations, funding for industry led research is generally available in Alberta - particularly for short or medium term projects that address a specific challenge and have a quantifiable financial benefit to the industry.
3. For new business start-ups related to supply of new beehive management products, financial support from ABC can often be matched by organizations.
 - Through Alberta Innovates, ABC can help support a third party (a new enterprise or new initiative for an existing company) to build a new business and supply a new product that will benefit the industry. For example, Alberta Innovates Voucher program funding can be as high as 3:1, with grants from \$10,000 for market research, and up to \$300,000 for new product development and trialing. Similar funding may be available from additional regional innovation networks, such as Grande Prairie Regional Innovation Network.
 - WD's Western Innovation Initiative (WINN) is a federal funding program for small and medium sized enterprises that has annual rounds of funding. Value added agriculture and biosciences are two focus areas that are well aligned with the honey producing industry.
 - NRC's IRAP program actively seeks out industrial research projects that it can support with matching funds. Technical advisors are based across Alberta to assist companies to define a project that can be supported by IRAP.
4. For general apicultural research funding, it is recommended that ABC work with its industry and federal partners to define projects that will specifically address the needs of Alberta beekeepers. The following are potential sources.
 - Western Diversification (WD) is a federal funding agency that has an annual round of funding targeting non-profit organizations. WD's focus is on cluster growth, which could include support for the existing bee health research community.
 - The Ag Funding Consortium (in Alberta) runs annual funding rounds for innovation and value added agriculture and food production. LOI's for 2020 funding are requested during 2Q 2019.
 - Alberta AF also offers the Strategic Research and Development Program (SRDP), focused on
 - a) enhanced productivity and efficiency
 - b) improved competitiveness

- c) differentiated products and new value-added products
- d) sustainable production to promote responsible use of resources and improve animal welfare, or animal or crop health, and
- e) addressing consumer demand and social license within Alberta's livestock or crop industry.

The next LOI to SRDP is due on January 25, 2019.