Literature Review on the Gypsy Moth (*Lymantria dispar dispar*) and *Bacillus thuringiensis kurstaki* (*Btk*) use

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Infestation and Lifecycle

Ministry of Natural Resources and Forestry. (2020). Gypsy Moth. Ontario. Retrieved from: https://www.ontario.ca/page/gypsy-moth.

The gypsy moth (*Lymantria dispar dispar*) is a defoliator moth that is native to Europe and the larvae (caterpillars) feeds on the leaves of many different tree species. It was first introduced to North America in 1869 in Massachusetts for the silk industry but was only detected in Ontario in 1969. By 1981, it had become an extensive issue for defoliation in Ontario. The preferred host species for gypsy moth caterpillars are oak (*Quercus*) and their distribution in Ontario coincides with mostly oak populations. Gypsy moths are generalists though, and feed on other hardwood trees, like sugar maple (*Acer saccharum*) and softwoods like eastern white pine (*Pinus strobus*).

Gypsy moths tend to have outbreaks every 7-10 years, with outbreaks lasting several years before they collapse. The moth overwinters on tree bark as an egg in masses yielding up to 1000 caterpillars. The eggs hatch in the spring (April-May) and the caterpillars ascend the tree they hatch on and begin to defoliate the leaves. They can travel to a neighbouring tree via wind on a strand of silk (“ballooning”). Most feeding is done at night, making early detection difficult. With about 40 days of feeding, caterpillars are done feeding by July, at which time they will create a cocoon to pupate inside. Adults emerge 10-14 days later and can no longer feed because they lack mouth parts. Their main priority now is to reproduce before they die naturally in another 10-14 days. Females cannot fly and usually stay close to the very cocoon they emerged from and release pheromones for males to locate them. By the end of July, egg masses are being laid on the tree bark to begin the cycle again the next year. Gypsy moths are such a successful invasive forest pest in North America because they are generalist feeders with many options, and they are very prolific and lack enough predation to control the population density.

Control Methods Overview

Nucleopolyhedrosis Virus (NPV) – the Gypsy Moth Virus

Michigan State University. (n.d.). Integrated Pest Management. A Virus and a Fungal Disease Cause Gypsy Moth Outbreaks to Collapse. Retrieved from: https://www.canr.msu.edu/ipm/invasive_species/Gypsy-Moth/virus-and-fungus-disease-cause.

- Gypsy moth populations in North America have always been affected by NPV. Since the virus is present in gypsy moth populations, it does not need to be introduced
- NPV damages the internal organs of the caterpillar, thereby killing it. Cadavers are left hanging off the bark in an upside-down “V”
- Sometimes gypsy moths can carry a sublethal dose that will make females produce smaller and fewer eggs
- NPV can become epizootic (many deaths over short period of time) during the second or third year of an outbreak
- NPV relies on population density (high population density of gypsy moth is regulated by increased spreading of NPV)

- NPV is used in the US as a product called Gypchek ® which is administered annually by a federal agency. In Canada, NPV is being used to develop a biological pesticide called Dispavirus (Invasive Species Centre, 2020)

Entomophaga maimaiga – the Gypsy Moth "Asian" Fungus

Michigan State University. (n.d.). Integrated Pest Management. A Virus and a Fungal Disease Cause Gypsy Moth Outbreaks to Collapse. Retrieved from: https://www.canr.msu.edu/ipm/invasive_species/Gypsy-Moth/virus-and-fungus-disease-cause.

- *Entomophaga maimaiga* is a fungus that is native to Japan and called the "insect eater".
- Introduced as a biological control in Northeastern US in early 1900's.
- Resting spores are thick walled spores that are present in winter in the soil and on bark. These spores germinate in May/June in favourable wet and humid conditions.
    - Spores stick to the foraging caterpillar and digests its way through the body. This will kill the caterpillar within a week.
    - Cadavers are left hanging straight, dried out, with their heads pointed downward
- Conidia spores ("dry" spores) rest on caterpillar cadavers and spread via wind action to other caterpillars.
    - Conidia spores can have 4-9 cycles per summer
- Weather impacts how well this fungus works to control Gypsy moths.
    - It needs moisture and high humidity, especially in May/June to effectively spread and prevent outbreaks
    - Drought years can lead to outbreaks

Bacillus thuringiensis kurstaki (*Btk*) – the Biological Pesticide

BC. (2014). What is Btk? Retrieved from: https://www2.gov.bc.ca/gov/content/industry/forestry/managing-our-forest-resources/forest-health/invasive-forest-pests/gypsy-moth/what-is-Btk.

- *Btk* is a bacteria subspecies that is targeted towards Gypsy moth caterpillars and is very potent for them. It also impacts other lepidopterans, especially those with similar lifecycles.
- *Btk* contains crystals that release a toxic protein. This protein dissolves in the moth's digestive system and causes them to stop eating and die within 5 days.
- Aerial sprays have been conducted in Toronto. A mixture of 3% *Btk*, 75% water, and 22% food grade inerts (additives to allow *Btk* to stick to foliage and last longer in sunlight) are used. This allows *Btk* to persist for 3-7 days before dissolving. The timing of administration is very important, and it is not effective to spray before caterpillars have hatched in May (Gov.Can. 2013).

Mating disruption and Pheromone trapping – Disparlure

BC. (2014). Alternative Treatments. Retrieved from: https://www2.gov.bc.ca/gov/content/industry/forestry/managing-our-forest-resources/forest-health/invasive-forest-pests/gypsy-moth/alternative-treatments.

- Disparlure is an artificial sex pheromone of the female gypsy moth (specific to gypsy moth)
- Disparlure is used in traps to attract males and capture them before they have a chance to mate. Females aren't caught because they cannot fly.
- The pheromone is also distributed as a bead or flake form that is released by aircraft over infested forests. This method is used to confuse males with too many sources of the pheromone.
- Use of disparlure is not registered for use in Canada but has been used in the US.
- This pheromone is specific to the gypsy moth species, so it doesn't interfere with other species. Doesn't require the introduction of a new species as a control method.

Impacts on Human Health

Otvos, I. S., Armstrong, H., and Conder, N. (2005). Safety of Bacillus thuringiensis var. karstaki Applications for Insect Control to Humans and Large Mammals. Natural Resources Canada. Retrieved from: https://cfs.nrcan.gc.ca/publications/download-pdf/28281.

A 2005 metanalysis was conducted by Natural Resources Canada of the risks that the use of *Bacillus thuringiensis* var. *karstaki* (*Btk*) may have on humans and large mammals. They focused on literature from urban, agricultural and forest environments. *Btk* has been the most widely used insecticide in Canada since the 1970's, with *Btk* being a subspecies of *B. thuringiensis* (*Bt*) that targets against over 200 species of Lepidoptera. Human exposure to *Btk* was studied in the 1950's in the field under realistic conditions using volunteers and found no health risks. In a laboratory setting, human volunteers in the 1950's consumed 1 g of Thuricide ® (the active ingredient in *Bt* var. *thurigiensis* (*Btt*)) or inhaled 100 mg of the powder for 5 days. Complete medical examinations were conducted before, immediately after the administration, and 5 weeks later. All volunteers showed no adverse effects from either consumption or inhalation of the *Btt* treatment. A previous toxicology study in 1989 from the US-Environmental Protection Agency (EPA) found no adverse effects of *Btk* exposure to mammals, including humans, when ingested. In 1984-1987, Quebec conducted aerial sprays of *Btk* for managing the invasive spruce budworm populations. People working directly with the spray program, and people living within 13 km of the area were monitored for health effects by collecting nasal and blood samples. Out of 484 nasal samples, 16 people (3.3%) had a positive culture of *Bt*, but no immunological responses. Out of 110 blood samples, only one person had an immunological response.

Since *Btk* registration in Canada in 1970, no health issues have been directly attributed to *Btk* use, including use on agricultural products for consumption. The US-EPA approves the use of *Bt* on food products, which is also true for Canada. *Bt* (maybe *Btk*) has been cultured multiple times from commercial vegetables both during and after gypsy moth eradication programs were administered in Vancouver in 1992. This has not caused any health issues for people, but consumers were told to properly wash their produce before consumption. Humans and other mammals will digest the toxins produced by *Btk*. Additionally, mammalian cells don't have the receptors required for *Btk* toxins to bind. With the available research in this metanalysis, *Btk* aerial spraying programs are considered safe to human health.

Sheila Van Cuyk, Alina Deshpande, Attelia Hollander, Nathan Duval, Lawrence Ticknor, Julie Layshock, LaVerne Gallegos-Graves, et al. (2011). Persistence of Bacillus thuringiensis subsp. kurstaki in Urban Environments following Spraying. Applied and Environmental Microbiology, 77(22), 7954–7961. United States: American Society for Microbiology.

This 2011 study, the persistence of *Btk* in urban settings was sampled after *Btk* aerial sprays in Fairfax County, VA and Seattle, WA in the United States. These two locations were mostly residential and commercial areas with <15% tree cover. In 2008, 245 samples were taken at each location for every sampling interval, which were taken immediately before and immediately after *Btk* spraying. They also sampled at 6, 12, 24, and 48 weeks after spraying. A control area in Washington State Department of Agriculture was also sampled for a baseline presence of *Btk* in a non-sprayed area. They sampled the soil, vegetation (grass and leaves), water, and urban surfaces ("wipes"). The researchers extracted DNA from the samples using real-time PCR and culture growth for analyzing *Btk* persistence. In Fairfax County, none of the samples tested positive by PCR or culture for *Btk* in the vegetation or wipe samples. Some water samples in Fairfax County had viable *Btk* cultures (50% culturable at 48 weeks). However, in Seattle there was no detection of *Btk* for culture or PCR in the water. The researchers found viable *Btk* in soil that was sampled 4 years after spraying in Seattle. They found that *Btk* can persist in the environment for years after administration in small amounts on vegetation, but mainly in soil.

TABLE 4. Percentage of pooled samples passing the *B. thuringiensis* subsp. *kurstaki* PCR screen and *B. thuringiensis* subsp. *kurstaki* culture for wipe, water, grass, and leaves for each location[a]

Location and time period	% Samples											
	Wipe			Water			Grass			Leaves		
	n	PCR	Culture	*n*	PCR	Culture	*n*	PCR	Culture	*n*	PCR	Culture
Fairfax County, VA												
Background	51	0	0	6	0	0	21	0	0	23	0	0
0 wks	42	7	0	7	0	14	15	80	7	21	48	10
6 wks	40	10	20	6	0	17	14	0	7	16	0	6
12 wks	40	0	13	3	0	0	17	0	0	20	0	5
24 wks	41	2	5	5	0	0	15	0	0	18	6	0
48 wks	39	0	26	6	0	50	13	0	0	19	0	0
Seattle, WA												
Seattle, control	39	0	0	3	0	0	0	NA	NA	0	NA	NA
Kent, 2007	40	23	3	1	0	0	0	NA	NA	0	NA	NA
Madison, 2006	40	5	0	0	NA	NA	0	NA	NA	0	NA	NA
Rosemont, 2006	39	0	0	0	NA	NA	0	NA	NA	0	NA	NA
Eastlake, 2005	43	0	16	1	0	0	0	NA	NA	0	NA	NA
Bellevue, 2004	44	0	0	0	0	NA	0	NA	NA	0	NA	NA

[a] The percentages of total pooled samples (*n*), samples passing the *B. thuringiensis* subsp. *kurstaki* PCR screen (PCR), and *B. thuringiensis* subsp. *kurstaki* culture samples (Culture) for wipe, water, grass, and leaves were determined for each location. NA, not applicable for samples not collected.

Figure 1. Btk persistence in wipe (urban surfaces), water, grass, and leaves (Van Cuyk et al. 2011).

Impacts on Non-target Species

Strazanac J. S. and Butler L. (n.d.). Long term evaluation of the effects of Bacillus thuringiensis kurstaki, gypsy moth nucleopolyhedrosis virus product Gypchek ®, and

Entomophaga maimaiga on nontarget organisms in mixed broadleaf-pine forests in the central appalachains. West Virginia University. 4 (34-79). Retrieved from: https://www.fs.fed.us/foresthealth/technology/pdfs/BtkNontargetStudy_v7.pdf.

A study from West Virginia University assessed the impact of three control methods (*Bacillus thuringiensis kurstaki,* the NPV product Gypchek®, and the fungus *Entomophaga maimaiga*) for the gypsy moth on non-target organisms over a seven-year study (1995-2001). The study sites were in George Washington National Forest and Monongahela Nation Forest, which are bordering wilderness areas. These sites had a total of 18 plots (nine for each) where gypsy moth egg mass surveys were conducted and birds, salamanders, and arthropods were monitored for the duration of the study.

For the arthropod studies, the researchers were able to assess the impact of biopesticide use (*Btk*, NPV, and *E. maimaiga*) and the following recovery time of impacted insect groups. Sampling of arthropods was done by using six different trapping techniques (including gypsy moth egg mass surveys) and hand collecting. Within arthropods, several insect groups were focused on as they make up a diverse and essential part of the forest ecosystem. Lepidopterans (butterflies and moths) were of primary concern since *Btk* impacts many of the species in this order, including the gypsy moth. Lepidopterans with the highest risk of *Btk* impact were those that shared a host plant with gypsy moth, had similar life histories, and are present and/or feeding during *Btk* treatments.

Their results showed that caterpillars sensitive to timing of *Btk* treatments, such as spring caterpillars, had large declines in the treatment years compared to control plots and Gypchek ® plots. This decrease was observed in the first post-treatment year as well. Species with more than one generation (multivoltine) in a year recovered faster from treatment than species with one generation (univoltine), such as the gypsy moth. The most abundant species of spring caterpillars surveyed were: the lesser maple spanworm (*Itame pustularia*) with no significant declines ($p<0.05$); forest tent caterpillar (*Malacosoma disstria*) with significant declines when combining treatment and post-treatment years; eastern tent caterpillar (*Malacosoma americanum*) with significant declines on *Btk* plots; a lymantriid species (*Dasychira dorsipennata*) with significant declines in post-treatment years.

The microlepidopteran family Tortricidae significantly declined in population during *Btk* treatment years but recovered in post-treatment years. There was no impact from Gypcheck ® for this group. Other insect groups adversely impacted directly and indirectly were: sawflies (Symphyta), who share similar larval characteristics to lepidopterans; and parasitoids (i.e. some Tachinids flies and Ichneumonid wasps) that specialize on Lepidoptera as prey and hosts. Pollinator insect groups including bees (Apoidea) and hover flies (Syrphidae) did decline, but not significantly, on *Btk* treated plots.

Btk seemed to mainly impact spring lepidopterans that are present during *Btk* treatments and share host plants with the gypsy moth. Natural enemies that target spring lepidopterans were also adversely impacted. Since *Btk* remains on foliage for a short time (several days) and is also naturally occurring, it doesn't appear to have long-term impacts on other insects. This study suggested applying Gypchek ® in isolated infestations and have *E. maimaiga* established for the long-term management as both methods target the gypsy moth and are the most environmentally friendly options.

For the bird studies, researchers evaluated bird populations that are dependent on Lepidoptera larvae for survival and food provisions for their young. This portion of the study focused on indirect effects of *Btk* on avian abundance and breeding ecology of forest songbirds. Indirect effects included changes in vegetation composition due to defoliation and changes in caterpillar abundance. They chose four migrant songbird species to monitor, who rely on caterpillars as a food source: red-eyed vireo, worm-eating warbler, wood thrush, and blue-headed vireo. They hypothesized that the reduction of nontarget lepidopterans would negatively impact the breeding ecology of birds with a lepidopteran diet and that there would be changes in abundance and species composition of birds. Both bird abundance and reproductive output were monitored over this seven-year study.

For species richness, there was no significant effect of *Btk* treatment, but there was a slightly decreased species richness on *Btk* treated plots during treatment years (1997 and 1998). The worm-eating warbler showed nestling weights that were 16% less on *Btk* plots and greater variability than in non-treatment plots. This species also showed decreases in clutch size and fledglings produced per nest on *Btk* plots.

They concluded that changes in avian abundance and diversity due to *Btk* treatment will not be evident until the following year after treatment for most songbird species. This seems to be because birds have already established their nests and territories by the time *Btk* is applied in the spring. If caterpillar abundance is low the following year, then bird abundance is reduced as well. Many species including rose-breasted grosbeak, acadian flycatcher, and the black-throated green warbler follow this trend and recovered as caterpillar abundance recovered.

Addison, J. A., Otvos, I. S., Battigelli, J. P., & Conder, N. (2006). Does aerial spraying of Bacillus thuringiensis subsp. kurstaki (Btk) pose a risk to nontarget soil microarthropods? Canadian Journal of Forest Research, 36(6), 1610–1620. Ottawa, Canada: Canadian Science Publishing.

In 1996, a field study done in a British Columbia, Canada forest explored the impacts of *Btk* aerial spraying on nontarget soil microathropods (which are small animals that live in the soil and are important decomposers and nutrient cyclers). *Btk* was being used for treatment of spruce budworm (*Choristoneura occidentalis*), which is another forest pest. In three replicate plots of 50 ha each, the *Btk* product Foray ® 48B was sprayed at a dose of 60 billion international units (BIU)/ha in 4.8 L/ha to determine the effectiveness of the treatment for spruce budworm. Sampling of microathropods was conducted once before the treatment, twice after treatment (3 weeks later and 3 months later). These sampling times were chosen to enable researchers to identify short and long-term impacts of *Btk* spraying on microathropods. The most abundant groups collected were springtails (Collembola) and mites (Acari). Using two-way RM-ANOVA statistical analyses, the researchers did not find a significant reduction in collembolan abundance due to *Btk* treatment. Similar statistical analyses for mite abundance revealed that *Btk* had no significant effect on the mites. Other taxa including tardigrades, proturans, symphylans, and others were not abundant enough to perform statistical analyses. The researchers also considered the depth of soil and found that even in the uppermost layer of soil (top 2.5 cm), there was no impact to abundance of any groups due to *Btk* treatments. The collembolan community (referring to the composition of different springtail species present) did not change after the treatment. When conducting gut content analysis, they found no changes in bacteria load within the ingested materials of collembolans. In conclusion, there were no found negative or positive impacts of *Btk* treatment on springtails or mites.

D'Urso, V., Mazzeo, G., Vaccalluzzo, V., Sabella, G., Bucchieri, F., Viscuso, R., & Vitale, D. G. M. (2017). Observations on midgut of Apis mellifera workers (Hymenoptera: Apoidea) under controlled acute exposures to a Bacillus thuringiensis-based biopesticide. Apidologie, 48(1), 51-62. Paris: Springer Paris.

A study from 2017 wanted to determine the effect of the biopesticide *Btk* on honeybees (*Apis mellifera*) with controlled exposure. Other studies had shown that *Btk* can have long-term, but sublethal, effects on honeybees at certain concentrations of the toxins from *Btk*. This study wanted to investigate this further by examining changes in the midgut of honeybees, which could influence behaviour and mortality. There were four trials to this study including mortality analysis, light analysis (LM), and electronic microscopy (SEM, TEM). Each trial used four different treatments of *Btk* at varying concentrations. These four concentrations included a control (no biopesticide), "field concentration" (the product-recommended field dose of 100.0 g/hL), "low concentration" (40.00 g/hL), and "very-high concentration" (24 400.0 g/hL). There were three replicates for each concentration group containing 10 honeybees each.

For the mortality analysis, researchers fed honeybees a solution of the biopesticide at the four different concentrations. The mortality analysis showed that "field concentrations" and "very-high concentrations" resulted in honeybee mortality 48 hours after treatment. Mortality especially increased at 72 and 96 hours after treatment for the "very-high concentration" treatment with the final mortality being 50 % of tested bees ($p > 0.05$). The "low concentration" and control group had 0% mortality. The behaviours of the bees changed in the "very-high" and "field" concentration groups to be inactive/motionless and decreased appetite. Bees in the "low concentration" group did not show symptoms of lethargy after treatment except for a couple specimens (not statistically significant). Bees in the "low" and "field" concentration groups gained back their usual activity level after 24 hours, but the "field" concentration group retained lower appetite than the control and "low" concentration group. Out of the surviving bees in the "very-high concentrations" group, they retained their inactivity and low appetite.

In the midgut analysis (LM, SEM, TEM), there was evidence of cell oncosis/necrosis (death) from microbial toxins at certain concentration groups, which is consistent with previous studies with exposure to *Bt* in other insects. The researchers hypothesize that the honeybee midgut cell death was related to the Cry-toxins that are found in the *Btk* solution. The "low" and "field" concentration groups recovered after 24 hours, whereas "very-high" concentrations did not recover (Figure 2).

The study concludes that their midgut analysis findings support the fact that biopesticides are less harmful than other agrochemicals and the *Bt*-toxins have high-specificity for which species of insects (especially larvae) that it impacts. They also state that bees are likely exposed to less than the "field concentration" realistically since they feed on pollen/nectar within a restricted time frame when flowers are blooming. Exposure to *Btk* in a natural scenario would more realistically be represented by the "low concentration" group and therefore no significant impact on honeybee mortality. The study does mention the need to further study long-term persistence of *Bt*-toxins in the environment (bioaccumulation) and behavioural changes of bees treated with *Bt*-toxins.

Table I. Overview table of the effects observed in the groups.

Effect on	Group Time	Control	Low concentration	Field concentration	Very-high concentration
Symptoms	4 h	Absent F.C.: 50.41 %	Sporadic (S.p.) F.C.: 47.81 %	All the bees (S.p./L.F.C.) F.C.: 30.85 %	All the bees (S.p./L.F.C.) F.C.: 30.00 %
	24 h	Absent F.C.: 47.10 %	Recovery F.C.: 46.90 %	L.F.C. F.C.: 33.75 %	(S.p./L.F.C.) F.C.: 30.83 %
	48 h	Absent	Recovery	Recovery	Worsening
	72 h	Absent	Absent	Recovery	Worsening
	96 h	Absent	Absent	Absent	Worsening
Midgut changes	4 h	Absent	Absent	Absent	Absent
	24 h	Absent	Some	Some	Several
	48 h	Absent	Few	Some	Widespread
	72 h	Absent	Sporadic	Few	Widespread
	96 h	Absent	Sporadic	Sporadic	Widespread

s.p. Stationary phase (Medrzycki et al. 2003); *F.C.* lower food consumption

Figure 2. This table summarizes the findings observed in controlled Btk exposure to honeybees (D'Urso et al. 2017).

Broderick, N. A., Robinson, C. J., McMahon, M. D., Holt, J., Handelsman, J., & Raffa, K. F. (2009). Contributions of gut bacteria to Bacillus thuringiensis-induced mortality vary across a range of Lepidoptera. BMC biology, 7(1), 11-11. England: Springer Science and Business Media LLC.

In a 2009 study, researchers assessed the susceptibility of six lepidopteran species to the bacteria *Bacillus thuringiensis* (*Bt*). The study species included the gypsy moth *Lymantria dispar (Lymantriidae), Manduca sexta* (L.) (Sphingidae), *Vanessa cardui* (L.) (Nymphalidae), *Pieris rapae* (L.) (Pieridae), *Heliothis virescens* (F.) (Noctuidae), and *Pectinophora gossypiella* (Saunders) (Gelechiidae).They focused on the larval stage and wanted to determine susceptibility to *Bt* based on the presence or absence of their original gut bacteria. The researchers previously found that when rearing gypsy moth larvae (*Lymantria dispar*) on antibiotics, mortality decreased after *Bt* exposure. When they restored a species of gut bacteria in gypsy moth larvae, the susceptibility to *Bt* came back. Based on those findings, the researchers created two treatments: larvae fed antibiotics and *Bt* treatment (2×10^2 CFU/gut), and larvae fed only the *Bt* treatment (1.6×10^2 CFU/gut).

The treatment group without antibiotics was susceptible to *Bt* in all six species (63-100% mortality), while the treatment group with antibiotics had significantly reduced mortality in five species (0-10% mortality). The species *P. gossypiella* experienced 33-75% mortality even with antibiotics. This species also had a different diversity of gut bacteria than the others. The diversity of gut bacteria in the study species usually consisted of two bacteria types. Enterobacteriaceae was always one of the types, but *P. gossypiella* did not have this type. When adding the original *Enterobacter* sp. back into the guts of the study specimens, mortality rose significantly in *M. sexta* (from 0% to 61%), *V. cardui* (from 8% to 86%), *P. rapae* (from 8% to 100%) and *L. dispar* (from 11% to 44%).

This study concluded that certain larval species who have their original gut bacteria are more susceptible to *Bt*. This is because the *Bt*-toxins perturbs certain strains of gut bacteria that are normally harmless into having pathogenic effects. Having diversity of gut bacteria is normally beneficial, but perturbations can cause the microbiota to become pathological, causing septicemia and host mortality. There are many different lepidopteran species who may have gut bacteria that will make them more susceptible to *Bt*-toxins than other species. This isn't fully investigated since factors like feeding window, larval gut microbiota, and larval diet may change the species' ability to survive exposure to bacteria *B. thuringiensis*.

Weeks, D. M., & Parris, M. J. (2020). A Bacillus thuringiensis kurstaki Biopesticide Does Not Reduce Hatching Success or Tadpole Survival at Environmentally Relevant Concentrations in Southern Leopard Frogs (Lithobates sphenocephalus). Environmental toxicology and chemistry, 39(1), 155–161. United States: Wiley Subscription Services, Inc.

To quote the main findings of this article:

"Amphibians are in global decline, and anthropogenic activities are known leading causes of their demise. Thus, the interaction between agriculture and amphibian health has been examined for decades. Many facets of amphibian physiology and ecology place them at high risk among the nontarget organisms affected by agricultural byproducts. Research has shown that many chemicals and fertilizers affect amphibian growth, reproduction, and survival. The impacts differ based on the type of agricultural byproduct (e.g., chemical pesticide or nutrient-heavy fertilizer) and amphibian species, but the effects are usually negative. However, minimal research exists on how organic biopesticides interact with amphibian populations. Biopesticides utilize insecticidal bacteria as the active ingredient in lieu of synthetic chemicals. The inert ingredients present in biopesticide commercial products are considered safe to nontarget organisms. The present study tested the impacts of a commercial biopesticide on the survival of amphibian embryos and larvae. We found that expected environmental concentrations of the microbial biopesticide Monterrey *Bt* did not significantly reduce survival in embryos or larvae. However, the higher doses used to assess threshold toxicity levels caused significant mortality. Our data suggests that biopesticides are not directly harmful to amphibian embryos or larvae in concentrations regularly applied for pest control."

Conclusion

Overall, there is evidence of impacts of *Btk* on non-target species in the environment. In addition, there are many concerns about potential impacts that have not been studied sufficiently. The main findings in this review that do raise concerns about the impact of Btk in the environment are:

1) **Potential human uptake and unknown immunological response by humans.**
Bt was cultured in humans exposed to aerial spraying (3.3%), and it is possible for humans to have an immunological response to *Bt* in their body. The immune response of people with compromised immune systems that are exposed to *Bt* needs to be investigated. *Btk* has been cultured from agricultural products after an aerial spray, has not caused illness in humans. It is recommended to always wash your fruits and vegetables before consumption. *Btk* aerial sprays are safe to use for most humans but should continue to be explored since there are cased of illness due to *Bt* and because many of the experiments exposing humans to *Bt* is older than ten years (Otvos et al., 2005).

2) **Persistence in the environment.**
Btk can exist in the environment for much longer than 3-10 days. After aerial sprays, *Btk* was found in water up to 48 weeks later and in soil 4 years later (Van Cuyk et al., 2011).

3) **Impacts to non-target invertebrate species.**
Non-target insects impacted by *Btk* treatments include multiple spring caterpillar species, who share similar lifecycles and timing as the gypsy moth. Decreases in abundance were found during and after *Btk* treatments. *Btk* treatments also caused decline in some parasitoid wasp species, who are important predators and often a natural enemy of the gypsy moth. Other control methods for the gypsy moth should be considered to reduce the known and especially the unknown impacts on non-target species and ecosystems. The use of an NPV product and *E. maimaiga* could be beneficial as an alternative (Strazanac and Butler, n.d.).

4) **Impacts to the micro biota of the soil and soil health has not been fully studied.**
When assessing soil microarthropods, no significant reductions in abundance was found. Researchers did not collect enough specimens from many taxa to be able to conduct analysis. They only had enough data to analyze Collembolans (springtails) and Acari (mites) species. These two groups were not impacted by *Btk* aerial treatments, but the impacts on other important soil organisms should be investigated. The health of soil is the basis for all other terrestrial life, and should be evaluated and monitoring before, during, and after *Btk* treatments (Addison et al., 2006).

5) **Impacts to honeybee health and viability.**
There are proven adverse impacts to the midguts of Apiodea (bees). Honeybees were impacted by *Btk* solutions under field concentrations, which is the expected exposure to concentrations in a real *Btk* aerial spray. Their appetite and energy level would be diminished, and this effect increased with increasing concentration. Most bees were able to recover, but some retained their low appetites and lethargy. At higher concentrations, the honeybee mortality was high (up to 50% mortality). *Btk* caused midgut cell inflammation and septicemia, resulting in death. This is very concerning

for honeybees, and any other pollinators that could be exposed to *Btk*. A decrease of 50% in pollination would be catastrophic to the environment, but even decreases less than 50% are problematic as wildlife is facing more pressures from habitat loss, climate change, and pollution. Wildlife resiliency is weakened by these stressors, and we should avoid unnecessary pressures when possible (D'Urso et al., 2017).

6) **Impacts to non-targeted Lepidopterans (butterflies and moths).**
The bacteria Bacillus thuringiensis (*Bt*) can also damage the midguts of Lepidopterans (butterflies and moths). Under a controlled setting, six species of lepidopterans were exposed to *Bt* and found that species are more susceptible to *Bt* depending on their diversity of gut bacteria. Certain species of gut bacteria will become pathogenic, or cause disease, when exposed to *Bt*. We do not know the diversity of gut bacteria in every single invertebrate species that will be exposed to a chemical spray like *Btk*. This study stresses the fact that we do not always have all the information to assess how a chemical treatment is going to impact an ecosystem, so assuming it is safe means that we are ignoring the stress we are imposing on our environment (Broderick et al., 2009).

7) **Potential impacts to Avian species in subsequent years.**
There was a slight decrease in bird species richness on *Btk* treated plots during treatment years, however, it was not significant effect. The researchers concluded that changes in avian abundance and diversity due to *Btk* treatment will not be evident until the following year after treatment for most songbird species. This seems to be because birds have already established their nests and territories by the time *Btk* is applied in the spring. If caterpillar abundance is low the following year, then bird abundance is reduced as well. Many species including rose-breasted grosbeak, acadian flycatcher, and the black-throated green warbler follow this trend and recovered as caterpillar abundance recovered.

8) **Aquatic impacts are not well known and not studied.**
There are very few studies that assess the impacts to aquatic species.

Questions

1. What are the impacts that gypsy moth could have on forests if no control measures are used? Will the population self-regulate eventually?
2. How effective is the use of *Btk* (aerial sprays and spot treatments)?
3. What are the impacts of *Btk* on:
    a. Forest ecology
    b. Aquatic ecology
4. *Btk* targets Lepidopterans. Is there any current research or information of *Btk*'s impact on pollinators, specifically Monarchs?
5. Nucleopolyhedrosis Virus (NPV) occurs naturally in the environment and kills gypsy moths. Do we have evidence that NPV will control high densities of gypsy moth populations, or is intervention with other methods needed?
6. What control methods are being used in Ontario for the gypsy moth currently? Is a combination of treatment methods useful or being studied, such as *Entomophaga maimaiga* or NPV distribution?
7. Would you recommend the use of pheromone traps (Disparlure) for cottagers to use on their properties? How can they get access to appropriate, effective traps and how to properly maintain the trap?
8. What are other methods that home-owners can use to protect the trees on their property?
9. Are white pines more susceptible to dying from gypsy moth infestation because they already have other stressors (i.e. white pine beetle, climate change)? Are white pines preferred for laying egg masses (maybe due to their coarse bark that could provide more protection)?

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