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Agricultural Innovation Board Literature Review of Neonicotinoid Treated Seeds (NTS) Impact on the Environment

Annotated Bibliography

Alford, A. M., & Krupke, C. H. (2019). Movement of the neonicotinoid seed treatment clothianidin into groundwater, aquatic plants, and insect herbivores. *Environmental Science Technology, 53*(24), 14368-14376.

The objectives of the study were to: (1) use a field study to quantify the leaching potential of NTS throughout the growing season in Indiana corn and (2) use concentrations found during objective 1 and from other publications and conduct lab experiments with gibbous duckweed and water lily aphid to document nontarget macrophyte translocation and potential impacts on higher trophic levels. Water samples from tiles from three crop groups were analyzed: (1) corn plots (1.25mg/kernel clothianidin), (2) NTS-free soybean plots, and (3) NTS-free control plots (NTS-free variable annual crops). Clothianidin levels never exceeded 0.1 ppb throughout the growing season in the control plots. A maximum of 3.37 ppb clothianidin was collected after rainfall event 4 weeks after planting from the corn plots. This concentration is about 10x higher than previous research where clothianidin was measured in surface water where it can be diluted and subject to photodegradation. Duckweed grown in clothianidin-contaminated water showed rapid uptake of clothianidin, but aphids were unlikely to suffer acute mortality when fed on duckweed for 48 hours.

Grout, T. A., Koenig, P. A., Kapuvari, J. K., McArt, S. H. (2020). Neonicotinoid Insecticides in New York State: Economic benefits and risk to pollinators. Section 6.3 Environmental fate of neonicotinoid insecticides *Cornell University Extension,* 195-198.

The environmental fate section of this extensive review of neonicotinoid research is summarized here. Non-target exposures to pollinators may occur if they are present at a site during application via transport of the insecticide away from application site or if they are exposed after the application occurs via persistence at the application site. In 2013, USEPA mandated pollinator protection language be present on all products labelled for outdoor foliar use. Therefore, when the label is followed (as is required by law) risk to pollinators is likely to be minimized, but exposure is still possible. Seed coatings account for the majority of neonicotinoid insecticides in New York and abrasion to seeds during transport, loading, and planting can create insecticide contaminated dust. The amount of dust produced depends on how the seeds are coated and cleaned, the lubricating agent used at planting, type of planter used and environmental conditions during planting. A standard test (Heubach test) measures the amount of dust produced per set number or weight of seeds by simulating potential mechanical stress. Talc and graphite used as lubricants can contribute to dust drift, but advanced seed lubricants, such as Bayer's Fluency Agent Advanced for corn and soybeans have been shown to reduce dust due to abrasion by more than 88% over talc. However, the advanced seed lubricants are more expensive and therefore are used less commonly. There is no publicly available data on the percent of growers using advanced seed lubricants. Dust drift can also be affected by planter technology. Mechanical-type planters produce less dust during planting than vacuum-type machines. Overall, production of dust drift can be mitigated by using appropriate seed coating formulations and lubricants, redirecting or filtering exhaust of planters, and avoiding planting during dry and windy conditions.

One advantage of NTS is they require less active ingredient than an equivalent soil drench or in-furrow granule, and the active ingredient is more precisely targeted for uptake by the germinating plant. However, the plant only absorbs between 1.6-20% of the active ingredient from the seed coating, depending on the crop and environmental conditions. The remainder of the active ingredient can persist in soils or move from the site via leaching or transport in surface or ground water. This persistence and movement in soils can result in direct soil exposures to pollinators (the majority of New York's 417 species of bees are ground-nesting). Persistence in soil can also lead to nectar/pollen exposures in field margins via contaminated wildflowers that take up the neonicotinoids from the soil. Persistence of neonicotinoids in soil depends on pH, temperature, moisture content, organic matter, root systems, and soil structure and texture. Half-lives of neonicotinoids in soil ranged from fewer than 90 days (dinotefuran) to several years (over 8 years for imidacloprid and 19 years for clothianidin). Persistence of neonicotinoids in water depends on pH and UV radiation. When in surface water and exposed to sunlight the half-lives of imidacloprid, clothianidin and thiamethoxam are short (<3.5 days) and half-lives of thiacloprid and acetamiprid are slightly longer (8-68 days). Because sunlight cannot penetrate through deeper water and groundwater, longer half-lives are anticipated. It is important to note that breakdown of neonicotinoids in soil and water does not make them harmless because some breakdown products are more toxic or similarly toxic to bees. For example, metabolites of imidacloprid are more toxic than the parent compound and thiamethoxam breaks down in part into clothianidin.

Overall, since movement of neonicotinoids in soil and water are influenced by so many variables, it is difficult to predict the extent to which they will move through the environment. However, neonicotinoids are generally highly mobile compared to most other insecticides due to their high water solubility and other chemical characteristics. Numerous studies have found neonicotinoids in pollen and/or nectar of wildflowers in field margins despite evidence in another study suggesting that up to 90% of neonicotinoids in soil are not bioavailable to plants.

Hall, M. J., Zhang, G., O'Neal, M. E., Bradbury, S. P., & Coats, J. R. (2022). Quantifying neonicotinoid insecticide residues in milkweed and other forbs sampled from prairie strips established in maize and soybean fields. *Agriculture, Ecosystems & Environment, 325*, 107723.

This study wanted to understand pesticide exposures to pollinator habitat near crop fields. Quantified concentrations of clothianidin, thiamethoxam and imidacloprid in soil and leaf tissue in reconstructed prairies (established 3-4 years prior) within or adjacent to corn or soybean fields in Iowa. Samples collected April – August 2017-2018. 100% of soil, 80% of vegetation from blooming forbs, and 80% of milkweed leaf tissue had at least one neonicotinoid present above the detection limit (0.07-0.9 ppb). The maximum concentrations detected in milkweed leaf tissue are 10-130x lower than the chronic dietary LC_{10} values for monarch larvae, so it is unlikely that this route of neonicotinoid exposure will cause adverse effects. Exposure to monarch larvae is below the threshold of concern. Most likely exposure route to non-target plants is surface/subsurface runoff because did not see early season/post plant spike in foliar neonicotinoid concentrations indicating exposure due to dust drift at planting and neonicotinoids are highly water-soluble allowing for transport and availability for uptake by down-slope plants.

Hatfield, R. G., Strange, J. P., Koch, J. B., Jepsen, S., & Stapleton, I. (2021). Neonicotinoid pesticides cause mass fatalities of native bumble bees: A case study from Wilsonville, Oregon, United States. *Environmental Entomology, 50*(5), 1095-1104.

A case study of lethal impact of neonicotinoid, dinotefuran, on pollinating insect populations in suburban Wilsonville, Oregon. Dinotefuran was applied to an ornamental planting of European linden trees while they were in bloom for aphids (foliar application) and root weevils (soil drench application). Based on geographic information systems and population genetic analysis, authors estimated 45,830 – 107,470 bumble bees were killed during this event.

Hladik, M. L. (2018). Environmental Risks and Challenges Associated with Neonicotinoid Insecticides. Environmental Science & Technology 2018 52 (6), 3329-3335.

“While the use of seed coatings can lessen the amount of overspray and drift, the near universal and prophylactic use on major agricultural crops has led to widespread detections in the environment (pollen, soil, water, honey). Pollinators and aquatic insecs appear to be especially susceptible to the effects of neonicotinoids with current research showing that chronic sublethal effects are more prevelant than acute toxicity. Meanwhile evidence of clear and consistent yeild benefits from the use of neonicotinoids remains elusive for most crops.”

Research has shown neonicotinoids to be highly water soluble, persistent in soils and waters and easily shifted from their intentional locations to non-targeted locations. An average 5% of the neonicotinoid treatments applied get absorbed by the crop while the remaining approximately 95% either remain in soil or soil water with only an average of less than 2% lost as dust off during the planting process.

In soils of fields where treated seeds are planted, neonicotinoids increase during each subsequent planting year appearing to plateau off after approximately 5 years of use but persist several years after their use is discontinued. Water sampling for multiple neonicotinoids in the highly cultivated areas detected at least one neonicotinoid in 76% of all samples taken and was followed by a rate of 53% of all samples taken in mixed land use areas in a study of the Midwestern United States. Note the sources for these detections range from multiple sources such as overspray, particulates, seed treatments, soil applications.

75% of honey samples taken from around the world had at least one neonicotinoid present, 45% of these contained multiple neonicotinoids. A full field study conducted in Sweden “…where bumblebee colonies placed next to oilseed rape fields treated with clothianidin performed markedly more poorly than controls.” Adjacent to the treated fields, Osmia bicornis, mason bees “failed to breed entirely” while “honeybee hives showed no measurable effects.” Full field studies conducted in Germany, Hungary and the United Kingdom all presented similar results:” …clear adverse effects on bumblebees and mason bees (*Osmia*) and variable effects on honeybees….Some field trials have found no negative impacts” on honeybees. A United Kingdom study revealed predictions on honeybee colony losses could be made using "geographic and temporal patterns of imidacloprid use." In England predictions on declines in wild bee populations could be made utilizing "regional patterns of neonicotinoid use." Further studies in both California and the United Kingdom mirrored these findings. In aquatic insect species neonicotinoids have been shown to be particularly toxic to species such as mayflies, caddisflies, and midges that "support aquatic and terrestrial food webs." Direct consumption of treated seed by granivorous birds can induce lethal or sublethal effects but birds may avoid consumption of treated seed in cases where other food sources are present. Sublethal effects such as: impacts on reproductive ability, impaired orientation in flight or loss of body mass can be generated; in the case of reproduction, by the ingestion of a single treated seed. In insectivorous birds the potential exists for indirect effects by a depletion of their food sources.

Concerns exists on often detected groupings of multiple neonicotinoids together with their metabolites and environmental degradates as to their toxicity potential. Noted "degradation may not infer reduced toxicity" as "studies have shown neonicotinoid metabolites to be as toxic as the parent compound." Reviewers here pointed out that the seed treatments in use can include not only neonicotinoids but "multiple fungicides, herbicide safeners, nematicides, and plant growth regulators along with surfactants/adjuvants."

Prior to the use of treated seed only approximately 35% of maize acres in the USA were treated with an insecticide. Nearly 100% of maize crops planted in the USA and canola crops planted in Canada are coated with a neonicotinoid seed treatment. In Canada conservative estimation from 2009-2012 reveal an increase of 30% neonicotinoid treated acres. Neonicotinoid use has increased worldwide but yields are varied depending upon crop type. Timing of seed treatments may not coincide with the point of highest pest pressures. "…As currently used (seed treatments) are violating key principals in integrated pest management because prophylactic neonicotinoid treatments are targeting 'occasional pests' and there is evidence that pest resistance is increasing with increasing neonicotinoid use."

Hladik, M. L., Corsi, S. R., Kolpin, D. W., Baldwin, A. K., Blackwell, B. R., & Cavallin, J. E. (2018). Year-round presence of neonicotinoid insecticides in tributaries to the Great Lakes, USA. *Environmental Pollution, 235*, 1022-1029.

Study of 10 major tributaries to the Great Lakes, USA whose purpose was to detect the movement of neonicotinoid insecticide through the ecosystem. Monthly sampling was done from October 2015 through September 2016 from 10 tributaries of the Great Lakes. 74% of all monthly samples contained at least one neonicotinoid with as many as three neonicotinoids detected in up to 10% of samples. In decreasing frequencies researchers found imidacloprid (53%), Clothianidin (44%), thiamethoxam (22%), acetamiprid (2%) and dinotefuran (1%). No samples were found to contain thiacloprid. In areas cultivated crops were present concentration of clothianidin and thiacloprid increased significantly while in urbanized areas detections of imidacloprid were increased. Concentrations detected were greatest during the Spring and Summer months coinciding with seed treatments (Spring) and broadcast spraying (Summer). Neonicotinoids were detected at highest levels during the summer but were detected throughout the year.

Maximum individual neonicotinoid level detected in a sample: 330 ng L^{-1}

Maximum total neonicotinoid levels detected in a sample: 670 ng L^{-1}

Median detected neonicotinoid level detected: 7.0-39 ng L^{-1}

United States EPA acute level in aquatic invertebrates range: 385-17500 ng L^{-1}

United States EPA chronic level in aquatic invertebrates range: 10-1,100 ng L^{-1}

Other research (Morrisey et al. 2015) has suggested acute impact range: 200 ng L^{-1} and chronic impact range of 35 ng L^{-1}

By EPA standards no samples were found to be at acute levels and 33 samples were at chronic impact levels. By Morrisey et all standards 4 samples were found to be at acute levels and 26 were at chronic impact levels.

Researchers conclude their study shows "…evidence of potential for chronic toxicity impacts through the near constant neonicotinoid exposure to individual taxa as well as on ecosystem functions….More research is needed on the potential effects of year-round neonicotinoid exposures."

Hitaj, C. S. (2020). Sowing Uncertainty: What We Do and Don't Know about the Planting of Pesticide-Treated Seed. *BioScience*, 70(5), 390-403.

This overview article highlights the limited availability of accurate data when it comes the use of treated seed in the United States. It postulates there is a lack of data available for stakeholders to make informed choices on the cost, benefits and the off-target impacts of the utilization of these products. The bulk of available data centers on the use of field applied pesticides rather than targeting the use of treated seeds. The reviewers have attempted glean out pertinent data from what is currently available and expose the knowledge gaps.

The Food Quality Protection Act (1996) charged the USDA with collection of data on the use of pesticides but in 1988 regulations were passed to exempt treated articles. In 2015 private entities discontinued gathering information on seed treatments and despite the federal government beginning conducting voluntary surveys farmers for this data in 2015 a lack of knowledge as to the treatment products applied to their seed is deemed to have rendered such farmer surveys of little value. What has been gathered makes it difficult to parse out seed treatments from other pesticide usage and exact chemical components of treatments applied.

Treated seed sales have seen an increase from $200 million in 1990 to over $1 billion in 2008 as many seed and chemical companies have combined forces. These pairings broadened use of seed treatments, particularly treatments applied to GE seed with highly sought after traits This bundling of treatments and desirable GE traits made it difficult for buyers to find untreated versions of GE seed. Reviewers generated a snapshot of the average percent of popular crops grown with pesticide treated seed from data collected in 2012-2014 which revealed 90% of corn acres, 76% soybean acres, 62% cotton acres and 57% of wheat acres were grown with treated seeds.

As the purchases of treated seeds increased it appears the farmer's knowledge as to the exact nature of the treatments on their seeds has decreased from treatments, they, hired labor or custom applicators had applied to their seed in the past. This gap in knowledge has led to incomplete, false negative and inaccurate reporting on the seed treatments farmers are utilizing. An example of a survey with likely high degrees of false negative reporting exposed this gap in knowledge. Despite fungicide seed treatments in corn being near universal a 2016 survey of corn fields had only 28% of farmers indicate they had utilized a fungicide seed treatment.

Such inconsistencies in the limited data available are flagged by the reviewers as an impediment to the deliberations by policy makers on pesticide policy and mitigation efforts. They also conclude some evidence for current overuse of pesticide can be presumed in not only the lack of availability of untreated seed but also in the farmers' inability to accurately report the pesticides applied to the seeds they used. "Opportunities for providing more information about pesticide seed treatments to farmers include improved labeling of pesticide-treated seeds and posting information about the active ingredients contained in treated seed products on public websites. In addition to surveying farmers, alternative methods to obtain data on pesticidal seed use rates could include collecting sales data from seed retailers and companied….These companies would be able to provide more accurate data on what kinds of seeds are purchased but would not show where exactly these seeds are planted which is the advantage of surveying farmers….Ultimately, there are tools available to improve data collection of pesticidal seed treatments."

Labrie, G., G. A., Vanasse, A., Latraverse, A., & Tremblay, G. (2020). Impacts of neonicotinoid seed treatments on soil-dwelling pest populations and agronomic parameters in corn and soybean in Quebec (Canada). *PLoS one, 15*(2), e0229136.

Trials were conducted across Quebec in soybean (2015-2016) and corn (2012-2016) fields evaluating the effect of NTS on soil pest densities, crop damage and yield. No significant differences in plant stand or yield between treated and untreated corn or soybeans. NTS were useful in less than 5% of cases because there were low levels of pest pressure and damage. Each site was sown with two alternating strips of treated (neonicotinoids and fungicides) seed and untreated (fungicides only) seed from the same seed lots, repeated three times. Sites were sown in the direction of the slope to prevent surface runoff contamination to untreated plots. Corn seed treatments varied during the study, either 0.25 mg/seed clothianidin or 0.25 mg/seed thiamethoxam in addition to fungicide treatments compared to fungicide only corn. Soybeans were treated with 0.25 mg/seed thiamethoxam and fungicides and compared to fungicide only soybeans from the same lot. In corn plots, the number of wireworms per bait trap did not differ significantly across locations or years and did not differ significantly between treated and untreated strips. In soybean plots, the wireworm population density from bait traps did not differ significantly between treated and untreated in 2015 and 2016. However, a higher number of wireworms were captured in soil samples from the treated strips in 2015 (no difference in 2016). No significant differences in corn stand or soybean stand were observed between treated and untreated across all sites and years. In 2013-2015 the percentage of corn seedling damage by soil-borne insects was significantly higher in untreated plots (13.0%, 1.6%, 12.1%) than in treated plots (7.0%, 0.6%, 7.4%). Corn and soybean yields were not significantly different between treated and untreated strips. Overall, the low abundance of pests (wireworms was most prevalent) in most fields could explain the lack of yield differences observed between NTS and control plots in corn or soybean.

MacDonald, A. M., Jardine, C. M., Thomas, P. J., & Nemeth, N. M. (2018). Neonicotinoid detection in wild turkeys (Meleagris gallopavo silvestris) in Ontario, Canada. *Environmental Science and Pollution Research, 25*, 16254-16260.

Research objective to test for presence of neonicotinoid insecticides in terrestrial vertebrates that consume NTS, such as wild turkeys in Ontario. Tested neonicotinoid levels in 40 wild turkeys taken during hunting season in Ontario, Canada. Nine out of the 40 wild turkeys had detectable levels of neonicotinoids, the highest level of thiamethoxam detected was 0.16 ppm and clothianidin was detected at 0.12ppm. A fungicide, fuberidazole (used as seed treatment in cereals) was detected in two wild turkeys, the highest level was at 0.0094 ppm. Acute toxicity data (LD50 values) exists for clothianidin in select birds: 14 ppm for gray partridge, 31 ppm for Japanese quail, and 152 ppm for northern bobwhite quail. Data from this study help establish baseline data for southern Ontario wild turkeys. However, additional research is required to determine chronic health and reproductive effects on wild turkeys and other wildlife that may occur with repeated exposure and ingestion on NTS.

Main, A. R., Webb, E. B., Goyne, K. W., Abney, R., & Mengel, D. (2021). Impacts of neonicotinoid seed treatments on the wild bee community in agricultural field margins. *Science of The Total Environment, 786*, 147299.

Conducted a two-year field study (2017-2018, Missouri) to assess whether NTS and presence in environmental media (e.g. soil, flowers) influenced bee nest and diet guild abundance and richness. Planted 23 fields to soybeans with three treatments: untreated (no insecticide), treated (imidacloprid), and previously-treated (untreated but neonicotinoid use prior to 2017). Neonicotinoids were detected infrequently in both years within margin flowers (0%), soybean flowers (<1%), margin soils (<8%), and field soils (39%). Neither imidacloprid nor clothianidin were detected in soils of untreated fields in either year. Neonicotinoid presence in field soils was associated with significantly lower species richness (ground- and aboveground-nesting, diet generalists) of wild bee guilds. Long-term persistence of neonicotinoids in field soils may lead to reduced diversity in regional bee communities. Presence of neonicotinoids in field soils reduced richness of wild bee nest and diet guilds living in or near agricultural fields over the two seasons, but annual treatment did not show a significant difference.

McArt, S., & Grout, T. (2020). Notes from the Lab – September 2020 Neonicotinoid insecticides: When there's risk to bees, when there are economic benefits to users, and when there are viable replacements. *American Bee Journal, 160*(9), 1019-1022.

Summary of the main take-home messages from published comprehensive 432pg review of risk to pollinators and economic benefits to farmers (Neonicotinoid Insecticides in New York: Economic benefits and risk to pollinators, 2020)

What did we find regarding risk to pollinators?

There is better insight about risk in field crops compared to other settings like tree fruits, vegetables, and turfgrass & ornamentals. They used the Lowest Observable Effects Concentrations (LOEC) from literature on neonic impacts on honey bees as the standard for what level is defined as risk. In and near corn and soybean fields that are planted with NTS, 74% of exposures are likely to impact honey bee physiology, 58% of exposures are likely to impact honey bee behavior, and 37% of exposures are likely to impact honey bee reproduction. Thus, risk from neonics is often high in field crops settings. There is less data available to review to assess for risk in other settings. There are hundreds of studies that assessed hazard from neonics (i.e. how doses of neonics impact bee mortality, reproduction, behavior and physiology), but few studies assess exposure to bees in the settings where neonics are used. Risk from neonicotinoid used on cucurbits result in exposures that are likely to impact honey be reproduction in 85% of cases. In response the US EPA issued a recommendation to prohibit use of imidacloprid, clothianidin and thiamethoxam products on cucurbits between vining and harvest. Cornell's analysis extends this prohibition window to before the vining stage because applications before or during planting result in exposures known to impact honey bee reproduction. Exposures in ornamentals are likely to impact honey bee reproduction in 70% of cases (based on 18 exposure assessments). Not all neonicotinoids pose high risks to pollinators, for example acetamiprid (a cyanoamidine neonicotinoid) is three orders of magnitude less toxic to bees than clothianidin, imidacloprid, and thiamethoxam (nitroguanidine neonicotinoids).

What about economic benefits of neonics?

Data from over 5,000 paired neonic/control field trials that assessed impacts to pest populations, crop damage or yield were used to see if there were clear benefits from using neonics. The majority of trials conducted on fruits, vegetables and turfgrass find that using neonics reduces pest populations, limits crop damage and improves yield compared to untreated control plots. The benefits overcome the cost of the neonics and therefore result in direct economic benefits to these specific users. But benefits were not always observed when neonics are used, particularly in field crop settings. 83-97% of field trials find no significant increase or decrease in corn yield when NTS are used compared to chemical alternatives or untreated controls. Soybean observations were similar with 82-95% of field trials finding no difference in yield between NTS and chemical alternatives or untreated controls. Therefore economic benefits are infrequent for farmers. Nevertheless NTS are used by nearly all conventional field corn growers and the majority of soybean growers in part due to the insurance value of preventative pest control protecting growers against unpredictable, potentially severe, losses from early-season pests. The authors suggest that incentives and policies to reduce usage of NTS should address those products' value as inexpensive crop insurance as well as pest management tools. Further work to improve predictability of early-season pest outbreaks (i.e. degree-day modeling with site-specific characteristics) has potential the

sustainability and security of field crops production.
If neonicotinoids will be replaced, what should replace them?
Potential chemical insecticide replacements for neonics have risks of their own. IPM practices incorporating non-synthetic chemical, biological, cultural and genetic controls are likely to be effective, but alternative chemical insecticides are the most likely replacement to neonicotinoids in the short term. Alternatives exist for nearly all relevant pests, however switching to an alternative usually results in indirect or direct costs to users. When the user has chosen to use a neonic they have made the decision based on price, efficacy, safety, insecticide rotation pattern and other factors. In field crops the most promising alternative chemical insecticides are pyrethroids (e.g. tefluthrin) and anthranilic diamides (e.g. chlorantraniliprole and cyantraniliprole). Pyrethroids are not systemic and are less environmentally persistent so are likely less risky to pollinators compared to NTS, however they have greater toxicity to vertebrates including humans. Antrhanilic diamides are systemic, but less toxic to pollinators compared to neonics, and therefore show promise as alternative seed treatments, though they are currently more expensive. It is also of interest to point out that an alternative neonicotinoid, acetamiprid, exists that is significantly less toxic to bees than clothianidin, imidacloprid and thiamethoxam.

Parizadeh Mona, M. M. (2021). Neonicotinoid Seed Treatments Have Significant Non-target Effects on Phyllosphere and Soil Bacterial Communities. *Frontiers in Microbiology, 11*. doi:0.3389/fmicb.2020.619827

The purpose of this study was to: “(1) Characterize the drivers of variation in bacterial community structure of soybean and corn phyllosphere and soil and (2) identify the responses of the bacterial community composition variation and diversity to neonicotinoid seed treatment in a 3-year soybean/corn rotation” hypothesizing that these treatments would shift bacterial community composition, change variation, and change the diversity present. In both the phyllosphere and the soil.

Researchers utilized 16S RNA gene amplification sequencing of soil and phyllosphere samples collected from 2016 to 2018 in a soybean/corn/soybean no-till rotation in Quebec, Canada. The soil type in the test fields was a clay loam with a temperate climate and had not been sown with neonicotinoids in the preceding 3 years. Both the control and the neonicotinoid seeds were treated with a fungicide coating containing: difenoconazole, metalaxyl-M and sedaxane. The neonicotinoid seeds added thiamethoxam to these fungicide treatments. Study rows were set up in “four replicates of each non-neonicotinoid control and neonicotinoid-treated plots (100m x 3m)” and “were established alternately” and consisted of four rows a piece. “Two extra neonicotinoid-treated plots surrounded the experimental field.”

Two soil and phyllosphere samples were taken from each test plot three times each year in July, August, and September. Samples were sequenced and analyzed for bacterial composition and diversity and evaluated on the “relationships between bacterial communities and their host species, time and seed treatment...” Previous studies have indicated significant impacts on the diversity and community structure due to soil habitat, crop, time, and the interactions of these factors.

This study confirmed these findings; noting that “even in a rotation of annual crops the patterns of bacterial succession within and among years are an important driver of community structure.”

Neonicotinoids off target impact the "…bacterial community structure and diversity in a soybean/corn agroecosystem, in particular on the taxonomic composition of soil bacterial communities over the growing season. Phyllosphere and soil bacteria exhibit different patterns of community composition, alpha diversity, and temporal variation throughout the growing season and in response to neonicotinoid application." In the phyllosphere, bacteria host plant species and time were shown to be the primary drivers of "bacterial community variation" when viewed in comparison to the impacts of neonicotinoid treatments but that neonicotinoids do "influence" the bacterial composition in the phyllosphere in conjunction with those factors.

Soil bacteria displayed a greater change in its alpha diversity and community composition due to neonicotinoid application as well as displaying varying levels of change over time with the greater impacts on these parameters displayed mid-growing season. Researchers postulate this is due to neonicotinoid in the soil having a greater active period (persisting for years) than it has in plants. While there is potential for neonicotinoid accumulation and persistence in the soil researchers state they did not find "any significant inter-annual difference on bacterial diversity among years in interaction" with the neonicotinoid, postulating that this could be the result of leaching or degradation.

In bacterial communities that were more homogenous neonicotinoid seed treatment caused greater effect "(soil more than phyllosphere and corn phyllosphere more than soybean phyllosphere)." Some bacteria responded to the neonicotinoid by showing an increase in their population such as those bacteria that are believed to play a role in the degradation of neonicotinoids but among a number of bacteria that displayed a decline in population were species who are considered beneficial to plant health through their ability to form symbiotic relationships with plants. Several of the species listed as declining in population in response to neonicotinoid treated seed were *Bacillus, Bosea, Mesorhizobium, Rhizobacter, Bradyrhizobium, Microvirga, Ammoniphilus, Hyphomicrobium, Nitrospira, Nitrosospira and Rhodanobacter.* These bacteria play roles in the spheres of plant growth promotion, nitrogen fixation or the nitrogen cycle.

Researchers suggest that the effects seen could be the result of the "trophic interactions between bacteria and invertebrates (e.g., free-living nematodes and microarthropods) affected by the neonicotinoids. This insecticide may indirectly alter the bacterial community composition by affecting the top-down regulation of these communities through reducing the higher trophic levels that feed on bacteria." Researchers conclude neonicotinoid seed treatments show "…non-target impacts on soil bacterial communities of the phyllosphere and soil, especially the beneficial bacteria that are crucial for plant growth and health and soil fertility and quality."

Sánchez-Bayo, F. (2014). The trouble with neonicotinoids: Chronic exposure to widely used insecticides kills bees and many other invertebrates. *Science, 346*(6211), 806-807.

This article is a perspective published in Science magazine about how chronic exposure to sublethal doses of neonicotinoids that are systemic in the plant can have effects on bees such as: olfactory learning, memory and locomotory impairment, inhibited feeding, and immune deficiency. Seed treatment with neonicotinoids are the main source of soil and water contamination, but are often unnecessary and go against IPM principles.

Smith, J. L., Baute, T. S., & Schaafsma, A. W. (2020). Quantifying early-season pest injury and yield protection of insecticide seed treatments in corn and soybean production in Ontario, Canada. *Journal of Economic Entomology, 113*(5), 2197-2212.

A 4-year study was conducted comparing efficacy and value of fungicide-only, neonicotinoid insecticide + fungicide, and diamide insecticide + fungicide seed treatments for corn and soybean in Ontario, Canada. Experimental plots were assessed for early-season insect incidence and abundance. Wireworms and white grubs were frequently observed, but rarely above the legislated thresholds and injury levels rarely led to yield loss. Abundance differed by field site, but not by treatment. Field sites were chosen by partner farmers in fields where they had previously experienced injury by early-season soil insect pests or where fields were considered high risk to injury. 134 of the corn sites (92%) were planted with NTS with 0.25 mg clothianidin or thiamethoxam per seed. 7 sites planted NTS at 0.5 mg a.i./seed and 4 sites planted the highest rate of neonicotinoid on seeds at 1.25 mg a.i./seed. Soybeans were treated with either thiamethoxam (195 ml a.i./100kg seed) or imidacloprid (62.5-125 g a.i.100kg seed). A statistical yield difference between treatments was detected at 2 corn sites, but results were inconsistent: one site showed fungicide-only yield greater than neonicotinoid + fungicide; the other site the greatest yield was observed in neonicotinoid + fungicide treatment plots followed by fungicide-only, then diamide + fungicide. In soybean sites, the effect of seed treatment on plant stand was not significant and 84% of soybean sites saw no statistical yield difference between treatments. An overall positive yield response of 0.1 mg/ha was observed with neonicotinoid + fungicide treated corn seed, but an overall lower yield of 0.05 mg/ha was found in neonicotinoid + fungicide treated soybeans. Yield loss with NTS in soybeans has been observed in previous research and was attributed to increase slug feeding as a result of reduced ground beetle predation due to neonicotinoid toxicity. When analyzing the economic benefit of insecticide seed treatment the yield increase necessary to cover the cost of the seed treatment was calculated. This break-even yield increase was observed in 48% of corn sites comparing fungicide-only versus neonicotinoid + fungicide and 44% of corn sites comparing fungicide-only versus diamide + fungicide. The break-even yield increase required in soybean sites was achieved at 23% of the sites. Overall, the results of this study demonstrate: 1) early-season insect pests are not uncommon, but rarely reach economic injury levels (determined in legislature), 2) early-season injury, stand loss, and reduced vigor in sorn and soybean does not consistently translate into yield loss, and 3) there is no need or economic benefit for widespread neonicotinoid or diamide seed treatments in Ontario corn and soybean production.