AVIAN CONSERVATION & ECOLOGY

VOLUME 16, ISSUE 2, ARTICLE 26

Cornelius Ruhs, E., O. P. Love, L. Drainville, and F. Vézina. 2021. No common pesticides detected in snow buntings utilizing a farmland landscape in eastern Québec. Avian Conservation and Ecology 16(2):26. https://doi.org/10.5751/ACE-01979-160226

Short Communication

No common pesticides detected in snow buntings utilizing a farmland landscape in eastern Québec

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ABSTRACT. Many species of migratory birds are declining worldwide, including throughout North America. Some of the most cited causes of decline are linked to climate change, urbanization, and growth in agriculture. Across eastern Canada, a number of insecticides and herbicides are commonly sprayed before and during the grain growing season to control pests and foliage competitors. During wintering and migration, a declining Arctic-breeding songbird, the snow bunting (*Plectrophenax nivalis*), utilizes open farmlands of southern Canada; therefore, this could be a period when the species is most exposed to these pesticides. We tested snow bunting tissues (blood and liver) for the 4 pesticides most commonly used in grain agriculture in Canada: atrazine, chlothianidin, imidacloprid, and glyphosate, as well as a glyphosate derivative (aminomethylphosphic acid, AMPA). Although this species is thought to forage in grain fields during autumn through spring, we found no detectable traces of any of the five substances. Wintering buntings may either not be exposed to these pesticides during their presence in agriculture fields or, given the rapid turnover of these pesticides in the blood and tissues, be exposed to doses below detection level in samples.

Aucun pesticide commun détecté chez les Plectrophanes des neiges qui fréquentent des milieux agricoles de l'Est du Québec

RÉSUMÉ. De nombreuses espèces d'oiseaux migrateurs sont en baisse dans le monde entier, y compris en Amérique du Nord. Certaines des causes les plus citées pour expliquer cette baisse sont liées aux changements climatiques, à l'urbanisation et à l'expansion de l'agriculture. Dans l'Est du Canada, des insecticides et des herbicides sont couramment pulvérisés avant et pendant la saison de croissance des céréales pour lutter contre les parasites et les plantes compétitrices. Durant l'hivernage et la migration, le Plectrophane des neiges (*Plectrophenax nivalis*), oiseau chanteur en diminution qui niche dans l'Arctique, fréquente les terres agricoles du sud du Canada; par conséquent, ces périodes pourraient être celles où l'espèce est la plus exposée à ces pesticides. Nous avons testé certains tissus du plectrophane (sang et foie) pour les 4 pesticides les plus couramment utilisés dans l'agriculture céréalière au Canada : l'atrazine, la chlothianidine, l'imidaclopride et le glyphosate, ainsi qu'un dérivé du glyphosate (l'acide aminométhylphosphonique, AMPA). Bien que l'on pense que cette espèce se nourrisse dans les champs de céréales de l'automne au printemps, nous n'avons trouvé aucune trace détectable de l'une de ces cinq substances. Les plectrophanes hivernant peuvent soit ne pas être exposés à ces pesticides lorsqu'ils sont présents dans les champs agricoles, soit être exposés à des doses inférieures au niveau de détection dans les échantillons étant donné la dégradation rapide de ces pesticides dans le sang et les tissus.

Key Words: *Agriculture, atrazine, clothianidin, glyphosate, grain, imidacloprid, snow bunting*

INTRODUCTION

In Canada, the provinces of Ontario and Québec alone account for 91.9% of total corn production (Hamel and Dorff 2014). Across this crop type, regardless of geographic location, similar insecticides and herbicides are sprayed during the growing season (summer to early fall) to control pests and foliage competitors, respectively (Environment, Energy and Transportation Statistics Division 2015). A review of practices within the agriculture industry is ongoing to determine the biological significance of pesticide exposure on wildlife (Dion 2018, Langlois 2018); however, specific effects of these chemicals on birds, especially following chronic exposure, is generally unknown (but see Evans and Batty 1986, Wilhelms et al. 2006, Mineau and Palmer 2013, Gibbons et al. 2015, McGee et al. 2018). Some pesticides, like neonicotinoids (e.g. imidacloprid and clothianidin), atrazine, glyphosate, and glyphosate derivatives, have long-half lives in soil (Wilhelms et al. 2006, Battaglin et al. 2009, Hallmann et al. 2014). For example, imidacloprid has a field dissipation half-life of 26.5 to 229 days (Bacey n.d.), clothianidin is field persistent with a half-life from 56.4 to 1155 days (EPA 2005, Bonmatin et al. 2015) and glyphosate a half-life of 2-197 days (mean 47 days; WHO 1994, Giesey et al. 2000, Vencill 2002). Atrazine has a soil half-life of 35-75 days (Vogue et al. 1994, Workman et al. 1995); however, it has been found in the environment years after application (Jablonowski et al. 2010, Jablonowsi et al. 2011). Therefore, it is possible that birds could still be exposed long after the growing season, like during the wintering period when birds utilize agricultural fields.

Some of the most cited causes of declining avian populations are linked to climate change, urbanization, and agriculture intensification (Partners in Flight Landbird Conservation Plan

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2016). Growth in the agriculture sector undoubtedly leads to expansion of pesticide use and mounting evidence shows support for neonicotinoid toxicity in birds (Hallmann et al. 2014). It has also been suggested that these toxic effects are cumulative when birds are exposed chronically, even at small biologically relevant levels (Eng et al. 2017). For example, white-crowned sparrows (*Zonotrichia leucophrys*) gavaged with doses of imidacloprid (~0.2 treated corn seeds) over 3 days showed a decline in fat, body mass, and significant impairment in migratory orientation (Eng et al. 2017). Sparrows given chlorpyrifos also had impaired migratory orientation but showed no changes in body composition (Eng et al. 2017). Further, analysis of long-term datasets in The Netherlands revealed that local bird populations declined by 3.5% annually in areas where imidacloprid was used at concentrations >20 ng liter^{-1} (in Canada typical application rates are 50-320 g ha^{-1}, 240 g liter^{-1} imidacloprid for formulation Admire 240F; CCME 2007, Pest Management Regulatory Agency 2005, Hallmann et al. 2014). There have also been reported negative effects of atrazine on sexual maturation and food intake in Japanese quail (Wilhelms et al. 2006); and, similarly, of glyphosate on the breeding ecology of many marsh-dwelling avian species (Linz et al. 1996). Interestingly, neonicotinoid residues (clothianidin and thiamethoxam) were detected in wild turkeys (*Meleagris gallopavo silvestris*) sampled in Ontario, Canada during the spring hunting season (MacDonald et al. 2018). However, while we know some details about the impacts of exposure to pesticides, whether or not migrants can be exposed long after the growing season (e.g. winter, migration) is unclear.

The snow bunting (*Plectrophenax nivalis*) is a small circumpolar Arctic-breeding songbird. In North America, it winters in southern Canada and northern United States. Within central-eastern Canada, snow buntings have a migration route that runs along the St. Lawrence River shore in eastern Québec and up to Greenland (Macdonald et al. 2012, 2016). Snow buntings are cold-specialists (O'Connor et al. 2021, Le Pogam et al. 2021) and are primarily associated with open snow-covered agricultural areas in winter where they feed on a variety of weed seed plants (Gabrielson 1924) and on leftover crops (i.e., grain; Lyon and Montgomerie 2011). As alternative food sources are limited in winter, grain and seeds are their primary food source (Mckinnon et al. 2019) and are most often found in corn stubble fields (Cox 1958, Rosenblatt and Bonter 2018). As such, it has been proposed that these birds could be exposed to agricultural pesticides both in their south range (winter) and as they travel back-and-forth between the wintering and breeding grounds (Lyon and Montgomerie 2011). However, the risk of exposure during these time periods is not entirely clear. As birds become highly hyperphagic and accumulate fat for spring migration (Vincent and Bédard 1976, Le Pogam et al. 2021), it is likely that these agricultural foraging sites are exploited to provide fuel for this arduous migration and also to cope with the cold conditions on arrival to the Arctic. Although snow buntings are not currently threatened globally, recent estimates suggest that snow buntings have declined by over 60% in some parts of Canada over the last 40 years (Butcher and Niven 2007, Macdonald et al. 2012). Therefore, as this species primarily utilizes agricultural land during winter, it represents an ideal model to examine whether birds foraging in pesticide-treated agricultural landscapes during cold winters may accumulate agricultural pesticides.

In this project, we tested snow bunting tissues (blood and liver) for the four pesticides most commonly used in cereal crops, such as corn, in Canada. We focused on the spring migratory period as this is a time during which the birds accumulate large amounts of body fat for migration through hyperphagia (Le Pogam et al. 2021, Vincent and Bédard 1976, Power 2017) and might therefore be consuming grain-targeted pesticides while migrating along the St. Lawrence shore. The considered pesticides were the four most commonly used in grain agriculture in Canada: atrazine, imidacloprid, chlothianidin, glyphosate, as well as a glyphosate derivative (aminomethylphosphic acid (AMPA)). Our objectives were to (i) assess whether these chemicals were present in tissues that represent short-term (blood) and longer-term exposure (liver) to these pesticides and (ii) if so, at what relative concentrations.

METHODS

The eastern Canadian population of snow buntings spends the winter from western Ontario to eastern Québec before migrating north-eastward following the Gulf of St. Lawrence (Macdonald et al. 2012, 2016). Therefore, birds (n=10) were collected in March 2018 as this corresponds to the period of hyperphagia and pre-migratory body mass gain in outdoor captive birds (Le Pogam et al. 2021, Vincent and Bédard 1976, Power 2017). The historic range of snow cover during March in Québec can vary but ranged from 72-136 cm depth in March 2017 (data from 2018 unavailable; https://climate.weather.gc.ca/climate_data). Birds were captured in Saint-Joseph-de-Lepage, Québec, Canada (Lat: 48.575779, Long: -68.169576). All birds were captured on the same day (March 14th) within three hours (9:52 am to 12:20 pm). The capture site was an area with an open cornfield and snow cover. Birds were baited for a few days prior to capture using a certified organic corn product which therefore was not contaminated with the pesticides of interest (Fig. 1). Thus, any level of contamination would have to be resulting from previous exposure. Upon capture, a small blood sample (<1% total body weight) was collected. We then transported birds back to the avian research facilities at the Université du Québec à Rimouski where they were euthanized using CO_2 asphyxiation. Birds were then dissected to retrieve liver tissue samples. Liver was used because it (1) accumulates toxins and (2) is the primary source for metabolizing toxicants (Klaassen and Watkins 2008). A total of ~5 g of tissue was required to analyze for imidacloprid, clothianidin, and atrazine; however, another ~5 g was required for glyphosate and AMPA. As such, only liver samples were tested for glyphosate and AMPA, as there was not enough blood collected to test for all five pesticides. Samples were then stored at -80ºC and prepared for shipment and analysis at Brookside laboratories in Ohio, United States. All procedures were approved by the UQAR Animal Care Committee (CPA-72-18-199) and were conducted under a Canadian Wildlife Services scientific permit (SC-75).

Laboratory analysis

To determine the presence and levels of clothianidin, imidacloprid, and atrazine in samples, each sample was homogenized and weighed into a 50mL polypropylene centrifuge tube, recorded to the nearest 0.001 g. Five mL of water was then added to the tube. Ten mL of acetonitrile was added to the tube and shaken for 2 minutes. A packet of QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) salts was added to the tube

Fig. 1. Snow buntings feeding on organic (pesticide-free) corn bait during the winter in Québec, Canada. Photo by François Vézina.

(4 g MgSO4, 1 g NaCl, 0.5 g disodium citrate, 1 g trisodium citrate) and shaken for 1 minute. The top layer was then decanted into a cleanup tube containing 1.5 g MgSO4, 0.5 g C18, and 0.5 g PSA. This tube was shaken for 30 seconds and the remaining solvent filtered through a 0.2 um PVDF filter. Four mL of the extract was blown down under a gentle stream of nitrogen to near dryness and then reconstituted with 1 mL of methanol and 1 mL of 0.2% formic acid in water. If the sample contained precipitates it was filtered again with a 0.2 um PVDF filter into a screw-cap HPLC vial. The sample was then injected into a Thermo TSQ LC-MS/MS. One parent mass and two daughter masses were monitored for each compound. Matrix-matched calibration standards were produced using chicken liver as the matrix. These analyses had a minimum detection limit of 0.01 mg/kg for imidacloprid and clothianidin and 0.1 mg/kg for atrazine.

For glyphosate and AMPA, samples were homogenized and weighed into a 50 mL polypropylene centrifuge tube, recorded to the nearest 0.001 g. Five mL of 0.05% phosphoric acid was then added to the sample. The sample was tumbled end-over-end for 30 minutes. After this time the sample was centrifuged and then passed through a Waters HLB cartridge for cleanup. The collected liquid was diluted a further 5X and filtered with a 0.2 um PVDF filter into an HPLC vial. The sample was injected into a Waters Alliance 2695 HPLC with Fluorescence Detector and OPA derivatization unit. The limit of quantification was 0.8-2.05 mg/kg for glyphosate and AMPA.

RESULTS

There were no detectable amounts of atrazine, clothianidin, or imidacloprid in either the blood or the liver (Table 1). There were also no detectable amounts of glyphosate or aminomethylphosphonic acid (AMPA) in the liver (Table 1).

DISCUSSION

We found no detectable levels of four of the most commonly used grain pesticides in snow buntings during the spring pre-migratory fattening phase in eastern Québec. There are many possible explanations for this. Chemical degradation rates might prevent birds from becoming contaminated if pesticides and herbicides do not remain in the environment long enough after the last application for the birds to get contaminated (Klaassen and Watkins 2008). To shed light on this, future studies should test corn and grain remnants throughout the course of the year to establish whether these pesticides are still detected months after the application season. Physiological degradation of these chemicals by the animal could also be rapid, bringing contamination levels below detection limits within days after exposure, especially if contamination levels are low (Klaassen and Watkins 2008). For example, imidacloprid was found to degrade to ~22.5% of starting levels after 25 days (Liu et al. 2011) and ~50% after 60 days (Mahapatra et al. 2017) of application, depending on environmental factors. Additionally, the absence of pesticides in our birds is likely not simply resulting from the technical capacity to detect them in biological tissues, as our minimal detectable limit (0.01 mg/kg; atrazine, clothianidin, and imidacloprid) and limit of quantification (0.8-2.05 mg/kg; glyphosate and AMPA) is well below what has previously been shown to cause toxicity in birds (Fishel 2005).

Table 1. Results from the pesticide testing. There were no detectable quantities of atrazine, clothianidin, imidacloprid, glyphosate, or AMPA in any of the birds sampled. Limit of Quantification (LOQ) is the lowest concentration of analyte in a sample that can be detected, all samples for glyphosate and AMPA were between zero and the LOQ (0.8-2.05 mg/kg). Note that the minimal detectable limit (MDL) for the tested pesticides is well below concentration levels known to cause health issues in birds.

	Blood (mg/kg)	Liver (mg/kg)	Type	MDL or LOQ$^{\pm}$
Atrazine	ND	ND	Herbicide	0.01
Clothianidin	ND	ND	Neonicotinoid	0.01
Imidacloprid	ND	ND	Neonicotinoid	0.01
Glyphosate	.	ND	Herbicide	0.8-2.05 mg/kg$^{\pm}$
AMPA	.	ND	Herbicide†	0.8-2.05 mg/kg$^{\pm}$

†indicates an herbicide derivative

Previous studies focusing on the effects of pesticides in birds have been either experimental (Evans and Batty 1986, Mineau and Palmer 2013, Eng et al. 2017) or conducted during the crop growth (and pesticide application) season (Boutin et al. 1999). For example, a study in southern Ontario identified numerous farmland bird species that were at risk of pesticide exposure during the breeding season due to their high occurrence near agricultural landscapes and to the timing of that occurrence with pesticide application (Boutin et al. 1999). Our study aimed to determine whether snow buntings were contaminated during spring, long after the crop growth season. This period could be a very sensitive portion of their annual cycle due to important physiological changes occurring at that time for migration (Le Pogam et al. 2021, Vincent and Bédard 1976, Power 2017), but also due to pesticide contamination, which could increase as pre-migratory buntings become hyperphagic (Vincent and Bédard 1976, Laplante et al. 2019) and begin fattening for their long-distance flight (LePogam et al. 2021).

While our results are encouraging for this declining species, the lack of detection of these compounds during late winter is not necessarily a sign that these birds are not being exposed during other times of the year or in areas with higher pesticide use (e.g., Warner et al. 2019). For example, in eastern Canada, snow buntings are wintering in a gradient of agriculture intensity, from southern Ontario (intense) to Newfoundland (low; Hamel and Dorff 2014) and are faced with increasing pesticide use over time (Agriculture and Agri-Food Canada 2016). Further studies are needed to determine whether snow buntings are contaminated in areas of greater agricultural intensity. More research is also required to define the potential effects of these toxicants and the ecological impacts long after the crop growing season, especially during sensitive life-history stages like pre-migratory fattening. More precisely, future studies should also aim to sample populations that (1) are from other wintering locations where pesticide use is more extensive and (2) across a wider geographical scale varying in their pesticide use intensity.

Since the implementation of this project, both clothianidin and imidacloprid have been restricted, but not for grain crops like corn (PMRA a and b 2019, Environment et Lutte contre les changements climatiques Québec 2021); while glyphosate has been reapproved for the next 15 years. Given that locations for growing grain will increase as temperatures continue to climb and growing seasons get longer (Almaraz et al. 2008, Kucharik and Serbin 2008), understanding the long-lasting effects of pesticide application to wildlife is paramount. Several migratory bird species are declining throughout North America (North American Bird Conservation Initiative Canada 2012) and agricultural expansion could be a factor in this decline, especially in birds associated with open farmland landscapes. Therefore, whether agricultural intensity and pesticide exposure impact population decline is a question of utmost importance.

Responses to this article can be read online at:
https://www.ace-eco.org/issues/responses.php/1979

Acknowledgments:

The authors would like to thank Justine Drolet (UQAR), Jessé Roy-Drainville (UQAR), Caroline Gay (Terre-Eau) and Francis Bordeleau-Martin (Terre-Eau) for their help and support in collection of the samples and for their agriculture advise. We would also like to thank the Centre de formation professionnelle Mont-Joli-Mitis (CFPMM) for allowing us to catch birds on their property. COMPLIANCE WITH ETHICAL STANDARDS Ethical approval All applicable international, national and institutional guidelines for the care and use of animals were followed. Procedures were institutionally approved by the UQAR Animal Care Committee (CPA-72-18-199). Funding This research was funded through a Natural Sciences and Engineering Research Council of Canada (NSERC) Engage grant awarded to O.P.L. Conflict of interest The authors declare that they have no conflict of interest.

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Editor-in-Chief: Alexander L.Bond
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Sponsored by the Society of Canadian Ornithologists and Birds Canada

Parrainée par la Société des ornithologistes du Canada et Oiseaux Canada