Title: Neonicotinoids, more than herbicides, land use, and climate, drive recent butterfly declines in the American Midwest

Authors: Braeden Van Deynze*[1], Scott M. Swinton[2], David A. Hennessy[3], Nick M. Haddad[4], Leslie Ries[5]

Affiliations:

*Author for correspondence: braeden.vandeynze@dfw.wa.gov

[1]Washington Department of Fish and Wildlife, Olympia, WA

[2]Michigan State University, Department of Agricultural, Food, and Resource Economics, East Lansing, MI

[3]Iowa State University, Department of Economics, Ames, IA

[4]Kellogg Biological Station and Department of Integrative Biology, Michigan State University, Hickory Corners, MI

[5]Georgetown University, Department of Biology, Washington, DC

Abstract: Mounting evidence shows insects are in decline globally. Habitat loss, climate change, and pesticides have all been implicated, but their relative effects have never been evaluated in a comprehensive large-scale study. We harmonized 17 years of land use, climate, multiple classes of pesticides and butterfly data across 60 counties in five states in the US Midwest. We find community-wide declines in butterfly abundance to be most strongly associated with pesticides in general and the use of neonicotinoid-treated seeds in particular. This included the abundance of the migratory monarch (*Danaus plexippus*), whose decline is the focus of intensive debate and public concern. Insect declines cannot be understood without comprehensive data on all putative drivers, and the 2015 cessation of neonicotinoid data releases in the US will impede future research.

One Sentence Summary: Shifts in insecticide use towards neonicotinoid-treated seeds are associated with an 8 percent decline in butterfly species diversity across the American Corn Belt.

Introduction

Like many other insects, butterfly populations are in decline [1]. Globally, declines of abundance and biomass for many insect groups have been reported at rates of 2-4% per year, rates that compound to as much as 30-50% of total abundance loss, depending on study duration [2,3]. Declines of these magnitudes have also been widely reported for butterflies, including throughout grassland habitat in Europe [4], in the western US [5] and the US states of Ohio [6] and Illinois [7]. With such steep losses come evidence for a decline in the many ecosystem services provided by insects [8-10] and the mounting risk of collapse [11]. Present-day bird declines in Europe have been linked directly to pesticide use [12], and the recent, estimated loss of three billion birds in America north of Mexico [13] has also been hypothesized to be linked to insect losses [14,15].

Despite documenting recent insect declines that are widespread, evidence on the primary *drivers* of these declines remains murky [1]. The three most widely recognized global drivers of insect declines are land conversion, climate change, and agricultural pesticides (insecticides and herbicides). Yet the relative impact of each has been shifting over time and also interacting in ways that make it hard to tease apart their individual contributions [16]. While land use conversion is generally recognized as the historical driver of most species loss, landscapes in North America underwent the most dramatic conversions decades before the onset of recent insect declines [17] Although climate change acceleration over the last 20 years is now a primary focus of biodiversity loss [18], taxon-wide studies for insects show species respond variably, depending on their thermal niche requirements, producing climate "winners" and "losers" [19]. In contrast, agricultural chemical applications are the only putative driver of herbivorous insect declines specifically formulated to be either directly lethal to insects (insecticides) or to reduce the cover of plant species (herbicides) in the immediate or surrounding landscape on which these insects depend [16].

Unfortunately, quantifying insecticide and herbicide applications (hereafter, "pesticide" when referred to in combination) and their effects over large scales and long time frames is particularly difficult. This is because, unlike climate and land-use, where global time-series spatial data layers are publicly available at multiple scales, pesticide applications cannot be sensed remotely. US data on pesticide use, in coarsened form, have become publicly available for years [20], and have recently been synthesized into large-scale ecological analyses of the impacts of pesticide use [21]. Unfortunately, data at this resolution fall short of demands of analysis across stressors. Required estimates of pesticide use come from proprietary data, such as commercial sales records or detailed surveys of individual farmers and the types and mixes of chemical treatments they use, which vary substantially, not only over time, but on a farm-by-farm basis [22,23,24].

Recent US and Canadian studies have shown a negative relationship between pesticides and monarch (*Danaus plexippus*) abundance [e.g., 25-27], dragonflies (*Odonata*) and bumble bees (*Bombus occidentalis*) [28,29], and lowland butterflies in agricultural regions of central California [30]. Large-scale UK studies on pesticides with a particular focus on seed-coated neonicotinoids have shown a negative impact of this class of chemicals on bees [31] and butterflies [32].

In our analysis, we include all major classes of present-day pesticides and group them into six categories based on their main active ingredient, degree of specificity, timing of applications, whether the treatment is applied prophylactically (every year) or reactively (in

response to pest outbreaks), and the tendency of each to spillover and persist in the surrounding environment (Fig. 1; see Supporting Information). We overcome an ongoing difficulty in estimating impacts of agricultural chemicals, which occurs when separate but major technological changes that happen in rapid succession have distinct effects on pesticide use. In particular, the commercial release of genetically modified (GM) crops and insecticide seed coatings during 1996-2003 (Fig. 1) sharply narrowed the set of pesticides used by farmers, with repercussions on local biodiversity [33-35].

Figure 1. Evolution of pest control technology in North American corn and soybean production. See Supplementary text for discussion of mechanisms of butterfly exposure to pest and weed control impacts.

Here, we present a comprehensive analysis of the drivers of insect declines, incorporating the impacts of all three dominant drivers of insect declines, land use, climate, and multiple pesticide use, over a 17-year period in 60 counties in the Midwest (Fig. 2A-D). This region is the US center of corn and soybean production [36], with most counties in our study region having >60% of land under agricultural production (Fig. 2A-C). The Midwest also happens to be the US region with the densest network of butterfly monitoring surveys [37] (Fig. 2D). This high density of surveys is due to the initiation of volunteer monitoring programs in four nearby states: Illinois (initiated in 1987), Ohio (1996), Iowa (2007), and Michigan (2011). These four programs all follow a similar protocol where directors establish fixed routes, generally in land managed as parks or reserves, and each route is surveyed multiple times per year by the same volunteer [37]. Analyses of some of these data have already demonstrated state-wide butterfly declines [6,7].

Figure 2. Data summaries for key variables. (A-C) shows land cover by county as percent of total land area in 2014 and (D) shows the number of years each county contributed butterfly population surveys; bold borders indicate USDA Crop Reporting District (CRD) boundaries while hatching shows counties not included in analysis due to lack of butterfly survey data availability. (E-G) shows area-treatments, the average number of times a pesticide or pest control treatment is applied to an acre of soybean or corn cropland, for each pest control technology group.

Methods

Data construction

We bring together data from several sources to construct a unique panel dataset. The unit of observation is a county-year: the base geographic unit in the panel is a county and the base temporal unit is a year. The panel includes observations from 60 counties and 17 years (1998-2014). The annual number of monitored counties ranges from 15 to 37 based on data availability.

Butterfly Abundance Data

For butterfly abundance, we use county-year aggregates of monitoring surveys conducted by four volunteer programs associated with the North American Butterfly Monitoring Network. Both the Illinois Butterfly Monitoring Network and the Ohio Lepidopterists provide data throughout the period of study, while the Iowa Butterfly Survey Network and Michigan Butterfly

Network provide data beginning in 2006 and 2011, respectively. Subsets of these data have been previously analyzed to assess overall butterfly population trends in Ohio [6,38], and Monarch population trends in Illinois [26,39].

Each butterfly monitoring network makes use of methods known as Pollard surveys (Pollard and Yates, 1994, Taron and Ries, 2015) [37,40]. Routes are designed by volunteers in coordination with network coordinators to (a) transect a variety of habitat types, (b) follow existing pathways so as not to disturb habitat, (c) be easily located by other volunteers, and (d) take between 30 minutes to two hours to complete. For each route, a single volunteer walks at a consistent pace along routes (called a "run") a minimum of six times per year during the months of June, July, and August, with additional runs during these months or others if possible. Runs are conducted between 10:00am and 3:30pm on days with (a) less than 50% cloud cover and (b) light to moderate winds. During the run, the volunteer records all individuals by species sighted within roughly 20 feet to each side of the route. Volunteers are instructed to include only species they can identify with certainty and not to guess.

Counts are summed across surveys conducted during June through August for each county-year. We analyzed the total number of species and abundance of all individuals across all species, and also the heavily-studied monarchs (*Danaus plexippus*).

Pesticide Use Data

We used a unique, granular pesticide dataset, consisting of proprietary farm-level pesticide application data, including six primary pesticide classes defined for this study (Fig. 2E-G), collected via paid phone interviews by Kynetec USA, Inc., a market research company (see Supporting Information). These data were collected via computer-assisted telephone interviews of soybean and corn growers. Lists of eligible growers were constructed from lists of growers who receive federal payments, membership lists of state and national growers associations, and subscription lists to agricultural periodicals. Sampling lists were constructed to ensure representativeness of applications at the level of the crop reporting district (CRD), USDA-designated groupings of counties in each state with similar geography, climate, and cropping practices. Non-respondents were recontacted at least eight times to reduce non-response error. Respondents were asked to detail their field-level pesticide, tillage, and seed choices during the previous growing season. Respondents were compensated monetarily upon survey completion and responses were crosschecked against realistic application rates and consistency with other reported practices. These surveys included neonicotinoid usage from 1998 until 2014 (Fig. 2e), at which time, surveys about this treatment were discontinued [41].

Pesticide use is measured in county-level area-treatments for each pesticide group (see Supporting Information). Area-treatments are defined as the average number of times a pesticide within a group is applied over a defined region (e.g., a field or a county) in a season [35]. Area-treatment measures are preferred over volumetric measures because their construction accounts for sometimes dramatic differences in application rates between different products [35,24]. By accounting for both the geographic extent and frequency of application, area-treatments better capture the degree to which a butterfly population may have been exposed to a particular chemical.

Land Cover Data

We measure land cover at the county-level as the proportion of land within each county under three land cover groups: forest, urban, and cropland. We use county-level pixel counts

from the USGS National Land Cover Database, which classifies the entire land area of the conterminous United States into one of several land cover classes at 30m resolution. The database is published for six calendar years over the course of our panel: 2001, 2004, 2006, 2008, 2011, and 2013. For years when the NLCD was not published, we assign the value from the nearest available year using a rolling join, assigning the value from the nearest available year. The assignment of NLCD land cover classifications to land cover groups is described in Table S2. Further details on the measurement of land cover variables is available in the Supporting Information.

Weather Data

Local weather patterns have been previously shown to affect butterfly distributions, abundances, and the timing of life history stages, although the strength of such associations varies by species and land cover [26,54,55,42,43]. To control for potential weather effects on annual butterfly abundance, we generate county-level measures of precipitation and temperature that capture variation between years and within seasons.

Daily weather data were gathered from NASA Daymet, a 1km x 1km spatial grid of daily weather conditions using data from a network of weather stations. We average values over each county's boundaries [44]. Temperature is measured in growing degree days (GDDs), which measures the number of degrees Celsius within a range in which butterflies can develop (11.5°C to 33°C). Precipitation is measured in millimeters. We partition each season into three intervals: early (March and April), mid (May and June), and late (July and August) season. Daily accumulation of precipitation and GDD are summed over each interval. The resulting variables measure accumulated precipitation and GDDs for each county during each interval for each year.

Analysis

To determine the net effects of multiple shifting and interacting drivers, we use counterfactual analyses, which compare modeled estimates of outcomes based on a scenario in which a focal action of interest (e.g., a shift in pesticide usage) had not occurred to a scenario where the action did occur (see Supporting Information). This approach allows for a clearer separation of the impacts of a change in a focal driver from all other factors included in the analysis and is an emerging approach to increase rigor in multi-stressor ecological and conservation research [45, 46]. In addition to counterfactual analyses, we report the effect sizes of the generalized linear model (GLM) that underlies these predictions (see Supporting Information). These are estimates of the individual effect sizes of each of our environmental covariates (six pesticide classes, three land cover types, and six weather variables) while holding all others fixed [40]. Our study covers the years 1998-2014, because butterfly survey density began to increase substantially in 1998 [37] and county-level neonicotinoid data are not available after 2014 [21].

Results/Discussion

Our counterfactual projections compare a scenario where pesticide use has been fixed at 1998 levels while weather and land cover measurements are allowed to vary through time as observed. By 2014 butterfly abundance in the median county was 8% lower in projections under observed conditions versus the counterfactual projections. Our results show that insecticides are associated with a much more negative impact than herbicides (Fig. 3). Although our counterfactual does not distinguish between the different classes of insecticides or herbicides (as

shown in our GLM effect size results), we note that declines related to insecticide use begin in 2003 (Fig. 3), coincident with the initial deployment and rapid adoption of seed-treated neonicotinoids in corn and soybean plantings in the Midwest (Fig. 2E). By contrast, the other two insecticide types saw relatively stable use during the same period (Fig. 2F). Responses to herbicides were more diverse, with species richness observed to be higher than expected and abundance remaining roughly stable compared to the counterfactual over the entire 17-year time series (Fig. 3). Here, we note an observed shift in dynamics for both richness and abundance starting around 2008 (Fig. 3), a breakpoint that coincides with the proliferation of weed resistance to glyphosate [48] that triggered a switch in the trajectories of glyphosate vs. non-glyphosate herbicide use (Fig. 2F, [49]). In combination, weed and insect control measures appear to interact with each other, resulting in negligible changes in species richness, but a dramatic decline in overall insect abundance (Fig. 3F).

Figure 3. Net effect of pest control system change over time. Net pest control effect is the percent change between predicted incidence rates under observed pest control adoption and synthetic counties where pest control adoption is fixed at 1998 levels. County-level net pest control effects over time are shown in gray lines and annual median net pest control effect in black lines; columns show effects computed holding all weed control, insect control, and pest control variables at 1998 levels. Results are presented for species richness (A-C), total abundance (D-F), and *Danus plexippus* (monarch) abundance (G-I).

To disentangle the individual contributions of each hypothesized environmental driver (six pesticide classes, three land-cover types, six weather variables; Fig. 2), we interpret effect sizes calculated from the GLM parameter estimates, separately for richness and abundance when all other factors are assumed to be constant at their mean values (Fig. 4). Pesticides had statistically significant, but mixed, effects on yearly species richness levels (Fig. 4A) and weak, non-significant effect on yearly changes in total abundance (Fig. 4B). In contrast, weather was the most consistent factor governing year-to-year variability in species richness and abundance; warmer, wetter years were associated with higher species richness (Fig. 4C), while cooler, drier years were associated with higher overall abundance (Fig. 4F). The dominant influence of weather on yearly population sizes is not surprising because most butterfly species' abundances are known to fluctuate substantially from year to year, and these fluctuations are generally governed by the yearly variability in weather [16]. Succinctly, "good" or "bad" butterfly years compared to other years are likely due to weather conditions that are favorable for caterpillar development and/or the availability and quality of their host plants.

Figure 4. Model effect sizes for a standard deviation change in explanatory variables. (A-I) show model effect size estimates from GLM models for the species richness (number of distinct species identified per hour, A-C), total abundance (number of butterflies counted per hour, D-F), and *Danus plexippus* abundance (monarch, G-I). Effect sizes are derived from GLM negative binomial; see Methods for details. For land use, categories are compared to an "open space" baseline. Lines show 95% confidence intervals based on heteroskedasticity-consistent standard errors (HC1). Solid points show statistically significant point estimates at the 95% level. For interpretation: A one standard deviation increase in neonicotinoid seed treatment area-treatments is associated with 22% lower county-wide abundance for monarchs (G) and 7% fewer distinct species observed (A).

Yet yearly effect sizes, by themselves, do not explain directional trends over time because GLM effects alone show how the underlying variable changes year-to-year [45]. Based on our counterfactual analysis, pesticides account for a steep decline in total butterfly abundance over our 17-year study period (Fig. 3), while the counterfactual scenario where weather variables are fixed at 1998 levels only reveals the yearly fluctuations, but with no directional change (Fig. S1). Even though not statistically significant, the effect size results show that the mean estimated effect of all four insecticides classes are negative, with neonicotinoids having the most negative estimated effect size (Fig. 4D). These small, but highly variable effects appear in our counterfactual to accumulate to a persistent negative trajectory (Fig. 3E). Further, as noted above, these effects appear to trigger the decline starting in 2003, the year that neonicotinoid-treated soybean seed became available in the Midwest (Fig. 2E). Taken together, our counterfactual (Fig. 3) and effect size (Fig. 4) results suggest that neonicotinoids are the most important factor driving the recent declines in butterfly abundance in the Midwest, at least through 2014, the last year for which neonicotinoid data were publicly available. Further, use of neonicotinoids has a strong and statistically significant negative impact on local species richness (Fig. 4A). However, these negative effects on richness appear to be counteracted by changes in herbicide use resulting in a net zero change in species richness (Fig. 3C).

The negative impacts on herbivorous insects of neonicotinoid penetration to non-crop settings and subsequent uptake in other plants in adjacent habitat [50] contrasts with the relatively weak effects of both i) sprayed insecticides and ii) genetically modified *Bt* seeds, farmers' only other systemic and prophylactic pesticide option. Although *Bacillus thuringiensis* is toxic to all Lepidoptera, including butterflies, direct feeding on *Bt* crops is the primary exposure mechanism, whereas species that do not feed on corn (all Midwestern butterflies and non-pest moths) could only be exposed through *Bt*-contaminated pollen spread to plants in adjacent fields [51]. *Bt* pollen is, at most, a negligible contributor to toxicity in the field [51]. *Bt* seed adoption is linked to sharp reductions in use of sprayed insecticides (Figure 2E, [24]). Although any quantity substitution of *Bt* seed for sprayed insecticides would be accounted for statistically in our analysis, it is also possible that *Bt* seed reduces butterfly exposure by shifting insecticide spraying to earlier in the year [52]. In summary, our results suggest *Bt* seed adoption lacks the harmful effect on butterfly abundance and diversity that is associated with neonicotinoids, supporting the ecological benefits of continued and expanded use, and efforts to protect it from evolved resistance in target pests [53].

Although our study's focus is on the response of the whole butterfly community, the effects that underlie these larger patterns are cumulative, integrated over all individual species. Accounting for individual responses requires grappling with the wide variety of life history details and ecological relationships that are unique to each species. Since that is beyond the scope of this study, we instead explore results relative to a single species, the eastern migratory monarch butterfly (*Danaus plexippus*). We chose this species because it is the most intensively studied butterfly species in the world, due to its remarkable multi-generational roundtrip migration between isolated mountaintops in central Mexico and regions as far north as southern Canada [54]. Further, 95% of the individuals that arrive in the Mexican colonies each winter originate in the Midwestern corn belt [55]. The monarch's primary host plant, *Asclepias syriaca* (milkweed), is closely associated with row crops and field margins, and thus is particularly vulnerable to agricultural practices [56].

Our study shows that neonicotinoids are the only pesticide that significantly impacts local monarch abundances from year to year (Fig. 4G), a result that is consistent with studies of

decline of the western population of the migratory monarch [57]. Most previous studies have focused on the increased use of glyphosates that occurred with the adoption of herbicide-resistant crops and have caused intense debate within the monarch research community [58]. We found no effect of either glyphosate or non-glyphosate herbicides (Fig. 4G). Our conclusions differed from another study [26], whose analyses encompassed a period before adoption rates of glyphosate-resistant crops reached 75%, a threshold above which glyphosate effects flatten. As our study period includes data after this threshold had been reached, a lack of a statistically significant effect size for glyphosate in our analysis is not inconsistent with previous studies.

In contrast to our findings for richness and total abundance across the entire butterfly community, studies of eastern migratory monarchs show climate is the primary driver of recent declines in the eastern migratory monarch [27]. This and other studies have shown that weather conditions in monarch's spring breeding grounds in eastern Texas are the primary driver of year-to-year population sizes. However, summer weather conditions in the northern breeding grounds are becoming increasingly important as the climate has warmed with the specific result that hotter summer temperatures are associated with lower local abundances in the Midwest [27]. This is consistent with our monarch-specific effect size results that show that hotter temperatures in mid-summer have strongly negative impacts on local abundances (Fig. 4I).

A major concern for analysis and policy formation is that studies of effects of modern pesticides on insect decline after 2015 is not possible. Our comprehensive study of multiple drivers across the Midwest US for 17 years ending in 2014 covers a period of rapid technological change in pesticides and crop genetics that has not heretofore been possible. Since that time, butterflies have continued to decline at the same rate [e.g., 6,5]. Pesticidal seed treatment survey data, including the raw data underlying widely available and well used USGS estimates, ceased to be collected after 2014, crippling the ability of the scientific community to expand the scope of these analyses [41]. These concerns are surmountable if seed companies or others along the seed and pesticide supply chain provide government agencies with summary data on *Bt* and neonicotinoid sales at, e.g., the county level. Further, as revealed by our effect size results, although yearly weather conditions strongly affect butterfly communities (Figs. 4C, 4F), trends in climate are not directional during this 17-year period of study, so they are insufficient to explain the decline in butterfly abundances (Fig. S1). However, since neonicotinoid data are not available post-2014 [21], an analysis to compare the importance of pesticides vs. weather during the seven hottest years on record is not possible. Our results expose an urgent need for publicly available, reliable, comprehensive, and consistently reported pesticide use data, particularly for neonicotinoid seed treatments, to fully understand the drivers of butterfly decline.

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Conceptualization: SMS, LR

Methodology: BVD

Investigation: BVD, LR

Visualization: BVD

Funding acquisition: SMS, DAH, LR

Project administration: BVD, SMS, LR, NH

Supervision: SMS

Writing – original draft: BVD, SMS, LR, NH

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Supporting Information

Materials and Methods, Supplementary Text, Figs. S1, Tables S1 to S2, Data S1

Fig. 1. Evolution of pest control technology in North American corn and soybean production. See Supplementary text for discussion of mechanisms of butterfly exposure to pest and weed control impacts.

1
Fig. 2. Data summaries for key variables. (A-C) shows land cover by county as percent of total
land area in 2014 and (D) shows the number of years each county contributed butterfly
population surveys; bold borders indicate USDA Crop Reporting District (CRD) boundaries
while hatching shows counties not included in analysis due to lack of butterfly survey data
availability. (E-G) shows area-treatments, the average number of times a pesticide or pest control
treatment is applied to an acre of soybean or corn cropland, for each pest control technology
group.

1
Fig. 3. Net effect of pest control system change over time. Net pest control effect is the percent
change between predicted incidence rates under observed pest control adoption and synthetic
counties where pest control adoption is fixed at 1998 levels. County-level net pest control effects
over time are shown in gray lines and annual median net pest control effect in black lines;
columns show effects computed holding all weed control, insect control, and pest control
variables at 1998 levels. Results are presented for species richness (A-C), total abundance (D-F),
and *Danus plexippus* (monarch) abundance (G-I).
9

Fig. 4. Model effect sizes for a standard deviation change in explanatory variables. (A-I)
show model effect size estimates from GLM models for the species richness (number of distinct
species identified per hour, A-C), total abundance (number of butterflies counted per hour, D-F),
and *Danus plexippus* abundance (monarch, G-I). Effect sizes are derived from GLM negative
binomial; see Methods for details. For land use, categories are compared to an "open space"
baseline. Lines show 95% confidence intervals based on heteroskedasticity-consistent standard
errors (HC1). Solid points show statistically significant point estimates at the 95% level. For
interpretation: A one standard deviation increase in neonicotinoid seed treatment area-treatments
is associated with 22% lower county-wide abundance for monarchs (G) and 7% fewer distinct
species observed (A).