

**Significant impact of Bt (*Bacillus thuringiensis*) corn on *Danaus plexippus* (monarch butterfly) mortality during their breeding season in Iowa, USA from 2011 to 2020**

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**Summary:**

**Abstract**

The following investigation examines the potential relationship between genetically modified corn containing the *Bacillus thuringiensis* (Bt) gene and monarch butterfly population and mortality rate. Prior literature has shown that in laboratory conditions, Bt corn pollen has an adverse and fatal effect on monarch butterfly larvae. Very little research has conclusively determined whether this holds true in real-world conditions. This paper looks at Iowa- the largest corn-producing state in the United States of America- and the monarch butterfly count using three databases in the state during their breeding season for ten years. Results indicate that for a level of confidence of 0.1, there is a significant negative correlation between the two variables suggesting that an increase in Bt corn significantly reduces the monarch butterfly population in Iowa. These findings suggest that populations of monarch butterflies which are of paramount importance for pollination and for enabling several ecosystems to thrive, are at risk because of Bt corn.

**Introduction**

Genetically modified crops are crops that have a new gene inserted into their genome, which guides specific protein formation giving it new or different characteristics. Bt (*Bacillus thuringiensis*) is a common soil bacterium whose genome contains genes for several proteins toxic to insects. Bt corn is corn that is genetically modified to have this gene to protect against pests (selectively targets caterpillars within the order of *Lepidoptera*). The United States of America is the largest producer and exporter of corn in the world, producing almost 346.0 million metric tons in the 2021-2022 season, the state producing the greatest quantity being Iowa. According to the U.S. Department of Agriculture, over 90% of the produced corn is genetically modified. The issue with genetically modified (GM) corn is off-target effects. Unfortunately, while Bt corn kills insects, it also has unintended effects on non-target species of insects like *Danaus plexippus* (the monarch butterfly), which are extremely important for pollination and, consequently, human food systems. Bt corn varieties produce their toxin in all parts of the plant, including pollen, and this plant generally grows near *Asclepias curassavica* (milkweed) - food for larvae of the monarch butterfly. When milkweed becomes dusted with the pollen of Bt-modified crops, the larvae get poisoned by the toxin resulting in

their mortality. The first study that demonstrated that transgenic pollen kills larvae of the monarch butterfly was a laboratory experiment ( Losey et al., 1999). This study received backlash from plant scientists and representatives of life science companies because it was not a field study. Consequently, many field studies were conducted, all producing contradictory results. The first study that showed transgenic *Bacillus thuringiensis* (Bt) corn pollen naturally deposited on common milkweed in a corn field, caused significant mortality of *Danaus plexippus* was conducted in 2000 (Hansen Jesse & Obrycki, 2000). One investigation demonstrated that in field conditions, 23.7% fewer larvae exposed to Bt pollen reached the adult stage. Additionally, exposure delayed the developmental time of larvae by 1.8 days and reduced the weights of both pupae and adults by 5.5% (Dively et al., 2004). Another study conducted two field experiments that suggested that Bt corn did not influence larval survival (Koch et al., 2003). The contradictory findings suggest that more studies need to be conducted, taking large datasets into account while isolating the requisite variables.

Despite all the prior research conducted, there is limited data available analyzing the quantity of Bt corn production in a particular state that has almost no environmental incidents affecting its production and monarch population during the same period of time for ten or more years. This paper, therefore, aims to investigate the extent to which *Zea Mays* (corn) that are genetically modified to contain the *Bacillus thuringiensis* (Bt) gene cause *Danaus plexippus* (monarch butterfly) mortality in Iowa, United States of America (Appendix A), during the breeding season (summer) from the years 2011 to 2020. Considering the extent of corn production in the United States of America and prior literature, it can be hypothesized that Bt corn does significantly affect monarch butterfly mortality. It can therefore be predicted that if Bt corn significantly contributes to monarch butterfly mortality in Iowa, then there would be a significantly lower number of monarch butterflies during the breeding season near Iowa, U.S.A, where Bt corn which is measured in millions of bushels (M bu) is grown the most and which is harvested around the same time as the breeding season of these butterflies.

## **Materials & Methods**

Data related to corn production in Iowa was collected from CropProphet- a service provided that has daily-updated county, state, and national crop yield and production forecasts in correspondence with the USDA. The website provides data regarding Iowa's corn production measured in millions of bushels (M bu) from the years 2011 to 2020. This data, however, doesn't specify if all the crops are Bt corn crops or not. The USDA's National Agricultural Statistics Service (NASS) provides percentages of total corn crops for each state that only have the Bt modification from 2000 to 2020. The product of this percentage with the data from CropProphet gives the total number of Bt corn crops for each year. The monarch butterfly data was collected from two databases- Butterflies and Moths of North America (BAMONA), eButtefly & iNaturalist. The verified data was obtained from May 1st to September 1st for each year from 2011 to 2020. The data obtained was then averaged, and the standard deviation was calculated. Monarch butterfly and Bt corn trends were then compared.

To determine if these findings were significant or not, the Pearson correlation test was used to determine whether there is a significant relationship between these two variables. Based on the results, we can then conclude whether our hypothesis has been supported or rejected.

### **Results & Statistical Analysis**

Table 1: Data from CropProphet and USDA showing Bt corn production (in M bu) from 2011 to 2020 in Iowa, USA.

<b>Corn Production (M bu) in Iowa</b>	<b>% of Bt corn</b>	<b>M bu of Bt corn</b>	<b>Year</b>
2296.20	3.00%	68.89	2020
2583.90	4.00%	103.36	2019
2499.00	3.00%	74.97	2018
2605.80	5.00%	130.29	2017
2740.50	3.00%	82.22	2016
2505.60	5.00%	125.28	2015
2367.40	4.00%	94.70	2014
2140.20	5.00%	107.01	2013
1876.90	12.00%	225.23	2012
2356.40	13.00%	306.33	2011

Table 2: Verified monarch butterfly count from BAMONA, eButterfly and iNaturalist database from 2011 to 2020 during breeding season (May 1st to September 1st) in Iowa, USA.

BAMONA monarch data	EButterfly monarch data	iNaturalist monarch data	Year	Average	Standard deviation
5.00	2.00	215.00	2020	3.50	122.12
3.00	18.00	103.00	2019	10.50	53.93
5.00	25.00	92.00	2018	15.00	45.57
4.00	16.00	26.00	2017	10.00	11.02
0.00	14.00	5.00	2016	7.00	7.09
3.00	23.00	12.00	2015	13.00	10.02
1.00	15.00	1.00	2014	8.00	8.08
0.00	0.00	6.00	2013	0.00	3.46
0.00	1.00	0.00	2012	0.50	0.58
2.00	0.00	0.00	2011	1.00	1.15

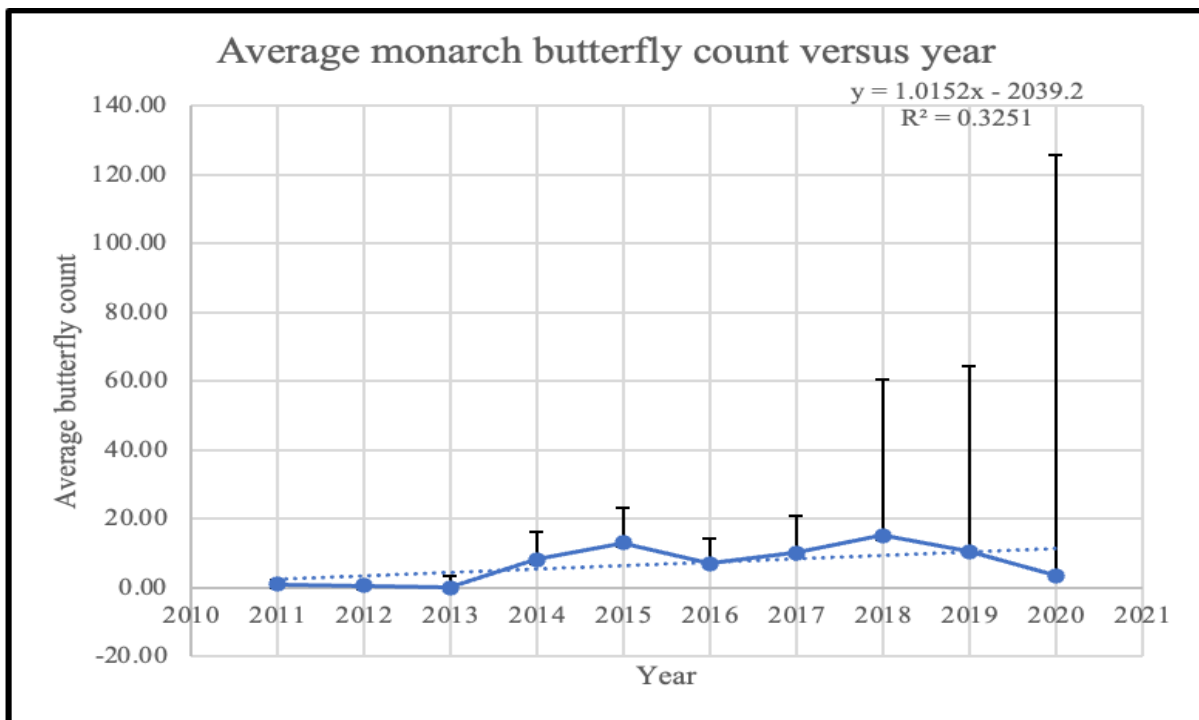


Figure 1: Average monarch butterfly count from 2011 to 2020 during breeding season in Iowa. Error bars represent standard deviation values calculated in Table 2.

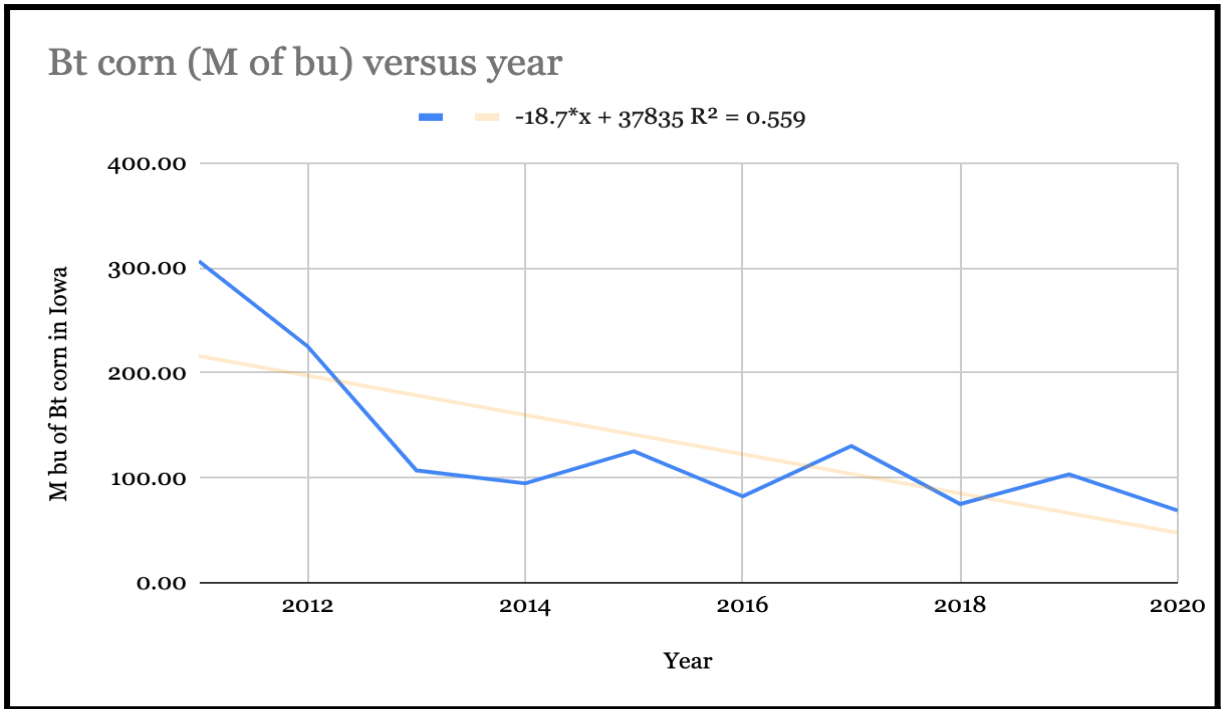


Figure 2: Production of Bt corn (M of bu) in Iowa, USA from 2011 to 2020.

Table 3: : Pearson’s product-moment correlation test. Variable X is the average monarch butterfly count mentioned in Table 2, and Variable Y is M bu of Bt corn in Table 1. N denotes the number of years. The squares of X and Y and the product of X and Y are also mentioned. The sum of all columns is calculated in the last row.

N	X	Y	$X^2$	$Y^2$	XY
1	3.50	68.89	12.25	4745.83	241.12
2	10.50	103.36	110.25	10683.29	1085.28
3	15.00	74.97	225.00	5620.50	1124.55
4	10.00	130.29	100.00	16975.48	1302.90
5	7.00	82.22	49.00	6760.13	575.54
6	13.00	125.28	169.00	15695.08	1628.64
7	8.00	94.70	64.00	8968.09	757.60
8	0.00	107.01	0.00	11451.14	0.00
9	0.50	225.23	0.25	50728.55	112.62
10	1.00	306.33	1.00	93838.07	306.33
SUM	68.50	1318.28	730.75	225466.17	7134.57

From here, the following equation is used to find the Pearson's correlation value:

$$r = \frac{n(\Sigma xy) - (\Sigma x)(\Sigma y)}{\sqrt{(n\Sigma x^2 - (\Sigma x)^2)(n\Sigma y^2 - (\Sigma y)^2)}}$$

$$r = -0.53$$

This value means that there is a reasonably strong negative correlation between the monarch butterfly count in Iowa from May to September and Bt corn production from the years 2011 and 2020. Furthermore, it is found that in a Pearson's correlation test, the critical value for a correlation with 9 degrees of freedom (found by the number of trials, 10 minus 1), and a level of confidence of 0.1, is 0.52 (Statistics Solutions, n.d.). The r value obtained from this investigation has a higher absolute value than this, meaning that the results are reasonably significant and that Bt corn negatively influences monarch butterflies.

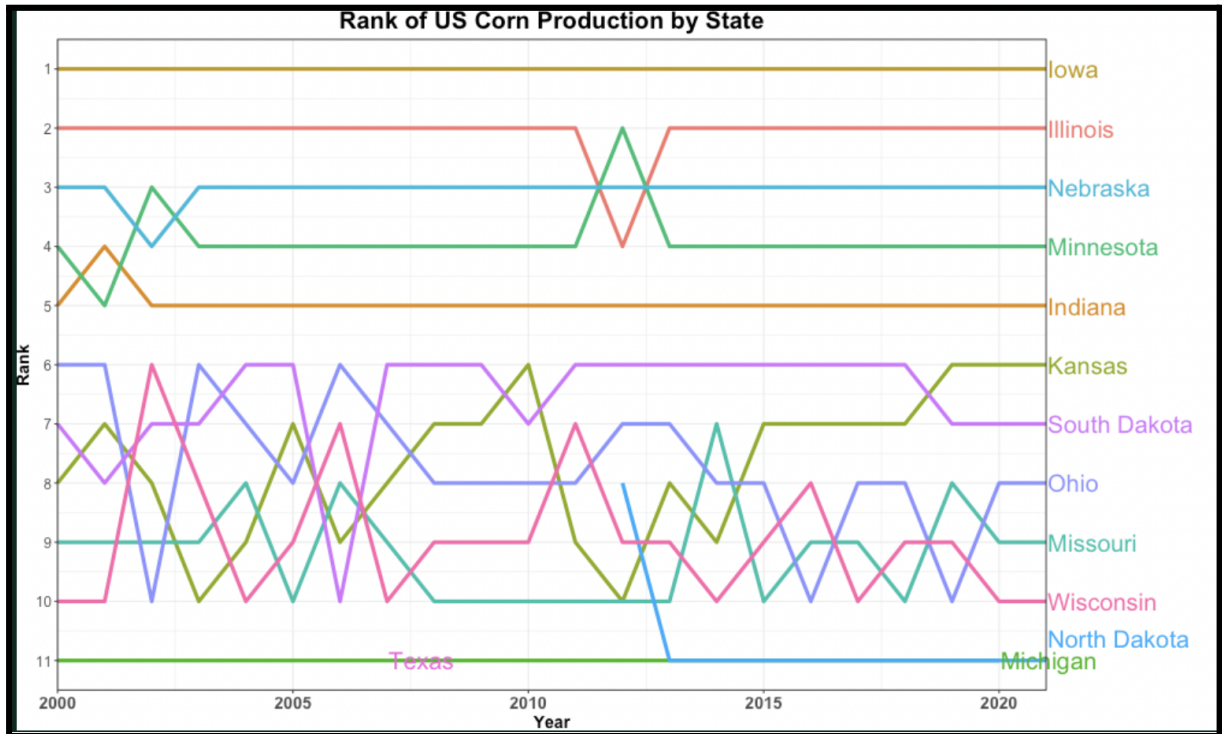
### **Discussion & Conclusion**

Findings suggest that there is a significant negative correlation between Bt corn and monarch butterfly mortality over time. More data related to monarch butterfly tracking over a longer period of time needs to be used to confirm these results. Furthermore, taking other variables into account like deforestation, flooding, and drought events would make findings more valid. Iowa has been consistently producing corn with no incidents within the past ten years affecting its production, making it ideal for this investigation. However, this does not mean that the state did not undergo other events related to monarchs and their habitats during this period which could have also affected their population. Additionally, Bt corn produces different types of insecticidal proteins called d-endotoxins, which predominantly comprise one or more proteins called Cry and Cyt toxins (Bravo et al., 2005). Investigating which of these toxins contributes most to the mortality rate would be particularly beneficial for life science companies that are continuing genetically modified crop production.

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## Appendix



Appendix A: figure showing consistency of Iowa corn production from 2000 to 2020 making it an ideal location to investigate the monarch butterfly population.