NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Butterflies in the Stomach: Is Genetically Modified Corn Harming Monarch Butterflies?

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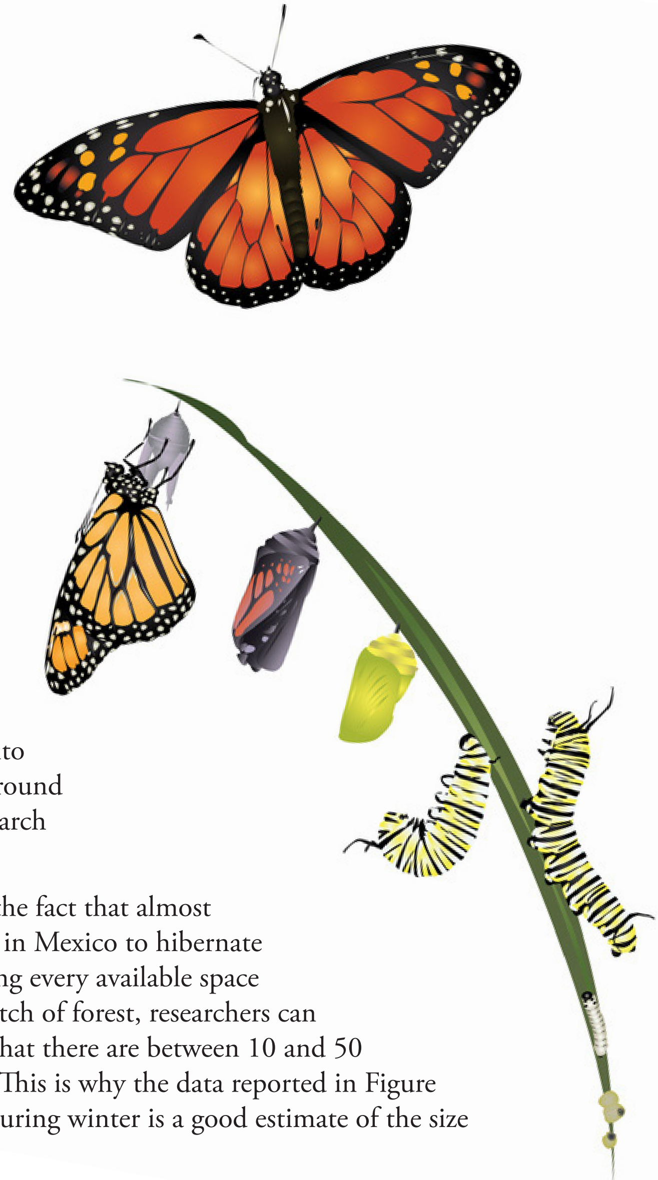

Part I – The Monarchs' Vanishing Act

Dr. John Losey, an expert in insect conservation biology, was concerned about the butterflies. Working at Cornell University in Ithaca, New York he noticed fewer and fewer iconic monarch butterflies every year. Curious about this observation, he plunged into the scientific literature and learned that this wasn't occurring only around Ithaca; it was happening all over North America. Why was the monarch population declining?.

To measure the monarch population, researchers take advantage of the fact that almost all monarchs in North American migrate to a small stretch of forest in Mexico to hibernate during the winter months. The butterflies rest on the trees, occupying every available space (Figure 1). Because the wintering grounds are limited to a small stretch of forest, researchers can measure the area taken-up by the butterflies each year. It's thought that there are between 10 and 50 million monarchs in every hectare where the butterflies overwinter. This is why the data reported in Figure 2, which is the area of forest occupied by the monarchs in Mexico during winter is a good estimate of the size of the entire North American monarch population.

Figure 1. Monarch butterflies overwintering on trees in large densities. *Credit:* Agunther, CC BY-3.0, <https://commons.wikimedia.org/wiki/File:Monarch-butterflies-pacific-grove.jpg>.

Monarch butterflies begin their life cycle in the northeastern U.S. and eastern Canada when eggs are laid on the underside of the leaves of a milkweed plant. Milkweed is a leafy plant found in meadows and along roadsides. It can grow in areas surrounding agricultural crops such as corn fields. The eggs are susceptible to predation by insects such as ants. Once the larvae hatch, they heartily feed on the milkweed leaves (and only on milkweed leaves—they are picky eaters!) (Figure 3). This stage of their life lasts for roughly two weeks (9–15 days); the larvae rapidly gain weight and increase their body mass up to 2,000 times. The larvae then form a cocoon where they undergo transformation and from which they emerge as beautiful butterflies. While in the cocoon they are vulnerable to predators such as ants, spiders and beetles, and only 5% of the

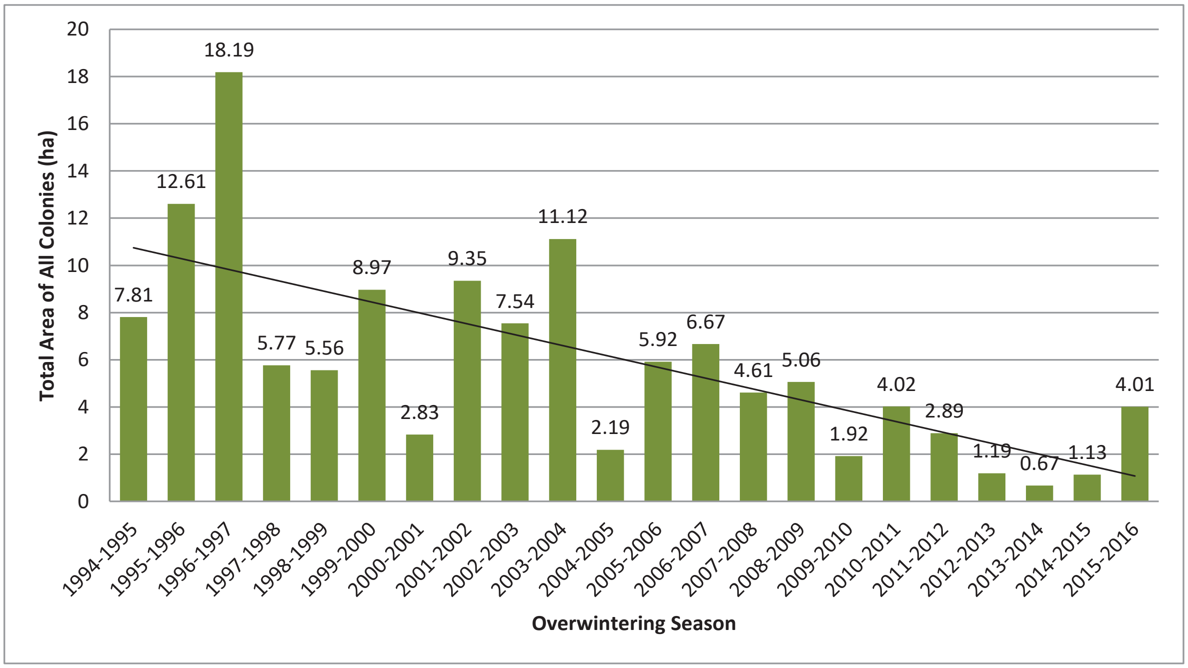

Figure 2. Estimates of the population of monarch butterflies found in Mexico in the winter months between 1994 and 2014. The x-axis shows the year when the data was collected, and the y-axis shows the total area of the overwintering colonies of monarch butterflies in Mexico. The line shows a regression model of the data. The average area occupied by the monarch butterflies over this two decade period is 5.9 hectares. Figure adapted from the following sources: Brower et al (2012) [Figure 1 of their paper]; Plumer (2014) [for data on 2012–2013]; Wade (2015) [for data on 2013–2014]; and *Monarch Watch* (2016) [for data on 2014–2016].

eggs that hatch survive to the butterfly stage. Butterflies typically live for about a month. During this time, they flutter around, feeding on the nectar of flowering plants and on the liquid from fruits. They are vulnerable to severe weather patterns such as cold snaps and droughts. When they are ready to breed, they lay their eggs under the leaves of a milkweed plant and a new generation begins.

This happens for several generations in the summer, but those born in the late summer are different. To escape the coming cold, they undertake a long 4,000 km journey to west central Mexico where they hibernate in the warm Mexican environment until the following spring. Mexico during the winter months can be home to 60 million to one billion butterflies—a spectacular sight! While the warm temperature protects the butterflies during the winter, they are vulnerable to predation. Up to 15% of the monarchs are typically eaten each year by mice and birds. In the spring, the overwintering butterflies migrate back to the U.S. and Canada, where they resume breeding.

Figure 3. Monarch larva on milkweed. *Credit:* Forest & Kim Starr, CC BY-3.0, <https://commons.wikimedia.org/w/index.php?curid=6126658>.

Questions

1. Examine Figure 2 showing the population of monarchs in Mexico for the past 20 years. Do you agree that the population has been declining? If so, to what extent? If you disagree, why?
2. Assume that the monarch butterfly population is declining. Why might this be? Propose at least three different hypotheses.

Part II – Maybe It's Bt Corn

Dr. Losey thought about the changes that had occurred in the monarch's environment in the past 20 years and zeroed in on one that might be involved: Bt corn. At the time, roughly a quarter of the corn planted in the US was Bt corn (USDA, 2015). Bt corn is a corn plant engineered to resist the European corn borer which plagues corn crops. To make the plant impervious to the common insect pest, a gene from the soil bacteria *Bacillus thuringiensis* (hence the name Bt) is incorporated into the corn genome. As a result of the genetic modification, Bt corn contains the Bt toxin in its tissues and is therefore toxic to insects that feed on it. (The Bt toxin affects insects but is safe for human consumption.)

While monarchs don't eat Bt corn, they do eat milkweed plants, which often surround corn fields. Dr. Losey proposed that the pollen of Bt corn contains the Bt toxin, and that it is carried by the wind and settles or nearby milkweed leaves, where it is eaten by monarch larvae. This proposed hypothesis is illustrated in Figure 4.

Figure 4. Dr. Losey's hypothesized mechanism of action for the effects of Bt corn on monarch larvae.

Questions

1. If Dr. Losey's hypothesis is correct, at what stage of development would monarchs be most susceptible to the adverse effects of the Bt toxin?
2. Where in the world would monarch butterflies be most susceptible to the effects of Bt corn?
3. What data would Dr. Losey need to collect in order to support his hypothesis that Bt corn is the reason for the declining population of monarch butterflies?
4. Propose an experiment to test Dr. Losey's hypothesis.

Part III – Dr. Losey's Experiment

As a test of his hypothesis, Dr. Losey worked with his colleagues to recreate field conditions in a laboratory setting. They dusted milkweed leaves with either the pollen of Bt corn plants, the pollen from corn that did not contain the Bt toxin, or no pollen at all. They dusted enough pollen to visually mimic the amount typically found on the leaves of milkweed plants found near corn fields.

Five monarch larvae were placed on milkweed leaves dusted with pollen from one of the three treatment groups (no pollen, pollen from corn, pollen from Bt corn). The researchers then recorded the number of larvae that were still alive each day for four days. They replicated this experiment five times. The results are shown in Figure 5.

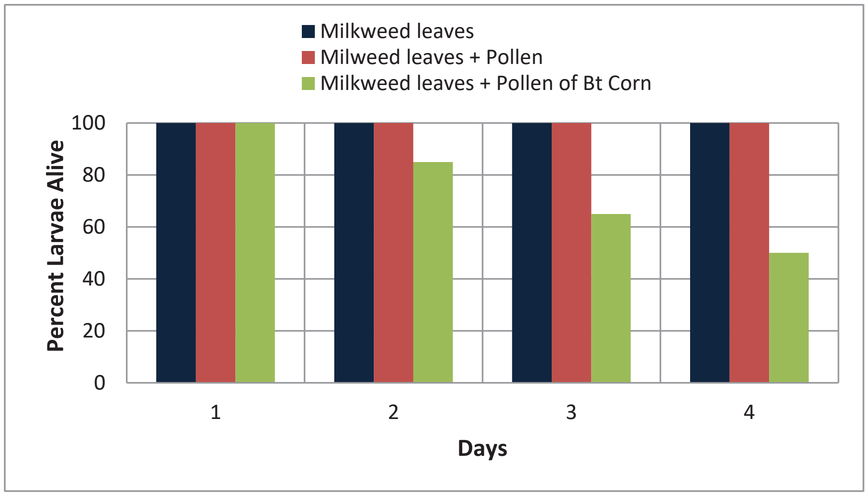

Figure 5. The effects of different types of pollen dusted on the leaves of milkweed plants on the survival and eating habits of monarch larvae.
The blue (first) bars show the results of the larvae feeding on milkweed leaves with no pollen dusted on it; red bars (middle) show the effects of the larvae feeding on milkweed leaves dusted with the pollen of corn that did not express the Bt toxin; and the green bars (last one) are the outcome of the larvae feeding on milkweed leaves dusted with pollen of Bt corn. Adapted from: Losey, J.E., Rayor, L.S., & Carter, M.E. (1999). Transgenic pollen harms monarch larvae. *Nature* 399(6733):214. Figure 1 of the paper.

To study the effects of the pollen on the eating habits of the larvae, Dr. Losey and his colleagues measured the average number of leaves consumed by each larva after 1, 2, 3 and 4 days. This is shown in Figure 6. John and his colleagues replicated each treatment five times.

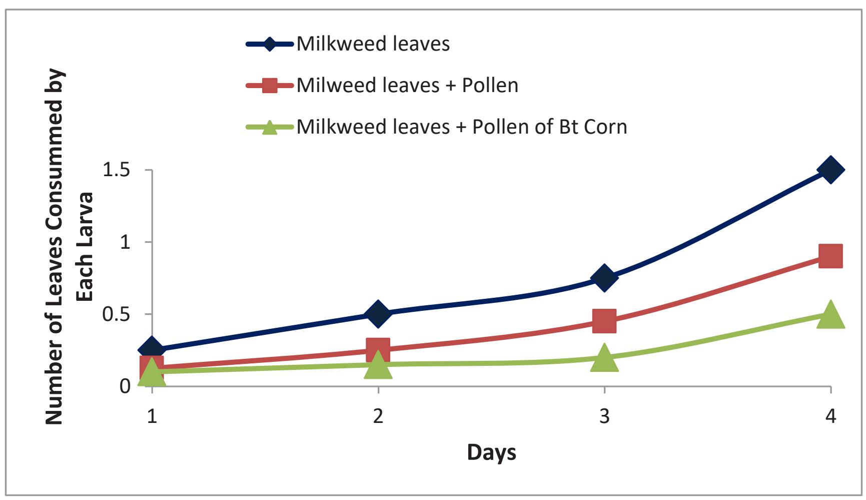

Figure 6. The effects of different types of pollen dusted on the leaves of milkweed plants on the eating habits of monarch larvae.
The blue line (diamond symbol) shows the results of larvae feeding on milkweed with no pollen present; the red line (square) shows the consumption of leaves by larvae eating milkweed leaves dusted with the pollen of corn that did not contain the Bt toxin; and the green line (triangle) shows the effects of a diet of milkweed dusted with Bt corn pollen. Adapted from: Losey, J.E., Rayor, L.S., & Carter, M.E. (1999). Transgenic pollen harms monarch larvae. Nature 399(6733), 214. Figure 1 of the paper.

Questions

1. Create a diagram of the experimental set-up. Be sure to identify the independent and dependent variables, the control(s) and the replicates. How many animals were tested in total? Below the diagram of each condition, summarize the results.
2. Does the presence of pollen (any pollen, whether it contains Bt toxin or not) on the leaves appear to affect the monarch larvae survival and/or appetite? Describe your answer and its implications.
3. Does the presence of pollen from Bt corn on milkweed leaves appear to affect monarch larvae survival and/or appetite in a manner that differs from that of pollen that does not contain the Bt toxin? Describe your answer and its implications.
4. What do you conclude from these results?
5. Critique the experimental design. What are some weaknesses of the way in which this experiment was performed?
6. Revisit your answer to Part II, Question 3. Which of the things that Dr. Losey needed to show in order to convince you that Bt corn poses a risk to monarch butterflies has been addressed by this experiment and which ones remain unknown?

Part IV – How Much Pollen Are Monarch Larvae Exposed to in the Field?

Dr. Losey's study was published in the prestigious journal *Nature* in 1999. The results were worrisome and garnered a lot of media and public attention. At the time, nearly a quarter of corn planted in the U.S. was of the Bt variety, so if it played a role in the monarchs' disappearance, we needed to know that as soon as possible.

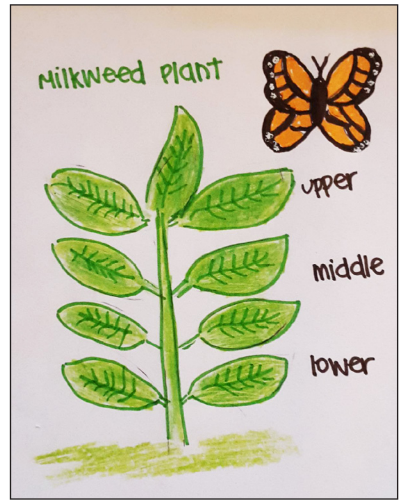

Figure 7. Diagram of milkweed to illustrate upper, middle and lower leaf positions.

Before jumping to conclusions, there is a need to verify the relevance of these *laboratory* results to what happens *in the field.* To properly assess the risks, the dose of Bt pollen that monarch larvae are exposed to in the field must be determined as well as the dose that has harmful effects on monarchs (i.e., there is a need for information about exposure and toxicity). A consortium of researchers from six universities, the US Department of Agriculture, and Environment Canada pooled resources to get to the bottom of this.

One of their priorities was to determine the amount of corn pollen that settles on milkweed leaves growing near a cornfield. Recall that Dr. Losey's experiment coated the milkweed leaves with an amount of pollen that "visually matched the amount observed on plants in or near cornfields." This is an approximation at best and more precise methods should be used. The researchers went into the field and counted the number of grains of pollen deposited on milkweed leaves during the 7–10 days that corn produces pollen each year. Noting that milkweed has several layers of leaves that may receive different amounts of pollen, they categorized the leaves into "upper", "middle", and "lower" parts of the plant (Figure 7).

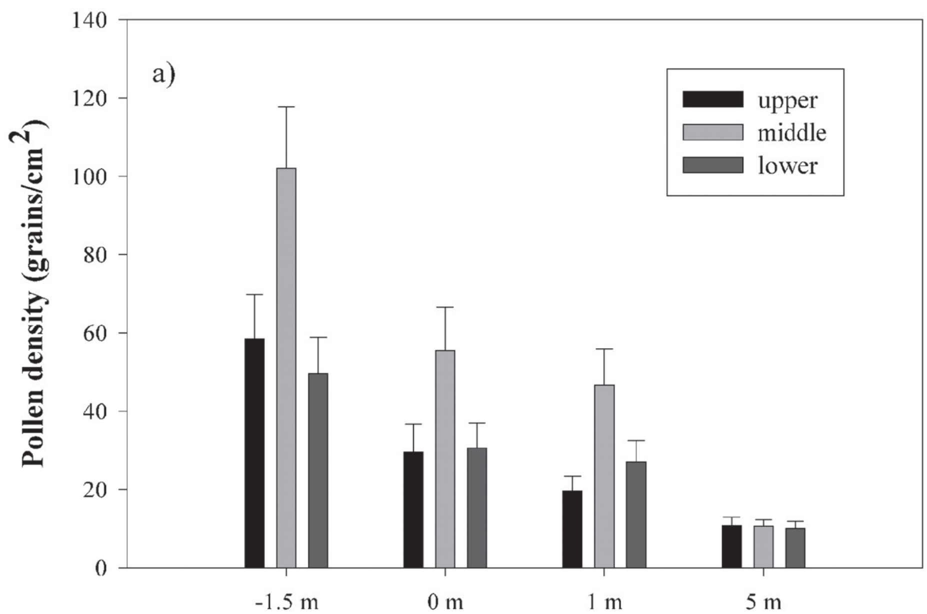

Figure 8. Amounts of pollen deposited on upper, middle and lower leaves of milkweed plants located around a corn field 6 days after the start of pollen production. The distance indicated on the x-axis is the distance of the plant from the edge of the corn field. –1.5m is within the cornfield. *Source:* Top panel of Figure 1 in: Pleasants, J.M., Hellmich, R.L, Dively, G.P., et al. 2001. Corn pollen deposition on milkweeds in and near cornfields. *PNAS* 98(21): 11919–11924. Copyright 2001, National Academy of Sciences, USA.

The results are shown in Figure 8. The x-axis shows the distance from the corn field where the milkweed leaves were examined; 0 represents the field's edge, -1.5m is inside the field, and the 1m and 5m represent plants sampled at those distances outside the field's edge. The y-axis shows the number of pollen grains found, on average, per square centimeter on the leaves of milkweed plants. Note that monarchs have a tendency to lay their eggs on the underside of upper leaves.

Questions

1. Explain the distribution of pollen on the milkweed plants. Why do you think the pattern is the way that it is?
2. Identify the range of pollen densities that monarch larvae are exposed to in the field. (*Hint:* this will be helpful for the next section.)
3. How might the data in Figure 8 be different if it was recorded a few days earlier or later? How might this impact the potentially toxic effect of Bt corn on monarchs?

Part V – How Much Bt Corn Pollen Does It Take to Harm Monarch Larvae?

In Part IV, Question 2, you determined a quantity of corn pollen to which monarch larvae are exposed in the field. The next step is to determine the toxicity of that amount of Bt pollen on monarch larvae.

The consortium of researchers that looked at exposure undertook this investigation. They noted that there are different types of commercially available Bt corn. These varieties differ in the amount of toxin that the plants express in their tissues (and presumably in their pollen) (Hellmich et al., 2001).

Dr. Losey used a variety called Bt176. Bt176 is not commonly used by farmers. It represented 2% of the Bt corn planted in 2001 and the company that developed it did not seek Environmental Protection Agency re-approval in 2001. It's therefore been phased out (USDA, 2015).

The researchers therefore assessed the toxicity of Bt176 as well as two other types of Bt corn more commonly used by farmers called Bt11 and Mon810. They replicated Dr. Losey's experiment and dusted pollen from several types of Bt corn onto milkweed leaves. This time, they monitored the precise amount of pollen placed on the leaves and assessed the effect of different amounts on the growth of monarch larvae. The results are shown in Figure 9.

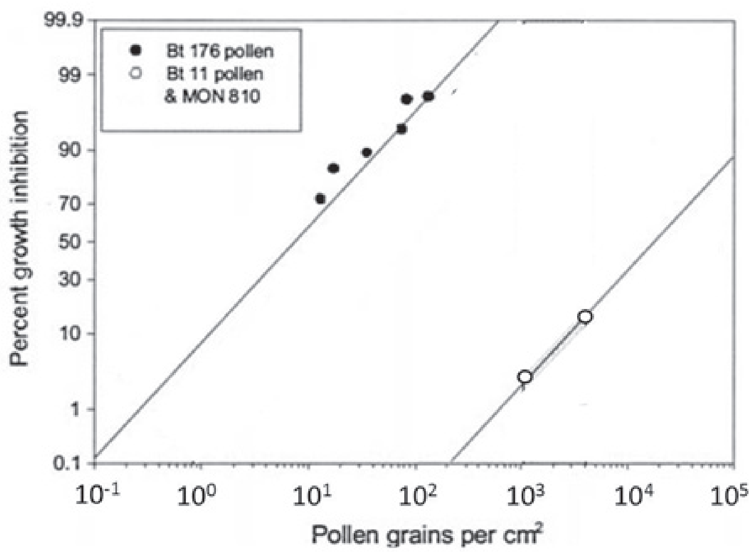

Figure 9: Effects of consuming milkweed leaves coated in varying amounts of Bt pollen on growth inhibition of monarch larvae.

- The x-axis shows the number of pollen grains present on the milkweed leaves. Note that the data is provided using a logarithmic scale, so the quantity increases by a factor of 10 at each notch.
- The y-axis shows the percentage of exposed monarch larvae that were affected by the presence of BT pollen after 4 days of exposure. For example, a 10% percent growth inhibition means that 10% of the insects that were tested did not grow and develop normally in the presence of Bt pollen. Note that the scale of the axis is non-linear.
- The filled circles represent the data obtained when Bt176 corn was used. The regression line shows the trend in the data.
- The open circles represent data obtained using Bt11 and Mon810, along with a regression line for that data. Note that for Bt11 and Mon810, the researchers could not detect any effect on growth at pollen concentrations between 100–1,600 pollen grains/cm^2. An effect was observable only at pollen densities >1,600 grains/cm^2, which are plotted on the graph. This is why there are only two data points for Bt11 and Mon810.

Adapted from: Figure 2 in: Sears, M.K., Hellmich, R.L., Stanley-Horn, D.E., et al. 2001. Impact of Bt corn pollen on monarch butterfly populations: A risk assessment. *PNAS* 98(21), 11937–11942. Copyright 2001, National Academy of Sciences, USA.

Questions

1. Re-examine your answer to Part IV, Question 2 about the amount of pollen on milkweed leaves near a corn field. At these pollen concentrations, do Bt176, Bt11, and Mon810 negatively affect the butterflies?
2. Is Bt corn the likely culprit of the decline of the monarch population?

Part VI – So What Is Killing the Butterflies?

While the initial results of Dr. Losey's laboratory were worrisome, further investigations clarified that under field conditions, Bt corn is unlikely to be the reason that monarch numbers are declining.

That still begs the question: if Bt corn is not to blame for the monarchs' decline, what is? Many monarchs do not survive the long migration to Mexico and the hibernation due to extreme weather conditions (Brower et al., 2012; Plumer, 2014). Cold winters, droughts, or heavy periods of rain are particularly hazardous to the butterflies' survival. And it doesn't help that the monarchs' overwintering habitat in Mexico is destroyed by illegal logging (Brower et al., 2012; Plumer, 2014).

However, the most likely culprit is the decrease in the amount of milkweed by up to 58% since 1999 (Brower et al., 2012; Pleasants & Oberhauser, 2012). Without food, the monarch larvae cannot grow. Thus, most conservation efforts aimed at restoring the monarchs' population focus on increasing the number of milkweed plants in North America.

Questions

1. Do a web search using keywords such as "Monarch butterflies" and "Bt corn." Is the evidence available online an accurate portrayal of the state of knowledge on this situation? Describe and comment upon your findings.
2. What are likely causes for the decline in the number of milkweed plants?
3. What might be done to restore the milkweed population?
4. How might we test that milkweed numbers are indeed the cause of the monarchs' decline?
5. Why should we care about the decline of a species of butterfly?

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