Case: Effects of Feeding Flossflower on Manduca Caterpillar Development

Background:

It has been observed many times that phytophagous (plant-eating) insects will attack some species but not others. Sometimes this is because the plant contains toxic compounds like nicotine (in tobacco), or cardinolides (in milkweed). Other plants contain “insect growth regulators” (IGRs), substances that disrupt insect growth and development. They affect physiological processes that are essential to the insects or disrupt development of their offspring. Unlike traditional synthetic insecticides (like organophosphates and carbamates) that are toxic to insects directly, IGRs reduce insect damage by blocking their ability to feed on the plant, or by causing abnormalities that impair insect survival and reproduction.

IGRs are attractive candidates for developing less hazardous insecticides. The problem is, which plants contain them? One way to identify sources of them is to look for plants that insects avoid eating. The example we will test today is blue flossflower (*Ageratum houstonianum*), which insects rarely attack.

Methods:

1. We selected twelve, 11-12-day old *Manduca sexta* caterpillars weighing 1.5 to 2 grams and 8-10 mm long and divided them into groups of 3 each.
2. We placed each caterpillar in its own separate rearing jar.
3. We fed each caterpillar 1 of 4 diets.

* Three received 10 g each of Generic Manduca Diet (GMD), prepared according to manufacturer instructions.
* Three received 10 g each of GMD diet with 1% (by weight) freshly ground leaves from blue flossflower (*Ageratum houstonianum*) added.
* Three received 10 g each of GMD diet with 5% (by weight) freshly ground leaves from blue flossflower added.
* Three received 10 g of GMD diet with 10% (by weight) freshly ground leaves from blue flossflower.

1. All caterpillars were kept in the same incubator at 28oC, in 12 hr dark: 12 hr light cycle.
2. After 7 days, we recorded the final weight and length of each caterpillar, and described how it looked.

Results:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **Observations taken 7 days after feeding was started** | | |
| **Treatment** | **Animal #** | **Weight (g)** | **Length (mm)** | **Observations** |
| 1. Control (GMD diet only) | 1  2  3 | 10.7  11.2  10.3 | 103  105  97 | Ate 2-3% of available food per day. #1 and #2 turning yellow-brown, moving less. |
| 2. Diet + 1% floss- flower leaves | 4  5  6 | 14.3  14.2  14.9 | 139  138  142 | Ate 4.5-5% of available food per day. All bright green, very active, and look swollen. |
| 3. Diet + 5% floss- flower leaves | 7  8  9 | 10.4  9.8  11.1 | 89  99  106 | Ate about 3-3.5% of available food per day. Not moving much. |
| 4. Diet + 10% floss- flower leaves | 10  11  12 | 7.1  6.6  na | 88  71  43 | Ate about 2% of diet per day. Animals 10 and 11 look thin compared to controls. #12 died on Day 5 |

Follow-Up Questions:

Discuss these with your group. Your instructor will then ask you to share your group’s ideas.

1. What can we say about the effects of flossflower based just on **direct observation**? (Don’t interpret the data, just summarize what you can **directly** observe or report.)
2. What can we **infer or conclude** from the direct observations? What is your **reasoning**, that is, the logic or thought process that connects your direct observations and your conclusions?
3. How would your group summarize the direct observations and inferences?

Instructor Notes

### Background

We intentionally do not give students the following additional information because we want them to think through the possible effects on their own first. This information is for instructors who are unfamiliar with insect endocrinology so they know which are reasonable hypotheses.

This is an excerpt adapted from a review by Tunaz (2004):

An emerging approach to insect pest control is the use of substances that adversely affect insect growth and development. These “insect growth regulators” (IGRs) affect certain physiological regulatory processes essential to the normal development of insects or their progeny. IGRs differ from traditional synthetic insecticides (for example, organophosphates and carbamates) that are toxic to insects directly. An IGR does not have to be toxic to its target, but leads instead to various abnormalities that impair insect survival (Siddall, 1976).

One of the earliest observations of IGRs was accidental. Researchers observed that cultures of linden bug (*Pyrrhocoris apterus* L.) had low egg hatch rates and failed to mature into adults. Their investigations traced the abnormality to the paper towels used in the rearing jars. Ultimately the cause was found to be juvabione, an IGR compound produced by balsam fir trees, which were the primary pulp tree used in the United States paper industry at that time. This discovery led to industrial interest in IGRs as possible alternatives to broadly toxic insecticides.

In addition to plant-derived insect growth regulators identified by traditional screening, synthetic IGR compounds have been created based on an understanding of the biochemistry and physiology of insect development. To maximize selectivity, research and development has focused on IGRs that target sites which do not exist in mammals. Thus, most currently known IGRs are either chitin synthesis inhibitors (which disrupt cuticle formation), or substances that either interfere with or over-activate processes regulated by the insect hormones (i.e. JHs, ecdysteroids.)

Tunaz, H. (2004). Insect Growth Regulators for Insect Pest Control. *Turkish Journal of Agriculture and Forestry*, 28, 377-387.

*A. houstonianum* is the original source from which the compound methoprene was first isolated. Methoprene and its synthetic derivatives are JH disruptors. Methoprene is an effective IGR because JH has such broad effects. At low physiological doses, JH inhibits energy storage, so more is available for growth. It also prevents moving into the pupal stage, so that molting produces extra larval stage instars. At supra-physiological doses, JH and its mimics can inhibit production of essential energy storage molecules. Conversely JH blockers prevent normal metabolic function. Either way, resultant metabolic imbalances can be fatal.

### Case Data Structure

The case data table is written as if a diet containing 1% *A. houstonianum* leaves is equal to a **small** increase in JH. As a result the caterpillars are eating more, and getting longer and heavier. Unlike the controls, they also are NOT browning, which means they are not preparing for pupation. At 5% and 10% added leaves, caterpillars are not eating as much and not gaining as much weight. This matches published observations that higher doses of JH analogues disrupt a normal life cycle, affecting multiple systems. That is why methoprene (the active molecule in flossflower) was ultimately developed into a commercial IGR insecticide.

Class Management

### Compiling the Group Responses

The simplest way to collect the responses of multiple groups is to ask each group to share ONE of their hypotheses, comments or thoughts for each question. Write a short 2-3 word summary of each on the board. Rotate between groups asking for responses until all groups’ thinking has been captured. Keep the data on the board for when students must revisit their original thinking at the end of the case.

**Be very careful not to lead students to a “correct” response.** The goal is for students to think broadly first, then refine their hypotheses based on observations. They continue this process as they design their own actual experiments.

### How to Encourage Quantitative/Statistical Thinking

The data table is organized to prompt students to start thinking about comparing group averages. The calculated means and standard deviations for each group are below.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Observations taken 7 days after feeding was started | | |
| Treatment | Mean±SD  Weight (g) | Mean±SD  Length (mm) | Mean±SD  Amt of food eaten (%) |
| 1. Control (GLM diet only) | 10.73±0.5 | 101.7±4.1 | 16.7+2.8% |
| 2. Diet + 1% floss- flower leaves | 13.13±0.9 | 130.3±2.1 | 33.3+2.8% |
| 3. Diet + 5% floss- flower leaves | 10.43±0.7 | 98.0±8.5 | 23.3+2.8% |
| 4. Diet + 10% floss- flower leaves | 7.5±0.7 | 89.5±21.1 (w/#12)  77.3±2.1 (w/o #12) | 18.3±11.5% (w/#12)  25.0±0%(w/o #12) |

As the class discusses how to make comparisons between groups, the instructor should focus on helping students understand:

1. The dose-response is not linear. Low levels affect caterpillars differently than high levels.
2. Not all comparisons are valid. For example:
   * A t-test would find a significant difference between average WEIGHT and average LENGTH of caterpillars in Group 1. However this comparison is experimentally meaningless; it would be pure coincidence if the length and weight were the same.
   * **Reinforce the point that students cannot rely on p-values to tell them which comparisons are important.** They must ask, “does this comparison make **logical** sense?”
3. Which data we include can impact our overall interpretation. For example:
   * One animal died (#12). Do we include its length and amount of food eaten in any averages or statistical comparisons?
   * Is the dead animal an outlier we should eliminate, OR does it tell us something about what methoprene does in high doses?
4. There are additional controls we could add to make this a stronger study. For instance:
   * Is it the flossflower, or a change in food composition? An additional control would be diet with a different flower species added.
5. We provide students in our labs with pre-made Excel templates for t-tests and other analyses. Use this opportunity to share your local strategy.

### Goals of the Follow-Up Questions

The four questions at the end of the case are designed to guide and clarify students’ thinking using a practical argumentation and logic model called the **Toulmin model**. Published in the early 1960s, it is widely used even though not many people know it by this formal name. Dr. Toulmin believed that robust arguments could be broken down into specific parts for analysis:

* **Claims** or conclusions
* **Evidence** or observations to support the conclusions
* **Reasoning**, inference, or rationale that connected the evidence and conclusions.

Toulmin’s full logic model has other elements, but for this exercise we only want students to be able to break a conclusion down and provide adequate support for it.

Focusing consciously on these specific elements

1. *What can we say about feeding based on* ***direct observation****? (Don’t interpret, just summarize the directly observable findings.)*

What is the **evidence** (direct observations) INSIDE the study itself? Don’t let students bring in outside data or knowledge yet. Focus on the evidence within the reported results.

1. *Can we summarize the results of this study more quantitatively or systematically? What would we gain from doing this?*

This is a skills-building question. Summarizing quantitatively points students in the direction of summary/descriptive statistics. We do not want to teach them statistics in detail just yet. We are simply introducing statistically oriented thinking.

1. *What can we* ***infer*** *from the direct observations? What is your* ***reasoning****, that is, the logic or thought process that connects the direct observations and your inferences?*

These two questions are asking students to identify and state what outside knowledge and pre-conceptions they are bringing to their conclusions. It is MUCH easier to evaluate reasoning for faulty logic when the reasoning is stated explicitly.

1. *What can we* ***conclude or claim*** *based on this study?*

The other point where students make frequent errors is making conclusions that are too broad given the data presented (over-generalization). We hope that by asking them to state their conclusions after presenting the direct observations and inferences, these details will be clearer in their minds.

It is PERFECTLY FINE for students to say, “Our conclusion can’t be correct because…” Self-correction means they understand that their original interpretation of the data or inferences they made were flawed. This is what students are most afraid of – being wrong.

Documenting the Case in a Notebook Entry

If students are required to summarize their weekly activities and document experimental data in a bound laboratory notebook, this case is a more realistic practice scenario than a checklist set of instructions. We ask students to write their first notebook entry during lab, using this case study. This has two goals:

* The TA/instructor is available to coach them through the process their first time.
* It reinforces our expectation that students complete notebooks DURING lab, before they leave for the day.

To keep the first entry down to a manageable size, students do not need to summarize the full case study. They can limit the scope of the entry to comparing Control (C0) and any one of the remaining three Treatment Groups (T1, 2, or 3). If students are ambitious and want more practice, they can compare all of the groups.

For this first entry, our students must:

1. Write an **Introduction** section using the background information on Manduca in the lab manual, and the excerpt from the 2016 eLife article.
2. Summarize the procedures from the handout in a **Methods** section.
3. For the **Results:**

* Recreate the handout table in their notebook as an example to follow later.
* Add another table with summary statistics (see p. 2 of this handout).
* Summarize their direct observations (Follow-Up Question 1)

1. For the **Discussion**:

* Summarize their responses to Follow-Up Questions 3 and 4.