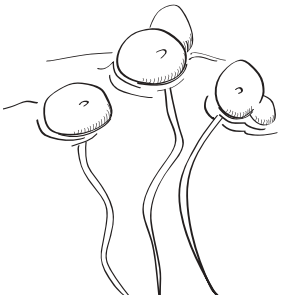
PROTOCOL 3. DOSE/RESPONSE EXPERIMENTS USING DUCKWEED

Objective

To conduct a dose/response bioassay using duckweed.



Background

A bioassay is an experiment that uses living things to test the toxicity of chemicals. One kind of bioassay is a dose/response experiment in which you expose organisms to various doses of a chemical and then measure their responses. In this protocol, duckweed is used as the bioassay organism. After placing duckweed plants in beakers containing various concentrations of a chemical, you monitor their growth and health over a five-day period.

Duckweed is a small aquatic plant that floats on the surface of ponds, wetlands, and nutrient-rich lakes. Worldwide, there are over 40 species of duckweed (family Lemnaceae), with 20 species found in the United States. *Lemna minor* is the species most commonly used for bioassays. Each *Lemna* plant consists of one or more fronds. The fronds look like little leaves but actually are a combination of leaf and stem, attached to a rootlet that dangles down in the water.

Although duckweed is a flowering plant, it rarely flowers. Usually it reproduces through budding—new fronds grow from buds on the parent plant. Eventually these new fronds grow their own roots and break off to become independent plants. When you conduct a bioassay using duckweed, you measure growth rate by counting how many new fronds develop over a five-day growth period.

In this protocol, you will carry out a dose/response experiment to test the sensitivity of duckweed to the serial dilutions you created in Protocol 1.

Materials (per student group)

Fluorescent or plant grow lights

105 duckweed plants

21 beakers or clear plastic cups

Miracle-Gro Liquid Houseplant Food Drops
or similar fertilizer solution (N:P:K = 8:7:6)

Eye dropper (for fertilizer)

Tweezers or paper clips (for handling duckweed)

Clear plastic film such as Saran Wrap

90 mL of each of the chemical solutions made in Protocol 1

90 mL spring water from source used in Protocol 1

100 mL distilled water (for rinsing)

Procedure

1. Label beakers or cups with your name, the date, and the solution concentrations listed in Table 2.1 (p. 41). Label the final three beakers "control" (three beakers per concentration).
2. Pour 30 mL solution into each of the beakers, following the labels for solution concentrations. In the control beakers, use spring water instead of a chemical solution. Add one drop of liquid fertilizer to each beaker.
3. Using tweezers or an unfolded paper clip, gently transfer five duckweed plants into each beaker. (Avoid using your fingers because that could introduce other chemicals into your culture solutions.) Choose only green, healthy-looking plants that have two fronds apiece and are approximately the same size.
4. Cover the beakers with clear plastic film, and place them under 24-hour fluorescent or plant grow lights. (Artificial lighting is optimal because it provides consistent conditions from one experiment to another. Indirect natural lighting is an acceptable alternative. Avoid placing the beakers directly in a sunny window because overheating may cause the duckweeds to get scorched.)
5. Let the beakers sit undisturbed for five days. Keep them covered with plastic, and do not add water to them during this time.
6. At the end of the five-day growth period, count the number of fronds in each beaker. It may be difficult to decide which fronds are real, and which are too small to count. The important thing is to be consistent so that your results will be comparable across treatments.
7. Record your data in Table 2.3 and make notes about any plants that are yellow, rootless, or sinking, or that otherwise appear unhealthy.
8. Using Figure 2.3, graph the mean (average) for each treatment. Then analyze your data using the guidelines below.

Analysis

Comparison to the Control

The first thing to check is your control (the beakers that contain just spring water and fertilizer solution). The purpose of the control is to identify how well the duckweed will grow under uncontaminated conditions.

You can expect the number of fronds to roughly double in the control beakers during the five-day incubation period. If your control plants did not grow much or do not look healthy, something may have gone wrong in your experiment. Perhaps the nutrient solution was too strong or too weak, or the plants were not healthy to begin with. Or maybe a problem developed with the environmental conditions. Did the solutions get too hot, too dry, or contaminated in some way?

Analysis of Trends

Looking at your graph (Figure 2.3), do you notice any trends? For example, does the toxicity of your test chemical appear to increase as the concentration increases, or does it stay the same from one concentration to the next? Are there any data that don't seem to make sense?

If so, make a note of these and try to think of any possible explanations for why they are different from your expectations.

A Look at Variability

The means for each treatment tell only part of the story. It is also useful to take a look at the individual data points (the number of fronds in each of the three beakers) to get an idea how much variability exists within each treatment. Try graphing individual data points for each treatment. The wider the spread between data points, the greater the variability within that treatment. The more variability there is within each treatment, the less confident you can be that one treatment is different from another, even if the means appear different on your bar graph (Figure 2.3).

Because of individual differences among organisms, you shouldn't expect each plant to respond in exactly the same way. However, it is reasonable to expect that the groups of individuals in each treatment will follow predictable trends. Did replicate beakers have similar numbers of duckweed fronds at the end of the five-day growth period? If your data are more variable than you think is reasonable, you could look into the potential sources of this variability. For example, did the plants appear to be healthy at the beginning of your experiment, or were they already stressed? Were the serial dilutions carefully made according to directions? Did one person do all the counting of duckweed fronds, or did two or more people share this task? Based on your experience with this bioassay protocol, what ideas do you have for reducing variability caused by measurement techniques?

Estimating the TC_{50}

The next step in your data analysis is to figure out how to answer the question:

How toxic is the solution or sample to the type of organism you tested?

In bioassays there are two ways to report results: LC_{50}, the lethal concentration that kills 50% of the test organisms, and TC_{50}, the toxic concentration that causes organisms to grow 50% as well as a control group. In duckweed bioassays, the plants don't necessarily die—they may just grow more slowly than they would in a less toxic solution. So in this case use the TC_{50} to represent the concentration at which the duckweed in the treatment grow approximately half as well as those in the control group.

Using Figure 2.3, you can estimate at what concentration the duckweed has grown roughly half as much as the plants in the control group. If none of your concentrations produce rates that are close to half those of the control, it makes sense to report the TC_{50} as a range rather than a single number. For example, you might have to say that the TC_{50} is greater than or less than all the concentrations you tested, or that it lies somewhere between two of your tested concentrations.

Drawing Conclusions about Toxicity

After you have estimated the TC_{50} for your experiment, you will be able to use this number to make a statement about the toxicity of the substance you were testing. Usually this statement will be something like:

The TC_{50} for chemical X and duckweed growth is in the range of __ to __.

If you have TC_{50} values for duckweed exposed to other chemicals, you can use these numbers to rank which chemicals are most toxic to duckweed. For example:

The TC_{50} for chemical X is a smaller number than the TC_{50} for chemical Y. This means that chemical X can affect duckweed growth at lower concentrations than chemical Y. Therefore, I conclude that chemical X is more toxic to duckweed growth than chemical Y.

It is important to remember that duckweed bioassays are not designed to help you reach conclusions about toxicity to humans because duckweed plants and humans are likely to respond very differently to chemical exposures. In order to use bioassays to predict toxicity to humans, you would need to use organisms such as laboratory rats that are known to provide a better model of human response to toxic chemicals.