Testing...testing...

Summary
Students perform an experiment to determine the feeding preferences of yellow admiral caterpillars.

Learning Objectives
Students will be able to:
- Explain why biocontrol agents are tested before release.
- Describe how biocontrol agents are tested before release.

Suggested prior lessons
What is a weed?
Cultivating weeds

Curriculum Connections
Science Levels 5 & 6

Vocabulary/concepts
Choice test, no choice test, repeated trials, control, economic threshold

Time
30-45 minutes pre-experiment discussion and set-up
30-45 minutes data collection and discussion

Materials
- copies of "What plant should I test?"
- leaves of nettle and other plants (see preparation instructions)
- moist paper towels
- small insect rearing containers (1 per caterpillar collected)
- flexible forceps or artists paintbrushes for picking up caterpillars gently
- copies of data sheets

Background information

Most weeds have a variety of natural enemies. Not all of these enemies make good biocontrol agents. A good biocontrol agent should feed only on the target weed. It should not harm crops, natives, or other desirable plants, and it must not become a pest itself. With this in mind, when scientists look for biocontrol agents, they look for "picky eaters".

Ideally, a biocontrol agent will be *monophagous*—eating only the target weed. Sometimes, however, an organism that is *oligophagous*—eating a small number of related plants—is also a good agent, particularly when the closely related plants are also weeds.

In order to test the safety of a potential biocontrol agent, scientists offer a variety of plants to the agent in the laboratory and/or in the field. They choose plants that are closely related to the target weed, as these are the most likely plants to be attacked. The non-target plants tested may be crops, native plants, ornamentals, or even other weeds. The tests are designed to answer two main questions:

1. Does the agent attack non-target plants (and under what conditions)?
2. Can the agent survive and develop on non-target plants?

The second question is important, because an agent may be able to eat a non-target plant, but if it cannot grow and develop properly on the non-target plant, its effect on the plant is likely to be very small which may still be acceptable.

Testing biocontrol agents often involves *choice tests* and *no choice tests*. In choice tests, the potential biocontrol agent is given the option of feeding on the weed or other non-target plants, to see which it prefers, given a choice. In no choice tests the biocontrol agent is given only the non-target plants, to see if the plants are at all edible to the biocontrol agent. A good biocontrol agent will not eat other desirable plants, even if it is starving. Sometimes in a no choice test, the agent is forced to eat something it would never naturally be attracted to, so tests may also be done in field settings to understand better how the agent will actually behave once released. If an agent does eat a non-target plant testing is extended to find out whether the agent can survive and reproduce on the plant.

Biocontrol agents released in New Zealand feed on serious weeds. The movement and deliberate propagation of these weeds is strictly restricted in most areas of the country, so it is impractical or impossible for students to conduct testing on these agents and their hosts. There are, however, many weeds of less importance in New Zealand, and insects that can be tested on these weeds. In this activity, students test the feeding preferences of the yellow admiral butterfly caterpillar, a native insect that regularly feeds on non-native nettles.

Activity

Please note that this lesson is best done during the spring/summer months, when nettles are abundant and yellow admiral caterpillars are readily available.

Preparation

Collect yellow admiral caterpillars from nettle plants. To avoid nettle stings, wear gloves, pick entire plants with caterpillars, and allow the plants to wilt before removing the caterpillars. Once the plant has wilted, it will not sting. Aim to collect at least 21 caterpillars. The more you can collect the better.

Collect plant material to test. Your material should include fresh European nettle (*Urtica urens*), and any of the following:

Native plants: (see plant ID sheet below)
- scrub nettle (*Urtica incisa*) (seeds available from Monarch Butterfly NZ Trust, www.monarch.org.nz; plants available from Oratia Native Plant Nursery, www.oratianatives.co.nz)
- matagouri (*Discaria toumatou*)
- NZ pellitory (*Parietaria debilis*)

* We **DO NOT** recommend using the native tree nettle (*Urtica ferox*) because of its particularly strong stings

Agricultural / horticultural plants:
- Mind-your-own-business (*Soleirolia soleirolii*) (sold as a pot plant or ground cover)
- hops (*Humulus lupulus*)
- common fig (*Ficus carica*)

• strawberries, raspberries, apple, pear, plum, peach, elm

Collect these plants as close as possible to the day you will use them in class. If you need to store them, keep them in a jar of water so they stay fresh. You may wish to collect plants with the students (see the "biocontrol agents in the field" lesson for additional activities and agents students can experience in the field), or have the students come up with a list of plants to test (see "Activity—choose plants to test" below).

Prepare a small insect rearing container for each caterpillar collected. Containers need to be large enough to accommodate two leaves of your collected plants. See the "Insect rearing containers for the classroom" sheet for ideas. You may want to have students create these containers themselves.

Introduction

Watch the video about how heather beetle was chosen and tested as a biocontrol agent for heather. Discuss how the beetle was tested, and why it seemed like it was a good biocontrol agent. Be sure to include in the discussion the idea of "choice" and "no-choice" tests for biocontrol agents. Ask students to come up with ideas about what sorts of plants scientists might have tested in the "choice" tests for heather beetle (plants closely related to heather that are native or economically important in New Zealand).

Explain to the students that they are going to be doing an experiment with yellow admiral—a native insect that eats the non-native weed nettle. The adult butterfly lays its eggs on nettle plants. Caterpillars eat the nettles and also use them for protection. Using silk, they curl a leaf into a small tube, which they sit in. They are going to perform some of the tests that scientists would carry out on a potential biocontrol agent to see if the agent would be a safe choice for release. Explain that you're not going to study actual biocontrol agents, because the weeds they eat are such a problem, you're not allowed to bring them to the classroom, even to study.

Activity—choose plants to test
Review biological classification to ensure students are comfortable with the concepts of order, family, genus, and species. Distribute "What plants should I test?" handout. Divide the students into small groups and have them read and discuss plants they could or should test if they were considering the yellow admiral as a biocontrol agent. Older students can source and collect plant material, or you can then introduce the plants you've previously collected, and explain why you chose each one for testing.

Activity—experiments
Depending on your students' level of experience with scientific inquiry, you may wish to have them decide on the experimental design. Otherwise, continue with the design below.

If you have enough caterpillars, divide the students into small groups and have each group conduct its own experiment. Set up the following for each experiment:

Place a moist (not soaking wet) paper towel on the bottom of each rearing container. For each plant you want to test, set up:

- 3 rearing containers containing 1 nettle leaf and 1 test leaf (these are your "choice" tests). Label each container "Choice (nettle + *test plant name*), Trial # (*1, 2, or 3*)"
- 3 rearing containers containing 2 leaves of the same test plant (these are your "no choice" tests). Label each container "No choice (*test plant name*), Trial # (*1, 2, or 3*)

** If leaf sizes of the test plants and nettle differ greatly in size, you may want to either cut nettle leaves smaller or add more test plant leaves, so that the amount of leaf material for each choice is the same.*

Also set up 3 rearing containers containing two nettle leaves (this is your control—it allows you to determine if a factor unrelated to food choice is affecting your caterpillars and influencing your results). Label these containers "Control (nettle), Trial # (*1, 2, or 3*).

Add a caterpillar to each rearing container. Place the caterpillar between the two leaves. Close the containers and place them in a location in the classroom where they will not be in direct sunlight.

Wait 1-2 days. Check the caterpillars and record where they are in the container, score the amount of feeding damage on the leaves, and note whether the caterpillars have made shelters on the Agent Testing data sheet. If few of the caterpillars have settled on a plant, wait another day and record your data again.

Discuss the results with the students.

- When given a choice to eat nettles or test plants, did the caterpillars eat the test plants? If so, how often? (e.g.: 1 out of 10 caterpillars ate a test plant) If they ate test plants, which ones?
- When given nothing to eat except test plants, did the caterpillars eat the test plants? If so, how often? (e.g.: 3 out of 10 caterpillars ate a test plant). Why is this test important to do? (biocontrol agents that successfully kill off the target weed might move on to harm other plants once the weed is gone). Could we accept a biocontrol agent that ate just a little of a plant we didn't want it to eat?
- Did caterpillars make shelters on the test plants? Why is this information important to understanding whether the caterpillars will harm non-target plants?
- Did any of your caterpillars die? Was there a difference in the mortality between tests? (e.g.: did more caterpillars in the no choice tests die than in the choice tests or control?) What can this information tell you about the biocontrol agent or your experiment?

- Do you think yellow admiral would be a good choice of biocontrol agent for European nettles? Why or why not?
- What further tests might you want to do before releasing yellow admirals?

Extension/discussion:

1. If the caterpillars feed on a non-target plant, test their growth and development on the plant to see if it is a good host. Use potted plants. Grow caterpillars on European nettle and on the non-target plant. Measure the time it takes them to complete development, their survival rate, their adult size, and whether adults lay eggs on the non-target plant. Evaluate whether you think the non-target plant is a good host or not.

2. In New Zealand, a new biocontrol agent may be approved for release, even if it may cause some damage to native or economically important plants if the benefits of the agent outweigh the harm it may cause. Discuss with the students how they feel about this. What social values affect these decisions? You might want to have students research, then debate the pros and cons of releasing biocontrol agents for banana passionfruit, which is closely related to commercially grown passionfruit.

3. Do the lesson, "To release or not to release", in which students role-play a variety of stakeholders in deciding whether to introduce a new biocontrol agent.

4. Have students choose a weed from the "weed biocontrol projects" (http://www.landcareresearch.co.nz/science/plants-animals-fungi/plants/weeds/biocontrol/research/projects) page on the Landcare Research website, and research the plants that would need to be tested for any biocontrol agent for their weed.

Curriculum Connections

Science—Nature of Science

Levels 5 & 6

Understanding about science

- Understand that scientists' investigations are informed by current scientific theories and aim to collect evidence that will be interpreted through processes of logical argument.

Investigating in science

- Develop and carry out more complex investigations, including using models.
- Show an increasing awareness of the complexity of working scientifically, including recognition of multiple variables.
- Begin to evaluate the suitability of the investigative methods chosen.

Communicating in science

- Use a wider range of science vocabulary, symbols, and conventions.

Participating and contributing

- Develop an understanding of socio-scientific issues by gathering relevant scientific information in order to draw evidence-based conclusions and to take action where appropriate.

Science—Living world

Level 5:

Ecology

- Investigate the interdependence of living things (including humans) in an ecosystem.

Level 6:

Ecology

- Investigate the impact of natural events and human actions on a New Zealand ecosystem.

Vocabulary/concepts

Choice test – an experiment in which a potential biocontrol agent is given a choice of two or more plants to eat, lay eggs on, or settle on. Usually, the two plants are the weed the agent is being introduced to control and a plant of economic or ecological importance.

Control – Part of a scientific experiment that involves no "treatment". A control detects and measures hidden factors that influence the results of an experiment.

Family – A unit of biological classification. A group of related genera.

Genus (plural: genera) – A unit of biological classification. A group of related species.

Monophagous – Eating only one species of plant.

No-choice test – An experiment in which a potential biocontrol agent is given only one plant to eat or lay eggs on. Generally the plant is closely related to the weed that biocontrol agents are being sought for. This test allows researchers to find out if a biocontrol agent will eat other plants once it has killed off the weed it was introduced to destroy.

Oligophagous – Eating a small number of closely related species of plant.

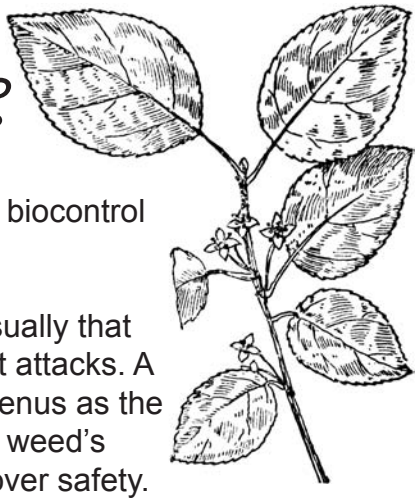
Order – A unit of biological classification. A group of related families.

Repeated trials – Doing the same test over and over. Repeated trials improve the reliability of a scientific experiment by averaging results, so unusual results of one trial don't cause researchers to come to erroneous conclusions.

Species – A unit of biological classification. A group of organisms capable of breeding and producing viable offspring.

Treatment – Part of a scientific experiment. A treatment involves the manipulation of one variable in a specific way.

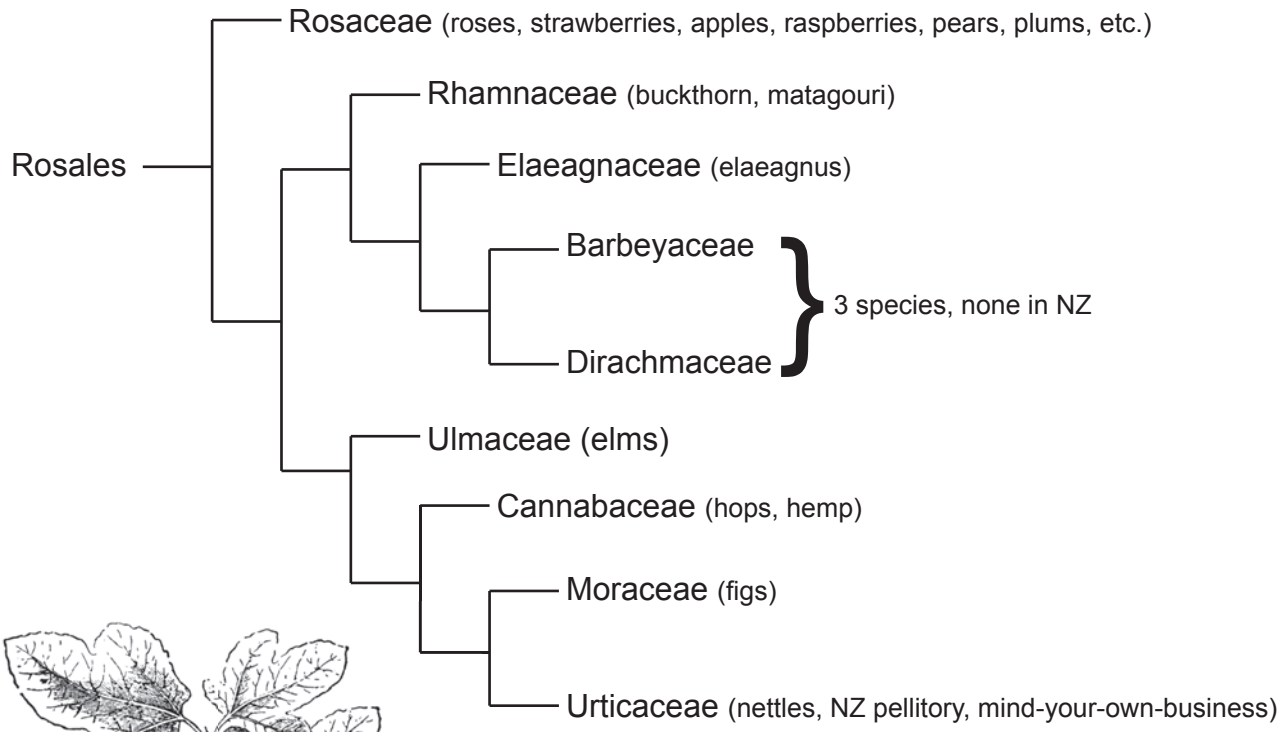
What plants should I test?



How do scientists choose which plants to test on a potential biocontrol agent?

They test the plants most likely to be eaten by the agent. Usually that means the plants most closely related to the weed the agent attacks. A biocontrol agent is most likely to attack plants in the same genus as the target weed. Scientists also often test other members of the weed's family, and more distantly related plants if there is concern over safety.

Below is a diagramme showing the relationships of plant families in the order Rosales. The closer two families are linked, the closer their genetic relationship.



Some genera of Urticaceae found in NZ

Urtica–nettles
- *Urtica urens* (European nettle)
- *Urtica incisa* (scrub nettle; NZ-native)
- *Urtica ferox* (tree nettle; NZ-native)

Parietaria–pellitory
- *Parietaria debilis* (NZ pellitory; NZ-native)
- *Parietaria judaica* (pellitory-of-the-wall)

Soleirolia
- *Soleirolia soleirolii* (mind-your-own-busness)

Landcare Research
Manaaki Whenua

Plant Identification

Native plants in the order Rosales

Scrub nettle
(*Urtica incisa*)

Photo: John Moss

Matagouri
(*Discaria toumatou*)

Photo: Sarang

New Zealand pellitory
(*Parietaria debilis*)

Photo: ©2012 P.B. Pelser; www.phytoimages.siu.edu

Landcare Research
Manaaki Whenua

Agent Testing

Record the status of each caterpillar and how much feeding damage is visible on the leaves by filling in the data table below.

Give feeding damage a score as follows:
1 = no damage, 2 = 1 or 2 small holes, 3 = many holes, more than half the leaf remaining, 4 = less than half the leaf remaining

Length of time in container: _____

Choice tests (nettle + test plant)

Test plant type	Trial #	On nettle	Feeding damage on nettle	On test plant	Feeding damage on test plant	Not on a plant	Has made a shelter	Dead
	1							
	2							
	3							
	1							
	2							
	3							
	1							
	2							
	3							

No choice tests (test plant only)

Test plant type	Trial #	On test plant	Feeding damage on test plant	Not on a plant	Has made a shelter	Dead
	1					
	2					
	3					
	1					
	2					
	3					
	1					
	2					
	3					

Control (nettle only)

Trial #	On nettle	Feeding damage on nettle	Not on a plant	Has made a shelter	Dead
1					
2					
3					

Example data chart:

Choice tests (nettle + test plant)

Test plant type	Trial #	On nettle	Feeding damage on nettle	On test plant	Feeding damage on test plant	Not on a plant	Has made a shelter	Dead
Scrub nettle	1	✓	3		0		✓	
	2		0	✓	2		✓	
	3		0	✓	3		✓	
matagouri	1	✓	3		0		✓	
	2	✓	2		0		✓	
	3	✓	3		0			
hops	1		0		0	✓		✓
	2	✓	4		0		✓	
	3	✓	3		0			