

Video Article

Testing Nicotine Tolerance in Aphids Using an Artificial Diet Experiment

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URL: <http://www.jove.com/index/Details.stp?ID=701>

DOI: 10.3791/701

Citation: Ramsey J.S., Jander G. (2008). Testing Nicotine Tolerance in Aphids Using an Artificial Diet Experiment. JoVE. 15. <http://www.jove.com/index/Details.stp?ID=701>, doi: 10.3791/701

Abstract

Plants may upregulate the production of many different secondary metabolites in response to insect feeding. One of these metabolites, nicotine, is well known to have insecticidal properties. One response of tobacco plants to herbivory, or being gnawed upon by insects, is to increase the production of this neurotoxic alkaloid. Here, we will demonstrate how to set up an experiment to address this question of whether a tobacco-adapted strain of the green peach aphid, *Myzus persicae*, can tolerate higher levels of nicotine than a strain of this insect that does not infest tobacco in the field.

Protocol

In this experiment, 4 adult aphids are enclosed in a small plastic cup. They feed from liquid diet pipetted between 2 layers of parafilm stretched over the top of the cup. After 3 days feeding, toxicity of diets containing different concentrations of nicotine is compared to control diets by counting adult and larval aphids in each cup.

1. Make solutions containing sucrose and 20 amino acids. Aphids can subsist on this diet, reproducing at normal levels, for at least 1 week. Please see attached file for the artificial diet.
2. Aliquot 1 mL of this diet into 6 different eppendorf tubes containing nicotine. Label them with respect to various concentrations of nicotine used, ranging from 0-500 μ M.
3. Select 4 aphids, either red or green, and put into these experimental cups.
4. When selecting aphids, it is important that they be of consistent age. Typically, look for aphids of similar size and color (the red aphids get darker when they are older).
5. Cover the cup with a piece of parafilm. Enclose the diet between 2 pieces of parafilm. Partially stretch the second parafilm level around the edges of the cup. Aliquot the liquid diet between the layers, and seal the top layer down.
6. Keep these cups in the insect room under lights in a long-day photoperiod for 3 days, after which time the data for the experiment can be collected.

Please visit [Annual Reviews of Plant Biology](#) for more information about this protocol and plant immunity to insect herbivory.

References