# *Part* X

# *Plant Form and Function*

**A hyperaccumulaor of toxic metal.** This plant of the mustard family, *Streptanthus polygaloides*, is unusual in that it accumulates large amounts of toxic nickel in its tissues. Does this serve to protect the plant?

## Why Do Some Plants Accumulate Toxic Levels of Metals?

All plants take up minerals from the soil. These minerals are used as cofactors in many important metabolic processes, helping enzymes carry out their catalytic duties. A few of the plant species that grow on soils that contain high levels of metals (so-called serpentine soils) are particularly interesting, as they have very high levels of metals in their tissues, even more than metal-tolerant plants do. These rare high-metal plants, termed hyperaccumulators, contain in excess of 1000 micrograms of metal per gram dry weight of tissue. Nickel (Ni) is the most accumulated metal—of 450 hyperaccumulating species of plants that have been described, 340 of them accumulate nickel.

What is going on here? Why have these hyperaccumulating plant species evolved ways to tolerate high metal levels that would kill an average plant? We do not know. Some researchers have suggested that hyperaccumulation may simply be a way to survive in a high-metal environment, with plants sequestering the metals by packaging them in vacuoles where their high concentration poses no danger to the rest of the plant cell. Other researchers have suggested that the metal taken up by hyperaccumulators makes the plants drought resistant. Still others suggest that the metal in fallen leaves and other litter may deter other plants from growing in the vicinity of a hyperaccumulating individual, lessening competition from too-near neighbors.

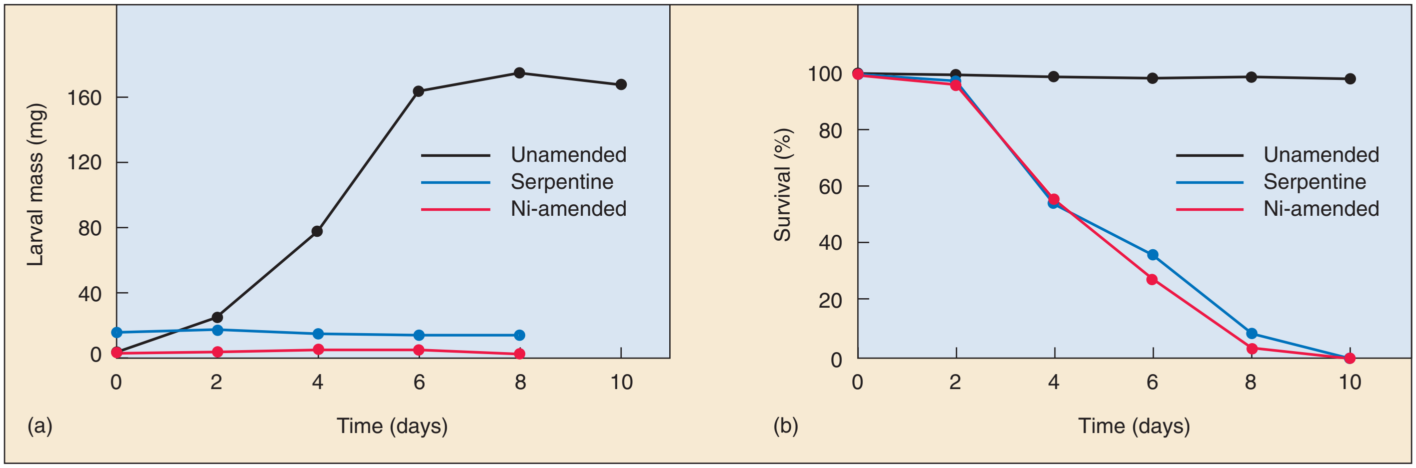
Few experiments have been carried out to test these hypotheses. There is one possibility that seems particularly attractive. Perhaps the metal in hyperaccumulator plants makes them toxic to herbivores. If that were so, it would be easy to see why natural selection would favor hyperaccumulator plants in heavy-metal soils: organisms which feed on these plants would die. Protection from herbivory thus offers a very tempting explanation for the evolution of hyperaccumulation in plants.

The hypothesis that hyperaccumulation of metals by plants deters herbivore consumption has been examined by Robert Boyd of Auburn University and Scott Martens of the University of California, Davis. In order to investigate this hypothesis clearly, the researchers needed a way to compare plants with and without high levels of nickel. First they selected a Ni-hyperaccumulating species of plant, a member of the mustard family that grows naturally only on Ni-rich serpentine soil. Then, in the laboratory, they attempted to grow the plant on artificial potting soil bearing no nickel. In parallel experiments they grew the plants on artificial potting soil to which they added nickel ("amended soil"). The plant grew just fine in either of these artificial soils, demonstrating that it had not evolved a *requirement* for nickel. Importantly, when they examined the tissue of the Ni-hyperaccumulator plant when it had been grown on Ni-free soil, the tissue still had the mustard oils and other chemicals characteristic of the species, but little nickel. Now they had what they needed for a successful experiment, a way to manipulate the level of nickel in plant tissue with nothing else being different.

One could imagine two ways that a plant might use high levels of nickel to protect against herbivores:

*1. Mortality.* Such a defense might result from insect mortality, the herbivorous insect being killed by ingesting toxic-metal-containing plant tissue. This is how the toxic elements selenium and fluorine work in defending plants that accumulate them. Selenium becomes incorporated into insect amino acids, making the proteins bearing them dysfunctional. Fluorine, incorporated into fluoroacetate, disrupts the insect's Krebs cycle. Unlike selenium and fluorine, nickel is not incorporated into an herbivore's metabolic chemicals. But its release from plant vacuoles after ingestion might directly poison many metabolic processes of a herbivore.

*2. Taste.* A hyperaccumulation defense against herbivores might also result from deterrence, the herbivorous insect finding the toxic-metal-containing tissue distasteful, and so learning to avoid eating the hyperaccumulating plant. The mustard oils characteristic of the mustard family (*Brassicaceae*)—you know them as the substances that give the pungent aromas and tastes to mustard, radish, and horseradish—signal the presence of distasteful chemicals to many groups of insects.



**Effects on *Pieris rapae* larvae of eating Ni-hyperaccumulating plants.** (*a*) Weight gain was only seen in larvae that fed on plants grown on unamended (nickel-free) potting soil and not in larvae fed on plants grown in Ni-amended soil (potting mix to which Ni had been added) or serpentine soil (which contains high levels of Ni). (*b*) Survival rates dropped dramatically in larvae that fed on plants grown in Ni-amended and serpentine soils.

## The Experiment

To test the hypothesis that the hyperaccumulation of nickel (Ni) in the tissues of certain plants acts as a defense against herbivores that attempt to use the plant as a primary food source, Boyd and Martens selected for study a plant from the mustard family, *Streptanthus polygaloides*. This small plant is a Ni-hyperaccumulating annual that grows only on serpentine soils in the foothills of the Sierra Nevada in California.

Three experimental protocols were set up: (1) greenhouse-grown plants in Ni-amended soil (i.e., a potting mix to which was added 1000 mg Ni per kg mix), (2) unamended soil (potting mix with no added Ni), and (3) plants collected from natural serpentine soil rich in Ni. In each protocol, leaves were removed from the plants and fed to one of three different insects known to feed on this family of plants. The responses of the insect herbivores to the three protocols were evaluated based on food choice, on survival rates, and on weight gain over the course of the experimental treatments.

## The Results

The larval stage of butterflies *Pieris rapae* and *Euchloe hyantis* were fed all three types of leaves, as were adult grasshoppers. All three insect herbivores fed in this way exhibited a disruption of growth, as determined by the measurement of body mass over the course of the experiments. The data for *Pieris rapae*, shown in graph *a* above, are representative. Weight gain was only seen in larvae and insects feeding on the unamended plants. Those herbivores fed Ni-amended or serpentine plants either maintained or lost weight.

*Confirming the Mortality Hypothesis.* Survival rates dropped dramatically in *Pieris rapae* larvae and grasshoppers feeding on Ni-amended or serpentine plants compared to those feeding on unamended plants (a parasitoid infection disrupted the experimental results of the *Euchloe hyantis* larvae). Again, the data for *Pieris rapae*, shown above in graph *b*, are representative.

To verify that Ni toxicity was sufficient to account for the data presented above, *P. rapae* was fed a synthetic diet containing various concentrations of Ni. Survival rates were not significantly affected with concentrations less than 500 ppm but dropped dramatically at Ni concentrations of 1000 ppm. In addition to determining the Ni toxicity in the insects, Martens and Boyd also examined the effects of Ni concentrations on plant fitness. After growing for 21 days, Ni-amended plants exhibited greater survival rates and weight gain than unamended plants which were almost completely defoliated by the larvae.

*Eliminating the Taste Hypothesis.* Leaves of all three plant preparations were consumed by the larvae and insects and all three species were observed eating the leaves regardless of Ni content. Based on this, Martens and Boyd could conclude that the results above were due to the Ni content in the plants and not due to feeding deterrence.

The researchers showed that the hyperaccumulation of Ni in plants can clearly serve as a defense mechanism in plants which can increase plant fitness. As with many defense mechanisms, some herbivores may have acquired counteradaptations that would allow them to continue feeding on hyperaccumulators. In order to become Ni tolerant, an organism would need to excrete or compartmentalize the Ni in its own system. An organism might also dilute the Ni concentration by consuming a mixed diet. Organisms may adapt to a diet of high Ni concentrations in other ways, one of many areas for further study.