

Getting to the root of protein production

As scientists identify greater numbers of natural proteins that have medical and industrial use, they continue to search for efficient ways to produce the molecules in bulk. Investigators have induced bacteria to churn out extra proteins, cloned animals that secrete novel proteins in their milk, and engineered plants to deposit proteins of interest in leaves or seeds.

Now, a research team has made tomato plants that secrete desired proteins from their roots. When such plants are grown hydroponically—in a nutrient-containing solution rather than in soil—researchers can collect the protein of interest from the liquid with relative ease.

"This may really change the economics and feasibility of protein production in plants by allowing you to 'milk' them in a way similar to milking a transgenic sheep or goat," says Ilya Raskin of Rutgers University in New Brunswick, N.J. He and his colleagues describe their new protein production technique in the May *NATURE BIOTECHNOLOGY*.

While roots are best known for drawing nutrients from the soil, Raskin notes that they also secrete various proteins, primarily to protect against bacteria and other disease agents. As scientists unraveled this secretion pathway, Raskin and his colleagues began to wonder if they could exploit it.

The investigators do so by changing the gene for any protein of interest. One alteration eliminates parts of the protein that signal a cell to retain the molecule. A second change adds a few amino acids

that tell the cell to secrete the protein. The researchers also place the modified gene under the control of a DNA sequence that makes the gene active only in root cells. Finally, they insert the tailored gene into a tomato plant's genome.

Raskin's group made such modifications on three genes and induced tomato plant roots to secrete significant amounts of each gene's protein. "We haven't spent any time optimizing the system, and we're already getting respectable rates of production," says Raskin.

Tests on the proteins, none of which is of commercial interest, showed that the

process didn't ruin their activity. The researchers are now introducing a gene into tomato plants to make their roots secrete a viral protein used in hepatitis B vaccines.

Collecting valuable proteins with this new technique wouldn't involve the harvesting of plants and the laborious, costly task of purifying the proteins from them, notes Robert L. Erwin of Biosource Technologies, a firm in Vacaville, Calif., that makes tobacco plants produce proteins in their leaves (*SN*: 1/30/99, p. 69). "You can harvest the protein [from the hydroponic solution] on a continual-flow basis. It would reduce what a lot of people have considered some of the disadvantages of transgenic plant protein production," he says. —J. Travis

Plants signal stress with a toluene burst

Toluene, a volatile organic compound often used as an indicator of human-made pollution, also emanates from plants, a new study finds. These emissions could confuse efforts to trace pollution from automobiles, factories, and other nonbiological sources.

Scientists at the Research Center Jülich in Germany found that sunflowers and Scotch pine trees give off small amounts of toluene under normal conditions and expel larger bursts under stress. Juergen Wildt and his colleagues report their surprise findings in the May 1 *GEOPHYSICAL RESEARCH LETTERS*.

Volatile organic compounds (VOCs) in the atmosphere react with other chemicals to form smog. Globally, plants emit about 90 percent of the VOC concentration in the air, says Alex Guenther of the National Center for Atmospheric Research in Boulder, Colo. In urban areas, however, human-made sources predominate.

"Emission of other VOCs from [botanical] plants is so large, having another one wouldn't be a big deal," says Guenther. However, the more interesting impact would be an undermining of measurements that use toluene as a tracer for air pollution generated by people.

"If toluene indeed turns out to be a significant emission from plants under stress," says Joost A. de Gouw of the University of Utrecht in the Netherlands, it will "have some implications for the interpretation of measurements of aromatic compounds in the atmosphere."

The Jülich researchers monitored toluene emission from a sunflower by enclosing the plant in a plastic bag and periodically taking samples of the gases inside. The scientists found that an empty bag didn't show measurable toluene, but the sunflower gave off the compound at a rate equal to a few percent of its emission of alpha-pinene, the VOC that gives pine needles their scent.

Putting stress on the plant turned up its toluene production. When the re-



When under stress, sunflowers and other plants emit toluene, a volatile organic compound previously thought to come only from nonbiological sources.

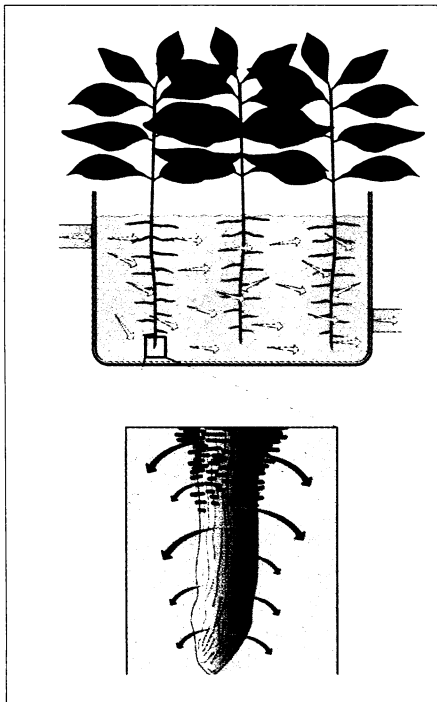
searchers deprived the sunflower of nutrients, it released more VOCs overall and doubled its discharge of toluene. When the researchers cut off a leaf, the injured plant responded with a VOC surge, in which toluene emission increased to 10 times its original rate.

In tests on Scotch pines, toluene emissions provided an early sign that the plants had been attacked by some unknown pathogen. "The plants were not wounded, did not suffer from insect attack, and were not exposed to high ozone concentrations," says Jülich's Michael Komenda. Yet their emissions of VOCs, including toluene, increased to 10 times the amount emitted by a healthy pine. After a few days, the green needles turned yellow from the mysterious ailment.

No one understands how the plants synthesize toluene, but the process is likely to be some kind of defense mechanism, says Guenther. VOCs are responsible for the familiar smells of certain plants, such as the scent of fresh-cut grass (*SN*: 4/3/99, p. 223).

The idea of toluene-emitting plants remains "slightly controversial," says de Gouw, but "the experiments seem to have been done carefully." Guenther agrees. Only further study, they say, can show whether plant toluene has an impact on atmospheric chemistry. —C. Wu

Bob Ciriimi/NATURE BIOTECHNOLOGY



The roots of tomato plants secrete designated proteins into a solution from which the molecules can be easily collected.