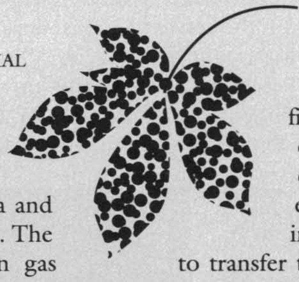
Nitrogen-Fixing Bacteria Find New Partners

Researchers have induced nitrogen-fixing rhizobial bacteria to form root nodules on nonlegumes, including rice



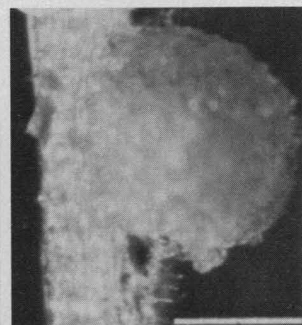
THE NITROGEN-FIXING RHIZOBIAL bacteria are a choosy lot. With few exceptions, they form nodules only in the roots of leguminous plants—soybeans, for example, and alfalfa and acacia. And the legumes thrive. The bacteria take useless nitrogen gas from the air and convert it to ammonia and other compounds needed for plant nourishment. In effect, the bacteria provide their host legumes with built-in nitrogen fertilizer factories.

So plant researchers would like to entice the persnickety rhizobia to infect nonleguminous plants and fix nitrogen for them, too. Now, within the past year or two, groups in the United Kingdom, China, and Australia have come a step closer to achieving this long-sought goal. They have induced the bacteria to form nodules in the roots of important crop plants, including rice, wheat, and oilseed rape, a plant that produces high-quality industrial oils, as well as canola oil, a current favorite of health-food aficionados because of its cholesterol-lowering properties.

"Demonstration of nodulation in non-legumes is very exciting," says Robert Haselkorn, a nitrogen fixation expert who works at the University of Chicago. He notes, however, that there is still a caveat. None of the groups has yet demonstrated that the bacteria in the nodules produce useful amounts of fixed nitrogen. "The real trick is to show that such nodules can feed the plant," Haselkorn remarks. If that can be done, it might be possible to get better yields from the crops with fewer applications of expensive, artificial nitrogen fertilizers, thereby reducing both the farmer's costs and the pollution caused by the runoff of the applied fertilizers.

That's why giving the major crop plants their own nitrogen-fixing capability has been the "Holy Grail" of researchers since they began working out the molecular basis of nitrogen fixation back in the 1970s. The original efforts attempted to do this genetically. During the early to mid-1980s, researchers in a number of labs attempted to transfer the set of about 20 genes responsible for nitrogen fixation from rhizobia and other nitrogen-fixing bacteria directly into plants. But transferring the needed packet of genes into novel species proved to be relatively easy compared to establishing a physiological environment in a plant that would allow these genes to function.

One problem the researchers encountered was the requirement that nitrogenase, a key enzyme in the nitrogen-fixing process, be kept free of oxygen, which rapidly inactivates it. The nodules normally provide an anaerobic environment that protects nitrogenase, but plant cells do not. Moreover, nitrogen fixation consumes vast amounts of energy, which most nonlegumes can't provide. Because these barriers to nitrogen fixation proved difficult, if not impossible, to overcome, most efforts to transfer the genes into nonlegumes have been abandoned and researchers have turned to other ways of achieving their goal.



Novel nodules. *Rhizobial bacteria can be induced to form nodules (shown in two views) on the roots of the oilseed rape plant (far left). Still unclear is whether they will fix meaningful amounts of nitrogen.*

Take Edward Cocking, whose group at the University of Nottingham, England, is at the forefront of current efforts to get rhizobia to nodulate nonleguminous plants. Unlike many of his colleagues, Cocking bases his research strategy on the assumption that nonlegumes would be genetically capable of supporting a symbiotic partnership with rhizobia if the bacteria could infect the plant roots and form nodules. He thinks that doesn't happen, however, because some surface property of the root tips prevents the bacterial entry.

Cocking says he originally got the idea that it might be possible to get nodulation of nonlegumes after unearthing an item in the botanical literature that showed that *Parasponia*, a semi-tropical shrub in Australia, has nitrogen-fixing nodules. This finding, made about 20 years ago, was then only of academic interest. But for those researchers who were aware of it, this apparently abstruse bit of knowledge overturned the then current view that nodulation was unique to the legumes, and convinced many, Cocking among them, that the quest to develop nitrogen-fixing nonlegumes was not hopeless.

Another factor that has influenced Cocking's research strategy was the evidence that has accumulated over the past 12 to 15 years showing that an interaction between the bacteria and the root tip surface is needed for initiating rhizobial infections. (Also see paper on p. 948 for a further discussion of what it takes to establish symbioses between legumes and nitrogen-fixing bacteria.) That spurred him to think that the barrier to nodulation of nonlegumes is at the root tip surface—an assumption that seems to have been borne out by his current results.

So far, Cocking and his colleagues have been able to achieve nodulation of both rice and oilseed rape with their new method.

They first treat the roots of seedling plants with an enzyme that breaks down the cellulose walls surrounding the root cells. Then the researchers expose the roots to rhizobia in the presence of polyethylene glycol, a compound that accelerates bacterial uptake by dissolving holes in the root cell membranes. The result: nodules that look very much like the nodules of legumes.

Cocking's method suffers from a disadvantage in that the seedlings require special handling. Since the plants are not genetically altered, the seedlings would have to be treated every year before planting. Although this is not necessarily a fatal handicap, translating the lab success into practice would certainly present a challenge to biologists and agricultural engineers. The work nonetheless demonstrates that rhizobia can nodulate nonlegumes, Cocking says.

Meanwhile, other researchers have been pursuing additional ways of obtaining nodulation of nonlegumes that would not require such unusual root treatments. For example, a group headed by Yuxiang Jing at the Institute of Botany in Beijing has developed a bacterial strain that will nodulate rice by exposing ***Rhizobium sesbania***, which normally infects an Asian bush called ***Sesbania cannabina***, to a mutagenic chemi-

The Name of the Rose, or Hunting for a Plant Database

Plant taxonomists from around the world are gathering at the Royal Botanic Gardens at Kew, just outside London, this week to examine two entities. In doing so they face the classic taxonomist's question: are the entities similar enough to lump as one? Or should they remain split and distinct? But the entities aren't species or families of flora; they are competing proposals for putting all the world's higher plants into a computer data base.

Although a digitized botanical "library" might seem a luxury—or a computer programmer's toy—it actually is a much needed tool in many areas of research. Improved species of plants are the key to many of the world's problems with fuel, food, and medicines. The pharmacologists, genetic engineers, and agronomists pursuing such challenges need to find out quickly what is known about a given plant. But there is no one source they can turn to, and different databases often conflict—especially in the matter of names.

The competing answers to this botanical Babel would provide a single, authoritative database, but each takes a different route to that goal. The Species Plantarum Project (SPP), brainchild of Dick Brummitt, a plant taxonomist in the Herbarium at Kew, would summarize all botanical data on every known plant. Spearheaded by Kew, the Missouri Botanical Garden, and five other institutions, SPP would offer the works: a taxonomic synopsis of all ferns and flowering plants, with names and biological relationships verified according to the latest scholarship.

The Global Plant Species Information System (GPSIS), which was discussed in detail at a conference held last month in the Greek town of Delphi, is less ambitious. GPSIS would be content with a "quick and dirty" checklist of acceptable plant names. But even that isn't a simple matter. The 21-volume *Index Kewensis* (started a century ago by Charles Darwin) lists a million names for flowering plants. Since botanists agree that there are only 250,000 plant species, a list that eliminates overlapping names would enable database users to communicate without confusion—especially internationally. Hence the attractiveness of GPSIS.

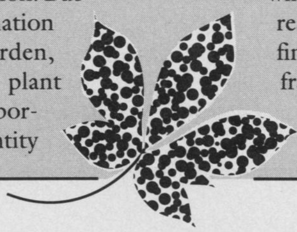
Indeed, Frank Bisby, a senior lecturer in biology at Southampton University in England and an organizer of the recent GPSIS meeting, stresses that users don't worry about taxonomy. "Ninety percent of users couldn't care less what system we use, so long as we all use the same one." He says they simply want a list of names—without confusion. But Nancy Morin, head of botanical information management at Missouri Botanical Garden, thinks just the opposite: "If you're a plant breeder, or an ecologist, the name isn't important. What's important is the biological entity you're dealing with. You want good biological information."

Until recently, this kind of point-counterpoint has characterized conversations between the proponents of each system. But after attending the Delphi meeting organized by his opponents, Brummitt, father of the ambitious SPP, seems to be approaching a compromise position: he has come around to the view that a checklist does have value—if only as a first stage. Still, he warns that if a checklist is to be prepared in two or three years, as GPSIS intends, "it can only be uncritical" and fail to incorporate recent taxonomic results. As a taxonomist, that worries him. "Once you publish a list, people place credence in it," Brummitt told *Science*. "I'm not against a list, I just want people to be aware of the limitations."

While the people who would construct the database struggle with these niceties, it does seem users want a list—and quickly. Chris Leon, an ecologist who used to run the Threatened Plants database of the World Conservation Monitoring Centre in Cambridge, spoke for many users of botanical databases when she told *Science*: "I just want a consensus.... There is no right or wrong in botany. It doesn't bother the users at all. We just want a set of names that will be fixed for 10 or 15 years." The lack of a list, explains Leon, who is currently working on a database of poisonous plants for Kew, "is holding up international conservation and it must be holding up other disciplines such as forestry and pharmacology too." An agreed upon checklist, which could be revised every 10 or 15 years, would form the backbone every database needs. To it could be appended additional information, for example on economic uses, or phytochemistry, or geographic distribution.

This logic has persuaded Rusty Russell, a collections manager at the Smithsonian Institution and coorganizer of the Delphi meeting: "SPP is a taxonomist's view and GPSIS is a plant-name user's view—but they're coming together." Direct evidence of that comes from Grenville Lucas, Keeper of the Herbarium at Kew. Anticipating this week's meeting, he told *Science* that SPP "will draw up a checklist of approved names." Indeed, most observers say that an atmosphere of cooperation is likely to prevail at the Kew conference, which is a considerable change from the situation that prevailed only a month ago. Brummitt has even come to say that SPP and GPSIS are "essentially the same project."

In the great plant database debate it looks as though lumpers will prevail over splitters. But both groups have some serious real world questions to face: there is, at the moment, no financing, no management structure, and no institutional framework for any plant database. Participants in the Kew meeting say those topics will be high on the agenda.



■ JEREMY CHERFAS

cal. The rice root nodules resemble those found on *Sesbania* and contain a small amount of nitrogenase, Jing says. Robert Burris, a veteran nitrogen fixation expert at the University of Wisconsin, Madison, is sufficiently intrigued by the result that he has accepted Jing's invitation to trek off to Beijing for a laboratory visit.

Barry Rolfe of the Australian National Laboratory in Canberra also has evidence that mutated rhizobia produce small nodules on rice. And at last September's meeting of the International Symposium on Nitrogen Fixation in Non-Legumes, held in Florence, Italy, Y. T. Chan of the University of Sydney reported that his group obtained nodule-like structures on wheat roots.

Yet, despite the promising results with nodulation in nonlegumes, no one has yet documented actual nitrogen fixation in these plants. Nitrogen fixation experts know that even with the presence of healthy nodules, there is plenty of opportunity for the system to go awry. The presence of air spaces in the new nodules may poison the oxygen-sensitive nitrogenase or the nodulated plant may not have the energy to fix nitrogen in a meaningful way.

For now, researchers working with nodulating nonlegumes have only demonstrated nitrogenase activity by measuring acetylene reduction, at best an indirect measure of nitrogen fixation. "The most sensitive way to assay for nitrogen fixation is to use the rare but stable isotope nitrogen-15, put it over the test material and then look for ^{15}N in proteins," Burris says. "This is the convincing test." He adds that, given the recent surge of interest in nodulation by rhizobia in nonlegumes, this key experiment should be attempted within the next 6 months.

Cocking, for one, agrees. His group now plans to try to improve the efficiency and frequency of nodule production. The nodules on the oilseed rape and rice plants are still pretty sparse by legume standards. But if all goes well, the researchers will, within the next year or two, do the more definitive assessment of nitrogen fixation, using the ^{15}N method. Then they will have a better idea about whether they are closer to the elusive goal of devising a general scheme for the design of self-fertilizing nonleguminous plants. ■ ANNE SIMON MOFFAT

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ADDITIONAL READING

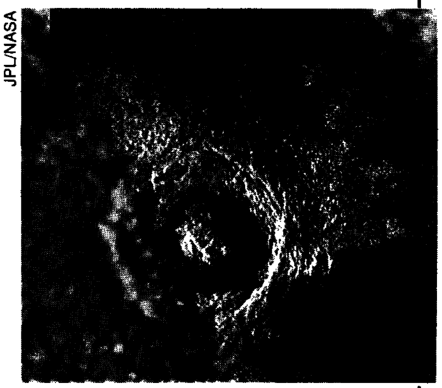
M. K. Al-Mallah, M. R. Davey, and E. C. Cocking, "Formation of nodular structures on rice seedlings by rhizobia," *J. Exptl. Botany* **40**, 473 (1989).

M. K. Al-Mallah, M. R. Davey, and E. C. Cocking, "Nodulation of oilseed rape (*Brassica napus*) by rhizobia," *ibid.*, in press in the December issue.

Venus Is Looking Too Pristine

The planetary geologists who are studying the radar images streaming back from Magellan find that they have an enigma on their hands. When they read the geologic clock that tells them how old the Venusian surface is, they find a planet on the brink of adolescence. But when they look at the surface itself, they see a newborn babe.

As the spacecraft's radar revealed one narrow strip of the Venusian surface after another during the first 2 months of its mission, Magellan scientists have been struck by the newly minted appearance of the craters formed by the impact of small asteroids and comets. Only one of the 75 craters identified on the first 5% of the planet mapped shows any of the typical signs of aging, such as filling up with the lava of volcanic eruptions or being torn by the faulting of tectonic disruption.



JPL/NASA

Venusian face-lift? *Magellan's sharper image (right) reveals the enigmatic freshness of this 34-kilometer impact crater.*

But by geologists' usual measure, these fresh-looking craters have had plenty of time to fall prey to the ravages of geologic change. Planetary scientists use the steady drizzle of asteroids and comets falling on a planet's surface to mark geologic time. Given some idea of the rate at which those impacts occur, a count of the number of craters on a given surface tells how long it has been exposed. According to this crater-count clock, the Venusian surface appears to be anywhere from several hundred million to a billion years old.

"We have been waiting for craters caught in the act of degradation by volcanism or such," says Magellan team member Sean Solomon of the Massachusetts Institute of Technology. "All of us are surprised we don't see a spectrum of states of preservation."

At the Division of Planetary Sciences meeting held last month in Charlottesville, Virginia, Magellan scientists strove to explain the paradox of young-looking craters on a relatively old surface. They raised the possibility that several hundred million years ago, a planet-wide volcanic outpouring wiped the slate clean, drowning any existing craters in a flood of lava. Then the flood would have had to turn off fairly abruptly so that the craters formed by subsequent impacts would remain pristine.

But such a global episode of volcanism generates another mystery. How could Venusian volcanic activity ebb so abruptly? Planetary physicists David Stevenson and Seth Bittker of the California Institute of Technology may have hinted at an answer when they recently speculated that just such a process may have taken place on Mars. Stevenson and Bittker said that the sources of Mars's lava might have become clogged with the residue of the melting that produced the lava in the first place—in essence, a self-sealing mechanism.

Other explanations for the uniformly youthful appearance of Venus's craters have been proposed, but researchers find them even less attractive than the perhaps far-fetched idea of volcanic episodicity. Could the planet have cooled so much that its volcanoes have turned off for good? Given its similarities to the still active Earth, this seems unlikely, Solomon says. Could Venus sport a version of plate tectonics? Then the planet would be continually turning out fresh crust whose craters would not be destroyed until the crust itself is consumed by sinking back into the mantle. Solomon, and most others, has yet to be convinced that such plate recycling is pervasive enough on Venus to explain the enigma (*Science*, 17 August, p. 742).

As Magellan continues to map Venus, team members await a resolution. Either many more degraded craters will begin to show up as Magellan works its way around the planet, and the enigma will evaporate, or it will be confirmed and, perhaps, enough clues accumulated to resolve it. That is, assuming the spacecraft is able to map the entire planet by next April, as scheduled. Magellan's early problems with its attitude control system (*Science*, 5 October, p. 27) appear to have disappeared as mysteriously as they began. And that's fine, Magellan team members say. They would much rather puzzle over the spacecraft's pictures than its machinery.

■ RICHARD A. KERR