news and views

New nitrogen-fixing symbioses *in vitro*

from John Postgate

THE truism that rhizobia, the symbiotic nitrogen-fixing bacteria, never fix nitrogen away from a leguminous host plant may seem, to an outsider, more of a dogma than an experimentally based fact. But a convention of scientific publication is that one rarely publishes purely negative findings, so the absence of documentation in the scientific literature of this failure does not do justice to the effort which has been put into the question: the truth is that many workers throughout the world have attempted, without success, to obtain fixation by pure cultures of rhizobium *in vitro* using all sorts of plausible conditions, such as nitrogen starvation at low oxygen tensions, presence of plant extracts, glutamine mutants and so on. A report in 1971 (*Nature*, **232**, 173) in which research workers from the laboratories of Dupont de Nemours obtained nitrogen fixation in a tissue culture of callus from a soyabean root, infected with *Rhizobium japonicum*, was greeted with excitement as a step in the direction of simplifying this nitrogen-fixing association. Fixation, though slight, was apparently taking place without the nodulation, leghaemoglobin and bacteroid formation seemingly necessary in the intact plant.

Though confirmatory reports gradually appeared from other laboratories, some scientists remained sceptical. Ethylene formation from acetylene, a diagnostic reaction for the nitrogen fixation enzymes, was the criterion used in these experiments; but ethylene is a normal product of plant metabolism. Were the controls for endogenous ethylene as reliable as they looked? In addition, persons working with such material know well that it is very difficult to rid callus cultures of bacteria completely and the contaminants which persist are often capable of nitrogen fixation alone. They also knew that it is difficult (because of the gummy nature of the colonies) to be wholly certain that cultures of rhizobium are pure. Finally, the critics knew that aerobic organisms—bacteria or plant cells—could scavenge oxygen from a microenvironment and enable anaerobes such as *Clostridium pasteurianum* to grow and fix nitrogen. Were the microbiological controls of the early experiments adequate? Certainly they were cursory as described in the earliest publications and sometimes even absent.

Considerations of this kind account for the admirable preoccupation with cultural purity noticeable in two important developments, reported simultaneously from Canada and Australia in this issue (pages 350 and 351). Both papers present evidence that rhizobia, at least of the slow-growing 'cowpea' type, can form associations *in vitro*, not only with callus from the leguminous plants with which they associate in nature, but also with callus from plants which do not normally nodulate and even with callus from quite unrelated plant groups.

Like the Dupont group, all the workers obtained their rhizobia from Dr Burton of Milwaukee. Child not only checked for contaminants carefully but used three different strains of rhizobium and callus from six different types of plant, thus diminishing the possibility that he is handling artifacts of contamination; Scowcroft and Gibson have also checked their material microbiologically and, in addition, with isotopic $^{15}N_2$, thus completely disposing of any possibility of errors due to endogenous ethylene production by the plant material. So, accepting that their meticulous negative tests for contaminating nitrogen-fixing microbes were adequate, the reality of effective nitrogen-fixing associations between slow-growing rhizobia and plants as remote from legumes as tobacco or the monocotyledenous wheat seems established, at least *in vitro*. It is entirely consistent with these findings, as both papers acknowledge, that Trinic recently discovered a natural nitrogen-fixing association between a slow-growing rhizobium and a non-leguminous plant of the genus *Trema* (*Nature*, **244**, 459; 1971). But the new experiments go further than this: their design is such that the plant callus is grown on a membrane, and this lies on an agar surface which has been infected with rhizobia. By removing the membrane the plant material can be removed from at least a proportion of its associated rhizobia. Yet the rhizobia remaining after the plant material had been removed continued to fix nitrogen for several hours. For the first time, therefore, rhizobia have been induced to fix nitrogen away from the plant, though the ability does not persist on subculture.

Physiological studies made it clear several years ago that rhizobia are the sites of nitrogen fixation in the nodule. Whether or not they possess the genetic information necessary to form the enzyme system is still not certain, but it is clear that, to use their information, they need something from the plant. The new experiments suggest that the material needed is diffusible and that its effect lasts for several hours. In the short term, biochemists, plant physiologists and geneticists will await eagerly the characterisation of the diffusible factor and the understanding of its apparent role in the regulation of the nitrogen fixation genes of rhizobium. In the longer term, these studies are a useful step towards the establishment of nitrogen-fixing associations between rhizobia and all sorts of agriculturally important crops and forage plants.

Elm bark disease in Australia

from Allan Rosel and John French

THE smaller European elm bark beetle (*Scolytus multistriatus*) was discovered in 1974 to be attacking elms in and up to 123 miles from Melbourne. This beetle is one of the main carriers of the Dutch elm disease fungus (*Ceratocystis ulmi*). It has not previously been recorded in Australia although the number of emergence holes in the bark of infested elms suggests that the beetle has been established in Victoria for several years. The beetle has been sought but as yet not found elsewhere in Australia. A survey is continuing.

Microbial examination of beetles and attacked trees has failed to reveal the presence of the Dutch elm disease fungus. Dr S. L. Wood of Brigham Young University, Utah believes that the disease, if present, would have been apparent by now. It seems that in Australia, as on the west coast of North America, the carrier is present but not the disease.