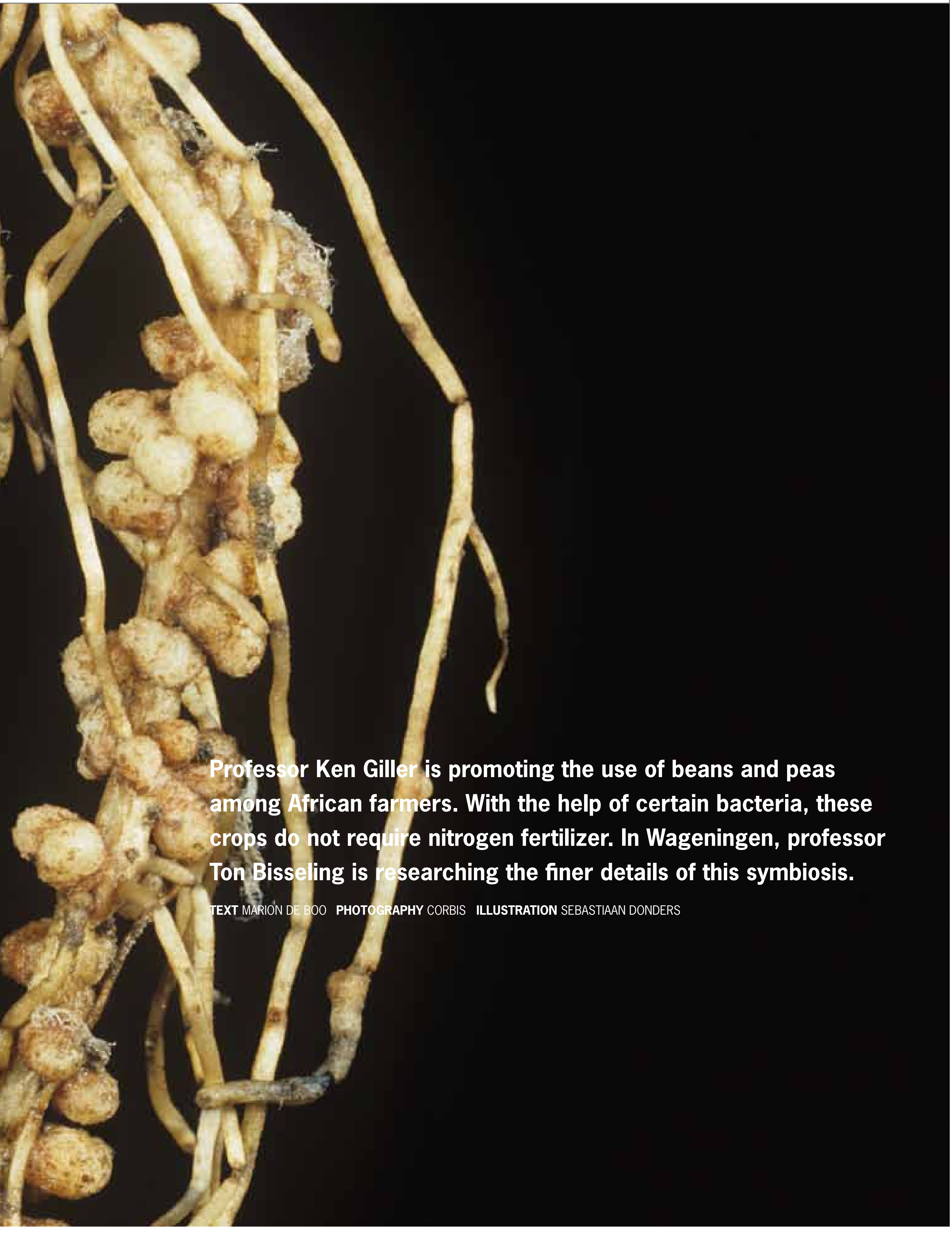
The promise of the nitrogen-fixers



Professor Ken Giller is promoting the use of beans and peas among African farmers. With the help of certain bacteria, these crops do not require nitrogen fertilizer. In Wageningen, professor Ton Bisseling is researching the finer details of this symbiosis.

TEXT MARION DE BOO **PHOTOGRAPHY** CORBIS **ILLUSTRATION** SEBASTIAAN DONDERS

It is as hot and humid as a tropical rainforest in this Wageningen greenhouse. The plants are being sprayed with a fine mist of water droplets and the thermostat is at 20 degrees. On a table stand pots of bright green saplings with stringy branches, ugly flowers and green, unripe seeds. They are exemplars of *Parasponia andersonii*, from the tropical rainforests of Papua New Guinea.
Parasponia is a true pioneer plant in the rainforest. It is the first plant to appear when a tract of forest has been razed to the ground, and it wastes no time, growing three to four metres per year. Its secret weapon –discovered in 1973 – are its root nodules inhabited by nitrogen-fixing Rhizobium bacteria. Their presence enables the tree to extract nitrogen, an essential nutrient, from the air: the same trick as is performed by leguminous plants such as beans, peas, soya and clover. 'Parasponia is the only non-leguminous plant that knows how to fix nitrogen', says professor of Development Biology Ton Bisseling of the Laboratory for Molecular Biology at Wageningen University, part of Wageningen UR. 'This extraordinary characteristic seems to have appeared in the plant kingdom twice in the course of evolution.' Just before Christmas, Bisseling managed to obtain a research subsidy of 2.5 million euros from the European Research Council in order to explore the deeper secrets of biological nitrogen fixing.
The Wageningen researchers are going to compare Parasponia's genes with those of a related species, *Trema tomentosa*. Trema bears a remarkable resemblance to Parasponia but once you uproot the tree, you see that it has to make do without root nodules and without nitrogen-fixing bacteria. So where exactly does the difference lie? Why can Parasponia fix its own nitrogen, while its relative Trema cannot? An answer to this question could eventually contribute to a sustainable world food supply, with crops that are able to meet their own nitrogen requirements without the help of expensive, energy-guzzling nitrogen fertilizer.


PHOTO GUY ACKERMANS

TON BISSELING,
Professor of Development Biology, Laboratory of Molecular Biology Wageningen University

'If we can optimize the symbiosis there is more chance of successfully creating green belts in the Sahel'

ESSENTIAL BUILDING BLOCK

Plans and other living organisms cannot grow without nitrogen, an essential building block in all kinds of molecules, including the amino acids that make up the proteins in plants and animals. Our atmosphere is made up of 79 percent nitrogen, but most organisms are unable to make use of that free nitrogen gas. The atoms in one molecule of nitrogen gas (N2) are very strongly connected with a triple bond. Only certain species of bacteria possess special enzymes with which they can convert that gas into ammonia (NH_4^+) and then into other nitrogen compounds that the plant can use. For roughly the last 100 years, we have known how to make nitrogen fertilizer out of free nitrogen. 'But it takes an awful lot of energy to do this. It is done under high pressure at a high temperature', says Bisseling. 'At least 30 percent of the energy costs in agriculture come from the production of nitrogen fertilizer.'
An alternative to nitrogen fertilizer is to grow leguminous crops, which are self-sufficient thanks to their root nodules. The nitrogen-fixing bacteria that lodge there provide the plant with the nitrogen compounds it needs in exchange for sugars manufactured by the plant. Because leguminous plants are so well supplied with nitrogen through this symbiosis, their seeds are exceptionally rich in protein.
Worldwide, there are more than 19,000 species of leguminous plants in a wide range of habitats, from the savannah to the tropical rainforest. Among the first crops that humans began to cultivate for their protein about 12,000 years ago were soya (in China), lentils (in the Middle East) and beans (in South America). The Romans knew that ploughing in the remains of legumes improved soil fertility. If you grew peas first and then wheat, you got a better wheat harvest.
In the course of evolution, genetic changes took place that made nitrogen fixing possible. 'Our first aim as researchers is to learn to understand that', says molecular biologist René Geurts, who will be leading the Parasponia-Trema research project in Bisseling's group. 'Once you have figured

‘We want to teach this tree to bind nitrogen itself’

out that mechanism, you might be able to transfer it to other plant species’, says Geurts. ‘As early as 1917, the idea was raised of providing non-nitrogen-fixing crops such as potatoes or wheat with root nodules full of nitrogen-fixing bacteria. In practice the development of genetically modified food crops is fraught with complications. Applications in forestry may be feasible sooner, perhaps in the form of new poplar species that generate their own nitrogen.’

COMPARING GENES

In the legume family, the characteristic of fixing nitrogen probably developed well over 50 million years ago, perhaps several times. In Parasponia it happened much more recently, perhaps 10 to 15 million years ago. According to Geurts, there are about 15 species of Trema, found in a wide range of habitats. Parasponia, by contrast, only grows on mountain slopes and volcanic ash in south-east Asia. ‘I think Parasponia is really a mutated Trema. The genetic mechanism may not be too complicated, and may consist of just a couple of changes. It will be very interesting to compare the genetic make-up of Parasponia with that of the legumes. We hope doing that will enable us to home in on what is useful and get to the heart of the symbiosis. Research on a tropical tree of this kind is not all that easy, but we have tissue cultures of Parasponia here in the laboratory, and we are doing tests on those. We hope to teach Trema to fix nitrogen itself within five years.’

Bisseling has his eye on another interesting nitrogen-fixing plant besides Parasponia. During an excursion in the Saudi Arabian desert for the University of Riyadh, >

PHOTOS ANP AND CORBIS

Planting nitrogen-fixing crops in Senegal. A farmer examines the plant’s nitrogen-fixing root nodules, to which Rhizobium bacteria have been added.

> **‘We look for the most promising crop for each region’**

where he is part-time professor, he discovered a fascinating leguminous pioneer plant called *Indigofera argentea*, which manages to survive in the burning hot, barren sand. This plant could well provide a key to making dry desert soils more suitable for trees and shrubs. This could make Indigofera very useful in the African plan for a kilometres-long ‘green wall’ of trees and shrubs to traverse the Sahel and stop the desert from spreading further southwards. Bisseling: ‘Indigofera appears to be very well equipped to withstand extreme desert conditions. The plant works together with drought-resistant Rhizobium bacteria which can apparently survive in the extreme heat and drought of the desert. If we can optimize the symbiosis under such extreme conditions as these, then there is more chance of success in creating green belts in the Sahel, where soils are generally nitrogen-poor.’

STAYING IN CONTROL

Symbiosis in the plant kingdom is an age-old phenomenon. Besides the plants that live symbiotically with Rhizobium bacteria in root nodules, there are also many plant species that cohabit with mycorrhiza fungi, which help the plant to extract water and nutrients from the soil in exchange for sugars. This collaboration is thought to be at least 400 million years old, going back to before the formation of plant roots even, according to fossil research.

In order to play the symbiosis game successfully, the plant must stay in control. Otherwise, fungi or Rhizobium bacteria would overrun the plant cells in no time and suck them dry. To avoid this, the plant protects itself with a specialized membrane. It controls precisely which substances get through this membrane: certain sugars are allowed out and certain nitrogen compounds are allowed in.

It is now known that free-ranging Rhizobium bacteria make their presence felt by giving off certain signal substances, the Nod (nodulation) factors, in the vicinity of the plant roots. The plant roots recognize these signals through their special Nod factor receptors, which trigger the production of root nodules in which the bacteria settle. Researchers can recreate these nod factors in the laboratory. Plants which are exposed to the signal substances in the laboratory immediately create root nodules. Bisseling: ‘By now we know all the main proteins and plant hormones needed in order to recognize and pass on the Nod signal.’

ANCIENT MECHANISM

The age-old mechanism triggered by the Rhizobium bacteria was already being used by mycorrhiza fungi half a billion years ago. ‘This mechanism and the genes that go with it are present in most plant species’, says Bisseling. ‘That gives us reason for optimism about the potential for using symbiosis with nitrogen-fixing bacteria in agriculture in future.’

Parasponia has just the one Nod factor receptor, which is still young in evolutionary terms. In leguminous plants, numerous different receptors have developed in the course of evolution, with very small changes every time. These extra accessories have brought about all sorts of optimal combinations, so that each plant species co-exists with its own specific strain of Rhizobium bacterium.

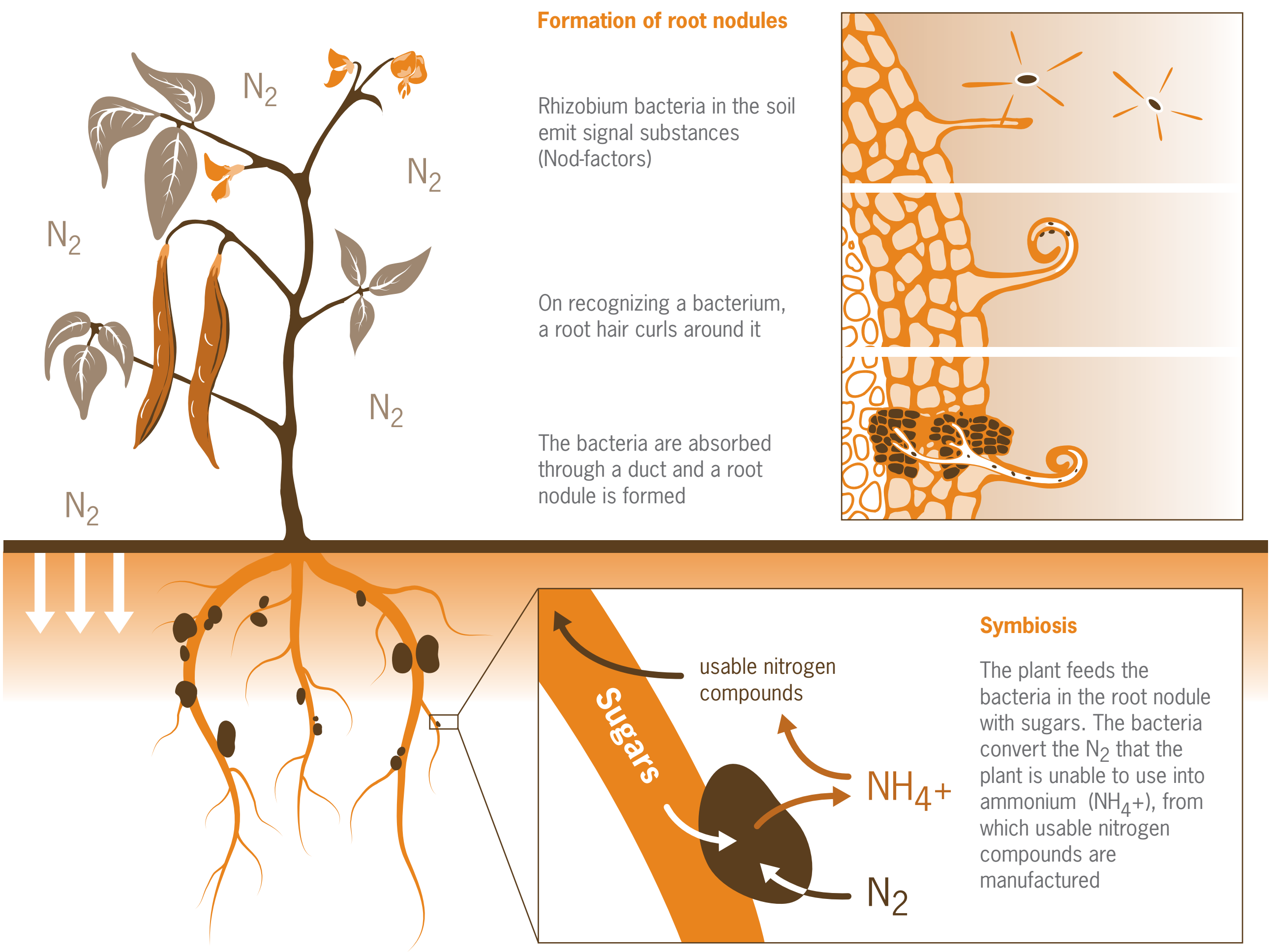
Bisseling has been doing research on Rhizobium symbiosis for 35 years. He expects a breakthrough soon. ‘Thanks to technical progress, the genome of many plants can now be mapped in fantastic levels of detail for a few hundred thousand euros. We already have the data on four legumes: soya, alfalfa, lotus and Cajun peas. But it will take us a little longer to interpret all the biological information that is now suddenly coming out of the genome research.’

BLACK MAGIC

Two storeys higher in the same university building, professor of Plant Production Systems Ken Giller’s research group is working on the N2Africa project to find out how leguminous crops could increase both food production and soil fertility for small-scale farmers in Africa. Giller himself has been working in Africa for 25 years. His N2Africa project is a consortium of researchers and development organizations from all over the world. The aim of the project is to improve the standard of living of at least 200,000 small-scale farmers in more than 10 African countries through the cultivation of pulses. The Bill & Melinda Gates gave the project 19.2 million dollars two years ago, and added another 1.3 million recently. The Howard G. Buffet foundation is also supporting N2Africa to the tune of 2 million dollars.

Much research goes into the selection of the right strain of Rhizobium bacteria for each crop. Giller puts a couple of plastic bags from Zimbabwe down on the table. Each bag contains 400 grams of finely ground turf mixed with enough Rhizobium bacteria to treat 100 kilos of seed. Using this ‘inoculant’ boosts harvests spectacularly. Local farmers talk of ‘black magic’. The method is very cheap and the inoculant

NITROGEN FIXATION IN LEGUMES



is produced locally. The turf stops the Rhizobium bacteria from drying out. Given the right inoculant and a little bit of phosphate fertilizer, a strong variety can easily double or even quadruple its yield. So the farmer's investment pays off very fast. What is more, the leguminous crops improve the soil, which means that after the bean harvest, the maize harvest will be better too.

'We are looking for the most promising crop for each area', explains Giller's colleague Linus Franke. 'In northern Nigeria and in the Sudan savannah, the climate is very dry and the soils are sandy. The growing season is only three months long, due to lack of rain. There we introduced the yardlong bean, a productive crop that >

PHOTOS N2AFRICA

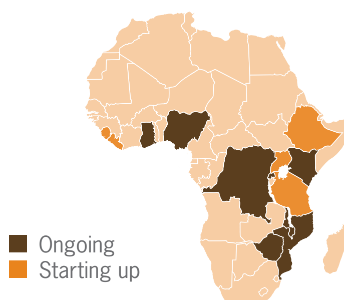
The use of extra Rhizobium bacteria leads to a spectacular increase in both nodule formation and yields.

N2AFRICA

Ken Giller works on the N2Africa project with a consortium of researchers and development organizations from all over the world. The aim is to improve soil fertility and food production among Africa's small-scale farmers.

The project addresses the entire value chain from producer to buyer. Its activities include identifying and introducing the most successful crop for each region, selecting the right Rhizobium bacteria strain for each crop, improving soil fertility by cultivating legumes, developing a market for pulses and introducing new processing techniques.

Giller works on the cultivation of pulses in eight African countries: the Democratic Republic of Congo, Ghana, Kenya, Nigeria, Malawi, Mozambique, Rwanda and Zimbabwe. Five new countries will also be included in the project: these are Ethiopia, Liberia, Uganda, Sierra Leone and Tanzania.



‘Those climbing beans are spreading like wildfire now’

flourishes under these dry conditions.’
In Northern Nigeria, new varieties of peanuts have also been tested, including in Kano State, one of the most densely populated regions of Africa. 23 percent of the children and 15 percent of the women in this state are undernourished. A household, which in this context means an extended family with an average of 15 people, has to eke a living from no more than a couple of hectares of land. There is a long tradition of growing legumes for home consumption, but they are now increasingly being grown for the market. The peanut varieties introduced by the project contain more oil and also produce many large seeds. The women are now producing their own oil by pounding the peanuts with a large pestle. The residue is fried as a snack. The peanuts provide not just protein but also several important minerals. Demand for the new varieties runs high, therefore, and people come from far and wide to buy them.
In northern Rwanda, farmers traditionally grew only low-growing bush beans. ‘Introducing climbing beans from cool regions of the Andes has doubled the harvest’, says Giller. ‘Climbing beans go on growing longer and grow higher, so they make better use of the growing season and the available light than bush beans do. But climbing beans do need canes to grow up, and where do you get those from? We’ve thought up all sorts of solutions to that one, including maize stalks, branches of trees or stalks of elephant grass grown for the purpose on the edge of the bean fields. Those climbing beans are spreading like wildfire now.’
Climbing beans do like fertile soils with good water retention and sufficient phosphate. ‘Most African soils are phosphate-poor, and without fertilizer the beans give very low yields on poor soils’, says Franke. ‘That is why we have demonstrated how much you can improve the bean harvest by using manure from the guinea pigs and the rabbits that are kept in the villages. Every little helps. A family of more than ten people here often only has a small plot of land of 0.2 hectares from which to scratch a living.’

WELL ORGANIZED

According to Franke, Africa is importing more and more soya, another legume, particularly for use as feed in the fast-growing chicken industry around the big cities. ‘I think Africa could become far more self-sufficient by increasing its own production of soya. But it is a major challenge to organize that properly so that African soya can compete better with imported soya. In Nigeria, we have been putting a lot of work over many years into breeding soya varieties that are adapted to local conditions. And with great success. All sorts of soya products are now for sale on the roadsides. At the same time, farmers are increasingly successful in supplying the ever-growing chicken industry with soya’, says Franke.
Giller: ‘Ultimately, we are primarily researchers. We work on institutional questions such as how you solve all the new problems that come up when you upscale a pilot to create a bigger project with 1,000 farmers. How do those farmers get access to the Rhizobium inoculant? How do you prevent their soils from being exhausted, and how do you make soils depleted by years of maize farming fertile again? How do the farmers get access to the market and how do you develop new markets for pulses? Our research looks at the money flows all along the value chain, from the farm to the customer.’
The key questions are: where do the opportunities lie for introducing pulses and the relevant new processing techniques, and how applicable is conventional knowledge under local conditions? ‘Large-scale projects such as N2Africa provide great opportunities to conduct comparative studies on the different regions of Africa. In the course of our fieldwork we developed a scientific model for the whole value chain. Now organizations such as the Bill and Melinda Gates Foundation are taking Africa’s problems very seriously. So I am definitely optimistic about the future of African agriculture!’ ■

PHOTO GUY ACKERMANS

KEN GILLER,
Professor of Plant Production Systems at Wageningen University

‘I think Africa can become more self-sufficient by raising its soya production’