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P. S. Nutman

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CENTENARY LECTURE

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[Plates 1 and 2]

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The discovery of symbiotic nitrogen fixation, announced a century ago in Berlin, and published in full two years later by Hellriegel & Wilfarth, ended 60 years of controversy and ushered in the modern era of intensive investigation, the extent of which was not then foreseen and which continues unabated.

Hellriegel & Wilfarth's great contribution was in proving that legume nodules fixed atmospheric nitrogen. They also showed that the nodule-inducing 'ferment' was to some degree specific, that it occurred in different abundancies in different soils, that it was killed by moderate heat and harmed by drought, that the nitrogen fixed by a nodulated legume was not immediately available to plants growing alongside and that small quantities of combined nitrogen did not affect nodulation, whereas larger amounts were inhibitory.

This remarkable achievement does not detract from the historic work done much earlier by Boussingault, Lawes, Gilbert and others, who showed by meticulous experiment that neither legumes nor other plants could fix atmospheric nitrogen, in spite of the capacity of the former to enrich themselves, and the soil in which they grew, with combined nitrogen. Earlier workers were so concerned with the need to exclude from their experiments any chemical contamination with ammonia, dust, etc., that they also excluded microorganisms and thus failed to discover fixation. Hellriegel & Wilfarth worked with the light infertile soils of North Germany, where addition of nitrogen was essential. This probably contributed to their success; differences in growth between legumes with and without nodules were quickly seen.

Advances in nitrogen fixation research over the past century encompassed many

areas in chemistry, biology and agriculture. That nitrogen fixation is among the key processes sustaining life was established by this work, but many fundamental aspects remain obscure, especially in the genetics and physiology of the functioning symbiosis. The catalogue of nitrogen-fixing organisms and associations, and their detailed description, is still incomplete; some nitrogen-fixing systems are in urgent need of conservation.

A deeper understanding of these matters will improve our ability to manage the cycling of nitrogen in agricultural and other ecosystems so as to increase protein yields of crops and avoid environmental and energy problems associated with intensive methods of production based wholly on fertilizer nitrogen.

1. Introduction

I propose firstly to describe in some detail Hellriegel & Wilfarth's historic experiments. This is necessary because most of us only know of their work through a translation of the brief report (*Tageblatt*) of the meeting in 1886 where their research was first described. This report, with Hellriegel as sole author, is more a set of conclusions than an informative summary account of the work and by no means does it sufficient credit, but rather reflects the modesty of the author. Indeed in the preamble to the full account of their work that appeared two years later, Hellriegel & Wilfarth were at pains to point out that the decision to present their data *in extenso* was to assuage criticism of the *Tageblatt* account, at the same time confessing that it was a striking but casual observation that led to their discovery ('*einige ganz gelegentlich gemachte auffällige Beobachtungen*').

The 1888 account (234 pages in length) appeared as a special issue (*Beilageheft*) in the *Zeitschrift des Vereins für die Rübenzucker-Industrie der Deutschen Republik*', under the joint authorship of Hellriegel & Wilfarth and also included, with the work summarized in 1886, that done in 1887. Rightly, Hellriegel & Wilfarth share the discovery of symbiotic nitrogen fixation, although by today's rules governing these matters, the date 1888 would have been given rather than 1886. However, the 1886 presentation was so convincing that it was immediately accepted by all but one of those present and whose opinions were recorded. Adolph Mayer (1905) described it as a sensational discovery, modestly reported, and he could not recall a greater impression being made at a scientific meeting (Glathe 1970).

From the spate of reports, generally confirmatory, that appeared over the next few years, it would appear that most of those who heard or were aware of the *Tageblatt* report were only too anxious to return to their laboratories and set up their own experiments. Nevertheless the criticism of the *Tageblatt* between 1886 and 1888 was an important spur to full publication.

After we have examined Hellriegel & Wilfarth's work, it will be instructive to look into its antecedents and also to survey the work of those who followed parallel but less successful paths. Finally, I shall attempt to outline the momentous consequences of their discovery, and to peer, however uncertainly, into the future.

2. The discovery

The occasion in 1886 on which we now need to focus attention was the 59th Conference of German Scientists and Physicians (*Versammlung Deutscher Naturforscher und Ärzte*) held in September in Berlin. The title of Hellriegel's paper was in the form of a question: 'What is the source of a plant's nitrogen?' (*Welche Stickstoffquellen stehen der Pflanze zu Gebote?*); Sir Henry Gilbert was in the chair.

Hellriegel gave an account of pot experiments done over the previous three years on the fertilizer requirements of different crops, especially their need for combined nitrogen. At the Bernburg Experimental Station, where they were done, and before this when Hellriegel was at Dahme (1862–4) he was very familiar with the problems of cropping light infertile soils, where manuring is essential. He and his early collaborators at Dahme (Drs Fittbogen, Fruhling, Sorauer and Marx) were particularly struck by the contrasted nutritional needs of cereals, root crops (Hellriegel was Director of a Sugarbeet Research Institute) and legumes. These differences were studied by measuring crop responses to N, P, K and Mg, and to liming to correct soil reaction. He repeatedly found that cereals responded in a regular manner to additions of combined nitrogen, usually calcium nitrate, whereas the responses of legumes were quite unpredictable, whether the tests were made in the light soils of North Germany or in controlled water culture or sand culture experiments, of which Hellriegel was one of the earliest practitioners. He described these methods in '*Beiträge zu den Naturwissenschaftenlichen Grundlagen des Ackerbaus*' (1883). The legumes' unpredictability could not be ascribed to variation in seed quality, soil type or its 'mechanical condition', or to sowing time, illumination, temperature or soil moisture.

I have summarized in figure 1 some of Hellriegel & Wilfarth's results to illustrate these differences between cereals and legumes, taken from extensive tabulated data in their 1888 paper. Total dry matter yields are plotted against increments in fertilizer nitrogen, showing individual yields as well as means. Their techniques were so good (of which more presently) that I have been able to plot all three years' results together. The first plot shows not only the

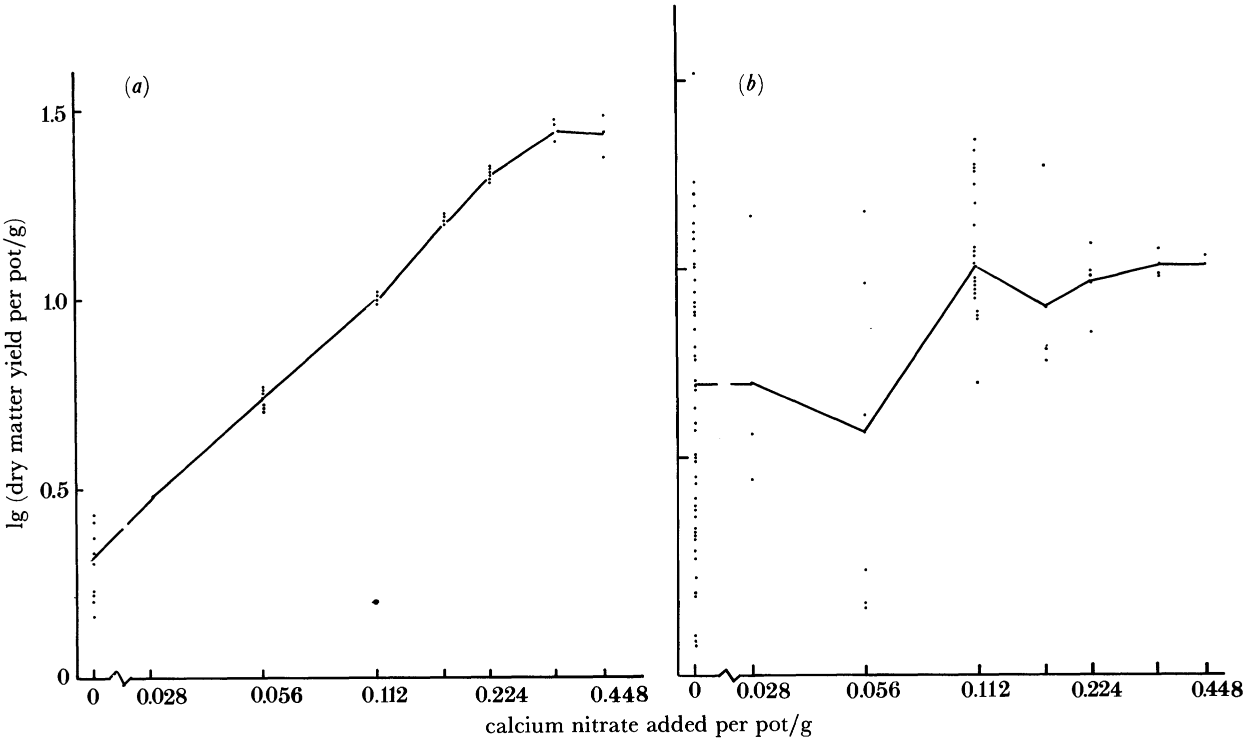

FIGURE 1. Responses of cereals and legumes to nitrogen fertilizer. Hellriegel & Wilfarth's pot experiments of 1883, 1884 and 1885. Means and individual pot yields are shown. (*a*) Response of cereals (barley and oats) to added calcium nitrate; (*b*), response of legumes (peas, lupins and serradella) to added calcium nitrate.

cereals' incontrovertible dependence on nitrogenous fertilizer, but also its precise predictability; even the values for individual plants hardly depart from the straight-line relation. In sharp contrast, legumes (peas, lupins and serradella) responded little to added nitrate and showed so large a variation from plant to plant as to make the mean responses meaningless. They made notes at frequent intervals of the condition of each plant. Without nitrate the legumes soon entered a yellow, N-starved condition from which some plants recovered and others did not. Recovery could take place at any time, heralded by a change in colour from yellow to green that could sometimes be detected between one day and the next. The indications of early poor growth then disappeared, the recovered plants having 'broad, juicy organs, dark black-green in colour and bursting with nitrogen excess' ('*Nachdem sie einmal den ursprünglichen Hungerzustand überwunden hatten, erinnerte nichts mehr daran, dass sie in einem stickstofflosen Boden standen, ihre Entwicklung war eine ungewöhnlich rapide, mit ihren breit angelegten, saftigen Organen und ihrer dunkeln schwarzgrünen Färbung stellten sie rechte Urbilder von Stickstoff-Uberschuss strotzenden Pflanzen dar.*')

In a closely argued discussion of these results, the authors put forward the hypothesis that the legumes' capricious responses must lie in some uncontrolled property of the medium for plant growth. They were aware of Boussingault's Liebig's and Lawes & Gilbert's experiments, which I will be discussing later, and of Berthelot's claim (1877, 1885) that nitrogen gas could be absorbed and combined in some way in the soil, possibly by chemical, electrical or microbial activity, but at this time root nodules were not suspected as being the sites for fixation; if they had any relevance at all it was rather as the centres for the accumulation of nitrogen in the form of proteinaceous granules taken up in other ways from the soil. Brunschorst (1885) proposed the term 'bacteroids' for these bacteria-like bodies in nodules. Hellriegel & Wilfarth considered that, if there exists in soil a nitrogen-fixing activity, it should be possible to transfer it experimentally, and if it is of 'mycodermic' origin it should be inactivated by heat. Though not so explicitly stated in their paper, this was clearly the rationale of the experiments they reported in 1886. To us, with the benefit of hindsight, they did the obvious crucial experiment: to pea plants growing in a sterile medium free of combined nitrogen, and without added fertilizer nitrogen, they applied either a suspension of raw soil or the same suspension sterilized by steaming for 1 h.

I have summarized their results in table 1 in terms of distribution of yield. Without soil present, yields were extremely variable, ranging from less than 1 g to nearly 20 g per pot. Soil counteracted this variability: with the addition of raw soil, yields were much higher and less variable and with sterilized soil they were all low. In making these comparisons it should be pointed out that the treatment without soil consisted of 22 pots whereas there were only four replicates of the other treatments. With greater replication some of the blank spaces in table 1 would have become occupied because of the probability of contamination; nevertheless, the results were clear and unambiguous.

Table 1. Hellriegel's first soil inoculation experiment with peas in 1886

(Percentage distribution of dry matter yield; no fertilizer nitrogen added.)

	mass/g				
	1	1–5	6–10	11–15	16–20
uninoculated (22 replicates)	9.1	18.2	22.7	36.4	13.6
raw soil inoculum (4)	—	—	—	25.0	75.0
sterilized inoculum (4)	100.0	—	—	—	—

Having obtained this exciting and promising result, and with the September meeting only two months away, Hellriegel & Wilfarth immediately set up a similar experiment with five species of non-legume and six leguminous crops, but on this occasion comparing yields with and without calcium nitrate (two levels) and with or without an added suspension of soil. They used the expression 'soil infusion' for their preparation, which having the connotation of extraction is also somewhat inappropriate (as indeed is the term 'inoculation' used today).

The fresh (or slightly air-dried) topsoil was stirred vigorously with five times its mass of distilled water and allowed to stand for up to 10 h, depending on the clay content of the soil. The decanted supernatant was added to the nutrient solution, at 25 ml per pot. This quantity of soil suspension had a nitrogen content of less than 1 mg.

Two sources of soil were used: a humose loamy marl taken from an experimental field near Bernburg (the L1 inoculum), or a glacial sandy soil from a field by the side of a railway station happily named '*Bahnstation Güterglück*' where lupins had grown that season (the S1 inoculum). Because the experiment was set up so late in the year it was frosted before it could be harvested. Nevertheless, careful observations were made that confirmed the good response of the cereals to nitrate and their indifference to the soil suspension. In contrast the legumes reacted irregularly to nitrate and strongly to inoculation with the soil suspension, depending on the species of legume and the source of the inoculum. Their results are summarized in table 2. The legumes fell into two clear groups: peas, sweetpeas, red clover and field beans grew well with the loamy soil inoculum and less well with the sandy soil suspension, whereas yellow lupin and serradella responded only to the sandy soil suspension. Evidently the loamy soil, as might be expected, contained *Rhizobium trifolii* and *R. leguminosarum* and the sandy soil mostly *R. lupini* and *R. meliloti*. This appears to be the first record of symbiotic specificity, although *Rhizobium* had yet to be isolated. Pots from this experiment were shown at the Berlin meeting.

TABLE 2. HELLRIEGEL & WILFARTH'S SECOND SOIL INOCULATION EXPERIMENT, AUTUMN 1866

(Because it was sown late, this experiment was frosted before it could be harvested. Pots from this experiment were demonstrated at the Berlin meeting on 20 September 1886. Qualitative growth responses: ++, very good; ±, intermediate or mixed; −, no growth.)

	barley, oats, summer rape, white mustard, buckwheat	peas, sweet peas, red clover, horse beans	yellow lupin, serradella
calcium nitrate...	++	±	±
loamy marl soil inoculum...	−	++	−
glacial sand soil inoculum...	−	− ±	++

Hellriegel & Wilfarth noted that only the well-grown legumes were nodulated, as in their earlier experiment, and rightly concluded that nodules were the organs where atmospheric nitrogen was fixed and not merely where nitrogenous compounds were stored.

In 1887, pot experiments were set up with oats, buckwheat, serradella, lupins and peas, with different sources of soil for inocula and several levels of nitrate, including a very small amount that we would today call a starter dose (7 mg N in 5 kg sand mixture). The experiments also included several other treatments that need not concern us. Hellriegel & Wilfarth used partial factorial designs, long before they were accepted in agricultural research. As a tribute to the excellence of their experimentation, it is of interest to describe in some detail the way in which a typical pot trial was set up.

Plants were grown in slightly tapered glass vessels, larger ones being used for the larger species. These were sterilized by rinsing in mercuric chloride (1:2000) and absolute alcohol. At the bottom of the vessel was a layer of quartz chips, used to adjust weights and improve drainage. The chips were pretreated with boiling hydrochloric acid. The layer of chips was separated from the main bulk of sand by a thin layer of cotton wool. Above the sand was a layer of sterilized cotton wool. The sand was from a tertiary quartz deposit which contained only small quantities of mica, hornblende and feldspar. It was sieved to retain the 0.2–0.4 mm fraction. The quartz chips and sand were sterilized in large shallow copper trays by dry heat at 150–200 °C for 2 h. The nutrient solutions, prepared in cotton-wool-plugged flasks, were boiled for 25 min, allowed to cool for two days and then heated to 100 °C in a steamer for 4 h. The nutrient solutions contained potassium monophosphate, potassium chloride, magnesium sulphate, calcium nitrate and calcium carbonate, depending on the requirements of the treatments. The volume of the medium and concentrations of these salts were so adjusted, by considering responses in preliminary tests with cereals and peas, that neither halving nor doubling the quantities of added salts appreciably affected growth; this was a novel way of achieving an optimum.

Great care was taken in selecting seed; all but the healthiest were first rejected, the good seed graded individually to a standard medium size. These were then pre-germinated and only uniform seedlings planted, usually at twice the intended density so that final selection could be made later. In the 1886 and 1887 experiments seeds were surface-sterilized by immersion in mercuric chloride (1:1000) for 2 min, and rinsing in boiled water.

Pots were provided with an aeration hole at the base; this was not required for drainage because each pot was watered daily to a constant mass with distilled water; the moisture content was not allowed to fall below 10%. To avoid contamination with atmospheric ammonia, the first $\frac{1}{3}$ of the distillate was rejected.

Pots were arranged on trolleys on rails so that they could be run under a glass roof when rain threatened, or into an open-sided shade house in very hot weather. The trolleys were netted against birds.

Some experiments had to be discarded because of mildew and aphid attacks. Much trouble was also caused by calcining the washed sand at too high a temperature, rendering it highly alkaline (the calcining was done to remove the last traces of nitrogen). The first experiment of 1887 was damaged by pollution, by the emission of a fine dust of Na_2CO_3 from a factory 1 km distant that prevented flower opening and discoloured the glumes of the cereals but without affecting yield.

The detailed description of methods and facilities, of which the above is a shortened account, even included the dimensions of the glasshouse and trolley wheels so that old photographs of the 'Vegetationshalle' at Bernburg (figure 3, plate 1) can be identified with confidence as the place where these historic experiments were actually conducted.

The scale of the work is such that it is impracticable to present all of their data, even in summary form. Accordingly the essential features have been abstracted and are presented as tables. The first three columns of table 3 show the anticipated response of oats to nitrate, which was unaffected by the addition of a suspension of a loamy marl soil taken from an experimental field at Bernburg on the right bank of the river Saale (the L1 'inoculum').

In terms of yield, serradella responded poorly to nitrate although the plants with nitrate were green and healthy in appearance. Serradella showed a strong response to the S1 inoculum

TABLE 3. HELLRIEGEL & WILFARTH'S EXPERIMENTS IN 1887 ON THE EFFECT OF ADDING DILUTE SUSPENSIONS OF SOIL TO OATS, SERRADELLA, LUPINS AND PEAS

(Plant yields per pot in grams (of tops only for oats) with and without added combined nitrogen (calcium nitrate at 0, 56 and 112 mg N per pot.)

	oats			serradella			lupins*			peas		
	0	56	112	0	56	112	0	56	112	0	56	112
0	0.6	5.1	11.8	0.1	2.9	6.5	0.7	0.6	0.7	0.8	7.3	12.9
L1	0.7	4.8	11.6	0.1	—	—	—	—	—	16.4	4.0	15.3
L1 sterilized	—	—	—	—	—	—	—	—	—	0.9	—	—
L2	—	—	—	—	—	—	—	—	—	23.5	—	—
S1	—	—	—	17.5	13.6	14.1	1.3[a]	0.8[b]	1.2[c]	18.0	—	—
S1	—	—	—	—	—	—	22.9[d]	25.2[e]	5.1[f]	—	—	—
S1	—	—	—	—	—	—	23.1[g]	—	—	—	—	—
S1 sterilized	—	—	—	0.1	—	—	0.7	—	—	—	—	—
S2	—	—	—	—	—	—	—	—	—	0.9[h]	—	—
S2	—	—	—	—	—	—	—	—	—	6.6[i]	—	—

Sources of soil: L1, humose loamy marl over deep limestone, experimental field, Bernburg; L2, humose glacial marl; S1, glacial sandy soil; S2, very infertile sandy soil from Dahme.

* Crop partly failed because nutrient solution was too acid.

[a] Pot no. 265; [b] pot 306; [c] pot 308; [d] pot 276; [e] pot 307; [f] pot 309; [g] pot 277; [h] pot 342; [i] pot 343.

at each level of nitrate, but not to the L1 soil. The stimulating effect of the former was eliminated by sterilizing the soil suspension by boiling. The absence of a response with L1 agreed with the preliminary trial in the late summer of 1886. (The authors incidentally mentioned, with respect to L2, that rape was grown before sugarbeet to trap eelworms; biological control is not a recent idea.)

The lupin results differed from the foregoing in two respects: nitrate did not affect growth, and the response to the addition of soil was irregular. Without nitrate, plants 'd' and 'g' responded to inoculation but not plant 'a'. With the lower level of nitrate plant 'e' gave a response but not plant 'b'. With 112 mg N per pot the responses to inoculation with soil were much smaller. The sterilized inoculum had no effect. These results indicate that the S1 inoculum was weak; this is not surprising, because it was made from air-dried soil.

The tests with peas employed four different soil treatments: the L1 inoculum from a marly loam over limestone that had grown sugar beet; the L2 inoculum from a humose glacial loam over standstone having previously grown sugarbeet and rape; and S inocula from glacial sands, S1 after yellow lupins and S2 from a poor soil at Dahme, never recorded as being manured, that had been occasionally cropped to lupins. Peas responded better than serradella or lupins to nitrate and grew very well when given the L1, L2 or S1 inocula but only partly responded to the S2 inoculum. Steam sterilizing completely removed the beneficial effect of L1, and later tests showed that holding the inoculum at 70 °C for 1 h destroyed its activity; pasteurization before it was used for milk.

TABLE 4. NITROGEN BALANCES OF PLANTS GIVEN NO FERTILIZER NITROGEN IN HELLRIEGEL & WILFARTH'S 1887 EXPERIMENT

	total N supplied in seed and inoculum/mg	total N in yield/mg	N lost (−) or N fixed (+)	percentage N
serradella				
no inoculum	23	1	−21	1.59
inoculated	23	377	+354	2.12
sterilized inoculum	23	1	−22	1.21
lupin				
no inoculum	64	12	−52	2.62
inoculated	64	1147	+1078	2.88
pea				
no inoculum	38	14	−23	1.54
inoculated	38	424	+386	2.53

Nitrogen analyses were done separately for grain, haulms and roots, with a modification of the Kjeldahl method devised by Dr Wilfarth. A few of the results for whole plants are entered in table 4. For each species without a soil inoculum, or where it was sterilized, the very small amounts of nitrogen found in the yields were even less than originally present in the seed and inoculum. With effective inoculation, however, each serradella plant fixed 354 mg N, each lupin plant, 1076 mg N and each pea plant, 386 mg N. Inoculated lupins had the highest percentage nitrogen and plants without inoculation the least.

Thus the 1887 experiments amply confirmed and extended the conclusions set out in the *Tageblatt*: without fertilizer nitrogen, only nodulated legumes were vigorous, indicating in the strongest manner that the root nodule was the site of the assimilation of atmospheric nitrogen.

In trials where sequential harvests were used it was noted that the end of the seedling's N-starvation phase corresponded to the start of nodule formation; this was often first seen in the greening of the cotyledons. Good growth was associated with abundant nodulation but not always *vice versa*, for which they were unable to offer an explanation, ineffective nodulation not having yet been identified. The 'cryptogamic growths' noted by Hellriegel & Wilfarth to occur sporadically on the insides of the glass pots and sometimes on the surface of the sand were considered to be irrelevant to nitrogen uptake because they did not affect the growth of the non-legumes without nitrogen or of legumes without nodules.

Hellriegel & Wilfarth were also interested in whether soil inoculation initiated a response in the whole plant or only in the part of the root exposed to the inoculum. This was examined by preparing pairs of culture vessels with a specially made common cover, which had a single opening through which the root of a pea plant could be threaded, half into each vessel. Both vessels of a pair were first inoculated with an active soil suspension and then one was sterilized by boiling for 15 min followed by steaming for 4.5 h. Four such paired plantings were made and in each only the vessel containing the raw inoculum bore nodules 'like pearls on a string' (*perlschnurartig*). Nodules developing in water culture seemed not be be so active as those forming in sand; figure 2 is drawn from a faded photograph of this experiment.

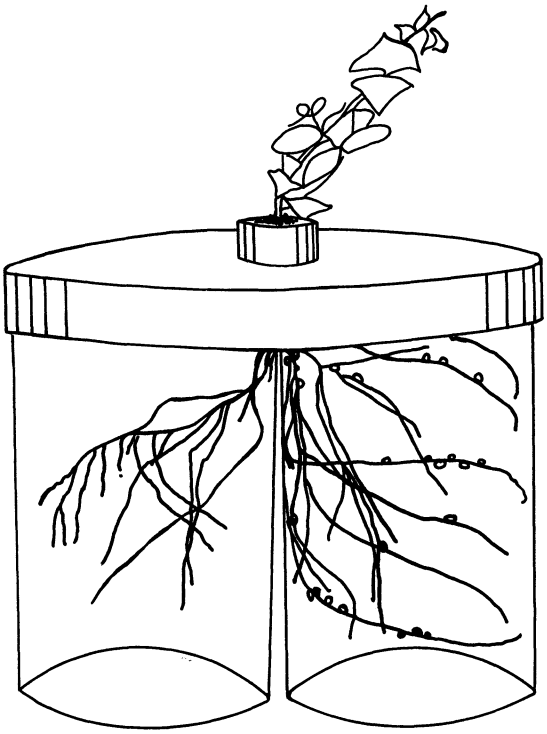

FIGURE 2. Hellriegel & Wilfarth's divided root experiment with a pea plant. Only the half-root inoculated with the raw soil suspension bore nodules.

Hellriegel & Wilfarth often remarked upon the difficulty of maintaining sterility in open-hot cultures over the long period of an experiment, more so with peas than lupins, a common experience of those working with these hosts. To control contamination more effectively (and building upon the experience of Boussingault, Lawes and Gilbert) they next set up two kinds of experiment in which either bell jars or carboys were used as growth chambers. Although they did not provide wholly satisfactory conditions, these experiments are important in reconfirming the essential role of nodules in fixation in the absence of casual contamination and in providing an estimate of fixation where the amount of nitrogen gas in the system was known. These experiments also give an interesting insight into how physiological experiments were set up a century ago.

The first system consisted of four tall bell jars (25 × 105 cm) mounted and sealed in grooves in a slab of slate that formed the roof of a small hut housing accessory apparatus. On the lower side of the slab, and corresponding in position to the bell jars, with which they were in open communication, were cemented glass pots for growing the plants. The pots were of a smaller diameter than the bells so that holes could be bored through the slab into the space of each bell jar. These were threaded with glass tubes sealed into the slab and so connected that a stream of air could be drawn through each of the bell jars in turn by means of an aspirator pump. Before entering the jars the air was scrubbed in a battery of absorption towers, one set containing H_2SO_4 to free the air of ammonia, and the other sodium bicarbonate. Flow rate was measured with a bubbler and was such that the air was in contact with the solutions for three minutes. A third tube was inserted into the first jar for adding measured quantities of carbon dioxide, judged to be sufficient because the plants in the last container grew as well as those in the first. From 2 July to 2 September, 722 m^3 of air passed through the system, from which 1637 mg N_2 gas was fixed, i.e. 2.27 mg N_2 from each cubic metre of air.

Early in 1888 the simpler system of Boussingault was used, consisting of a 44 litre carboy containing 4 kg of sterilized fine quartz sand with a nitrogen-free nutrient medium and inoculated with soil suspension (Bernburg) and sown with a single pea seed. The carboy was opened briefly twice to add a measured volume of carbon dioxide, to a maximum of 5% of the carboy's volume. The pea plant nodulated and grew well, although it was paler than when grown in the open; Hellriegel & Wilfarth attributed this, erroneously, to excess oxygen. At the time of the first opening one seed each of oats and buckwheat were sown in the carboy. These grew until their reserves were exhausted; although very small, each had formed a few seeds at the end of the experiment. Table 5 gives the nitrogen balance sheet of this experiment, showing the quantities of nitrogen analysed in the separate components of the system at the start and in the plants and medium at the end. The preponderant gain of nitrogen was in the pea plant, with insignificant amounts in the medium and possibly in the oats. The soil gains were in organic nitrogen, which it was thought may have come from root fragments left behind or possibly from root exudates.

TABLE 5. ANALYSIS OF THE NITROGEN (IN MILLIGRAMS) AT THE BEGINNING AND AT THE END OF HELLRIEGEL & WILFARTH'S CARBOY EXPERIMENT, 1887

	start	end
air	< 1.0	—
sand, etc	0.2	20.7
peas	8.1	233.5
oats	0.7	3.3
buckwheat	0.4	0.6
total	< 10.4	258.1
difference		+247.7

Hellriegel & Wilfarth's report in the *Tageblatt* does not do justice to the remarkable scope

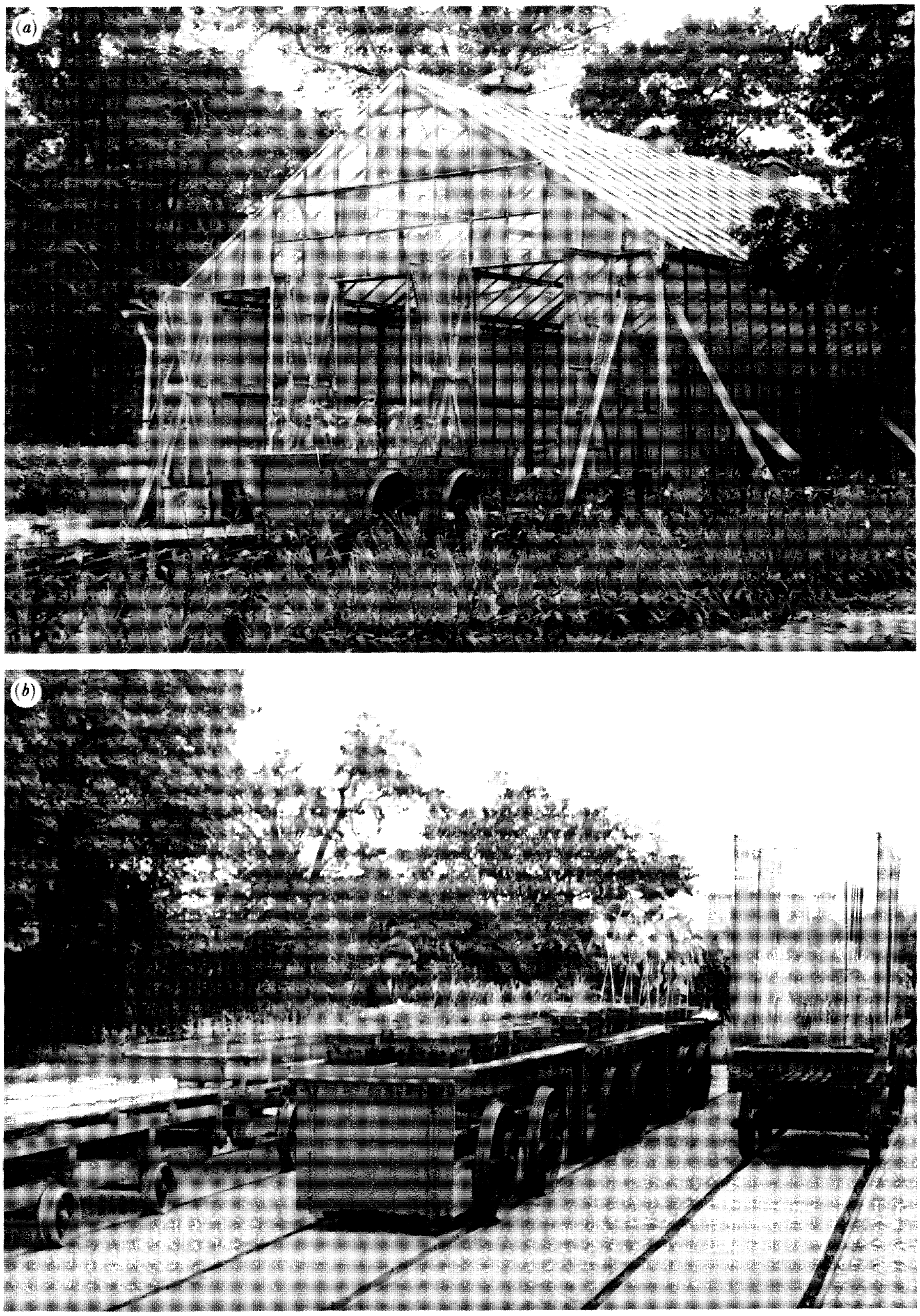

FIGURE 3. (*a*) The glasshouse at Bernburg where Hellriegel and Wilfarth conducted their historic experiments; (*b*) the glasshouse trollies at Bernburg.

FIGURE 5. Portraits of the major workers on nitrogen fixation in the nineteenth century: (*a*), Professor Hermann Hellriegel (1831–1895); (*b*), Bust of Hellriegel at the Institut für Getreideforschung, Bernburg-Hadmersleben; (*c*), Professor Hermann Wilfarth (1853–1904); (*d*), Jean Baptiste Boussingault (1802–1887); (*e*), Georges Ville (1824?–1897); (*f*), Baron Justus von Liebig (1803–1873); (*g*), Sir John Bennet Lawes (1814–1900); (*h*), Sir Joseph Henry Gilbert (1817–1901); (*i*), Dr Evan Pugh (1828–1864).

of the work and its sequel in the confirmatory studies made over the following eighteen months. Not only did they demonstrate unequivocally the fixation of nitrogen by nodulated legumes but they established it quantitatively and recorded for the first time the following important features of the symbiosis: that the nodule inducing 'ferment' was to some degree specific, that it occurred in different abundances in different soils, that it was killed by moderate heat and harmed by drought, that the nitrogen fixed by the legume was not immediately available to other plants growing alongside, and that small quantities of nitrate in the medium did not influence nodulation but that higher quantities were inhibitory.

This was a conspicuous achievement by the joint discoverers of nitrogen fixation. Reading Hellriegel's papers, one has the impression that he was the leading partner in the enterprise. His other work also demonstrated experimental prowess, meticulous technique and Teutonic thoroughness and persistence. One also gains some appreciation of his personality from his style and use of words, examples of which I have given. It was recorded by his biographer von Glathe (1970) that he was a kindly man, greatly respected by his colleagues, and an excellent lecturer with a sense of humour. This character can be glimpsed from the only portraits that we have of him, inadequate though they are (figure 5*a*, *b*, plate 2).

Little is known of his early career. He was a farmer's son who studied agriculture and forestry at the Academy at Tharandt under Professor Stöckhardt, qualifying as a chemist. He drew much of his inspiration for research from his close acquaintance with the practical problems of agriculture, spending much of his time advising farmers, and he published infrequently. Like everyone else in agricultural research he was much influenced by Liebig, but disagreed with Liebig's view that the mineral content of manures, rather than the amount of nitrogenous materials that they contained, was paramount. He was awarded Ph.D. (Leipzig) in 1854.

The Dahme research station where he first worked was set up by the Prussian Ministry of Agriculture, but initially was not well supported. It was housed in a school, Hellriegel's laboratory being the laundry. New buildings were erected in 1858 and a glasshouse in 1865. Hellriegel's interests from his earliest published work in 1861 was in general plant nutrition and physiology, the use of manures (farmyard, fish guano and sheep shoddy) and chemical fertilizers, the feeding values of forages and beet residues and technical matters such as the extraction of sugar from dried beet and the effect of pectins on the extraction processes. He devised equipment for soil sampling and temperature and light recording and was a pioneer in the development of water culture and sand culture and in applying simple statistical methods to pot and field experimentation. He was not a prolific writer, but to mark the jubilee of the Dahme Experiment Station he was persuaded to write an exhaustive distillation (796 pages) of his experiences in the use of sand culture (*Beiträge zu den naturwissenschaftlichen Grundlagen des Ackerbaus mit der besonderen Berücksichtigung der agrikulturchemischen Methode der Sandkultur*, 1883). Hellriegel was honoured by academic and agricultural bodies in Germany and abroad, his membership of the Royal Agricultural Society of England being proposed by Sir Henry Gilbert in November 1891.

Herman Wilfarth (born in Hamburg in 1853) trained as a chemist, graduating from Rostock University. At first employed in industrial work he was drawn towards the land and went to study agriculture with Kühn at Halle, soon becoming his assistant. From there he moved to Dahme to start a close and lifelong association with Hellriegel. Wilfarth was especially skilled in analysis and devised important improvements in the Kjeldahl process, and towards the end of his career wrote a comprehensive article on the mineral requirements of plants (1898). He succeeded Hellriegal as Director of the Bernburg Institute in 1895 and died at Bernburg in 1905 (Roemer 1905).

Neither Hellriegel nor Wilfarth extended their work on nitrogen fixation after 1888 except to present updated discussions countering Frank's renewed criticism of their work (1888, 1889) at meetings in Wiesbaden and elsewhere (Wilfarth 1887, 1893; Hellriegel 1889; Hellriegel & Wilfarth 1889). Hellriegel's last posthumously published work was on the nutrition of sugar beet, jointly with Wilfarth, Romer & Wimmer (Hellriegel *et al.* 1898). Hellriegel died in 1895 at the comparatively early age of 64. He may not always have enjoyed good health; at the 1886 meeting after the presentation of his paper he was unable the next day, through illness, to attend the meeting; his place as chairman was taken by Herr Settegast. At this time Boussingault was still alive and one may wonder whether he learnt of the solution of the problem that exercised him so seriously half a century earlier; he died the following year aged 85.

The antecedents of Hellriegel & Wilfarth's discovery are clearly seen in their papers. As they candidly pointed out, the discovery was accidental and indeed at first unwelcome (*zunächst unerwünscht*) in that they did not expect soil inoculation to lead to nodulation and consequent nitrogen fixation. (In racing parlance, they were outsiders who came from behind to win the race.) In writing up their results they painstakingly, and at great length, placed their work in the context of what had already been done, but their work was in no sense derivative.

Before considering in some detail the work of Boussingault, Lawes, Gilbert, Liebig and others, mention should be made of some of the people present and papers read at the 1886 meeting. There were two sessions on agricultural science at which were presented four other papers relevant to our subject: by Gilbert on the nitrogen nutrition of legumes, by Landolt on chemical transformations in soil mediated by microorganisms, by Thoms on measuring soil nitrogen and by Tacke on the evolution of gaseous nitrogen by schizomycetes. Gilbert (1886) summarized the earlier work at Rothamsted (again pointing out that soil can contain up to 20000 lb† N per acre,‡ some available to plants, and commented upon Berthelot's and Frank's claims that microbes influence nitrogen transformations in soil. His paper was read after Hellriegel's and one might wonder at his feelings on this occasion. As already mentioned Frank was alone in not accepting Hellriegel's explanation of their results. Nobbe was also present but did not appear to take part in the discussion.

3. STUDIES BEFORE 1886

Earlier work on the sources of nitrogen for plant growth was extensive, sometimes contentious, and often confused and misleading. Schneider's (1893) review quotes 274 references without even mentioning Boussingault! This review, the literature list by MacDougal (1894), the series of papers by Atwater & Woods (1886–1890) and Smith's (1911) article are valuable for those interested in the intricacies of the germinal literature.

Rather than attempting to guide you through this maze of work, I shall restrict myself to discussing the work of only the major investigators and the controversies that they engendered. Thomas Carlyle's 'Great Man Theory of History' will be my guide although I prefer Lord Blake's modern version that 'History is about Chaps'. (How the noble Lord has been able to

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† 1 lb = 0.454 kg.
‡ 1 acre = 4046.86 m² = 0.404686 ha.

sustain this view after the publication of his recent study, 'Conservative Prime Ministers from Peel to Thatcher' (1985, Fontana), has not been vouchsafed.) Today we are enjoined by our leader to embrace the virtues of a century ago and in following Carlyle one conforms to the sentiments of that quintessentially Victorian poet and educator, Matthew Arnold (1822–1888):

> 'The one or two immortal lights
> Rise slowly up into the sky
> To shine there everlastingly'.

(*a*) *Jean Baptiste Boussingault*

The first great man to claim our attention is undoubtedly Boussingault. He was born in Paris in 1802 into 'la petite bourgeoisie', his father, formerly an army storekeeper, keeping a tobacconist's shop. His education was rudimentary; he left school before taking his baccalaureat. Through a friend, a laboratory cleaner, whom he helped surreptitiously before being discovered, he obtained his first glimpse of a chemical laboratory. His mother encouraged this interest by buying him Thenard's four-volume work '*Traite de Chemie élémentaire*, *theoretique et pratique*'. Boussingault attended the excellent free public lectures then available in Paris; these were important in nourishing his wide interests.

After unsuccessful attempts to enlist in the Russian and French navies 'to see the world', he joined a school for mining artisans at St Étienne, attracted by the opportunity of working in a laboratory and using the library. A diligent student and tireless experimenter, he graduated to become a student demonstrator. At the early age of 19 he published his first work on a supposedly platinum–carbon alloy (later shown to be a silicate of platinum) in the prestigious *Annales de Chemie et de Physique*. Boussingault's first employment was at a lignite mine in Alsace, where he met the Le Bel family. He later married Mlle Le Bel, on whose father's farm at Bechelbronn he conducted his first manurial experiments.

A long-lasting desire to travel was satisfied when through the influence of Alexandre von Humboldt he was appointed Professor of Chemistry at the National School of Mines at Bogota, Columbia, which was not a sinecure. His most curious official assignment was an order to cast a statue of Simon Bolivar in platinum for the Plaza Mayor in Bogota, an impossibility because platinum could not then be cast; moreover, the total quantity of the metal available in Colombia was only a few kilograms.

On returning to Europe in 1832 with an established scientific reputation, he was nevertheless unsuccessful in applying for a post at the Musée d'Histoire Naturelle and at first in election to the Academie des Sciences. Eventually he was appointed to the chair of chemistry at the newly reopened Academie de Lyon, but not being provided with adequate facilities and disliking its bureaucracy and intrigues he neglected his duties there, spending much of his time in Paris and in Alsace where in 1836 he had started a series of field experiments on manuring on his father-in-law's farm; he resigned from the Lyons Academy in the same year.

In 1845 Boussingault was appointed to the chair of agriculture at the Conservatoire des Arts et Métiers in Paris.

Boussingault's interest in agriculture can be traced to his South American experiences where he arranged for the labourers of the Marmato gold mines to grow their own corn and vegetables instead of returning fortnightly to their villages to replenish their rations (McCosh 1984).

At Bechelbronn, Boussingault's trials on manuring and crop rotation (1837–1841) soon established that the masses of carbon, oxygen, hydrogen and nitrogen in a crop were greater than the masses of these elements provided in the manure. For example, in a five-course rotation comprising potatoes, clover, wheat, turnips and oats he measured a total gain in the yields of 47.5 kg N per hectare; the year-by-year analyses showed that this increase came from the clover. The hydrogen and oxygen could have come from water and carbon dioxide from the atmosphere, de Saussure having demonstrated its fixation in 1804. Only the source of nitrogen seemed to present a problem; did it come wholly from the soil or in part from the atmosphere? Earlier, before nitrogen was shown to be so unreactive, it was assumed that the nitrogen of plants and animals, first identified by Berthollet in 1785, came from the atmosphere.

The publication of Boussingault's findings immediately drew fire from the formidable Liebig, who promulgated with almost religious zeal the view that manures only needed to provide those elements that were to be found in the plant ash: 'the crops of field diminish or increase in exact proportion to the diminution or increase of the mineral substances conveyed to it in manure.' To which Boussingault made the rejoinder in '*Economie rurale*' that if this were so farmers should burn their manure heaps to reduce the cost of cartage! Boussingault at first disagreed with Liebig by showing in 1837 and 1838 that, whereas oats and wheat growing in open pots of sterile (calcined) sand did not gain in nitrogen, clover and peas did so, with increases ranging from 4 % to more than 50 %. However, he was not entirely satisfied that the legumes could not have absorbed nitrogen from organic dust or traces of combined nitrogen in the air. Accordingly in 1854–1855, after Liebig's criticism, he repeated these growth experiments in large closed glass containers: carboys of about 35 or 70 litres capacity, without an air flow, and in a 124 l capacity glass case. The latter was provided with a current of washed air enriched with carbon dioxide. These tests employed haricot beans (5 experiments), white lupins (7 experiments), oats (2 experiments) and garden cress (2 experiments). One cress test gave a small (5 %) gain in nitrogen but all the rest showed no changes or very small losses in nitrogen. These results are summarized in table 6 (Boussingault 1854, 1855, 1856). Boussingault (1860) concluded that there was no assimilation of free nitrogen from the atmosphere. Mène (1851) did similar pot experiments under bell jars and also found no evidence for nitrogen fixation.

Thus peace was re-established with Liebig but not with other protagonists.

(*b*) *Georges Ville and others*

Georges Ville was the issue of a liaison between Louis Napolean and a housemaid of his establishment at Port Saint-Esprit (*vive l'esprit!*) whose mother was later persuaded to marry a police commissioner of Lyon, M. Georges Ville. Georges Ville junior appears to have been flamboyant and easily offended and earlier had been in dispute with Boussingault on the salary paid to him at the Conservatoire, and on other matters.

Ville grew plants under large bell jars supplied with a continuous flow of clean air where he claimed increases in the nitrogen contents of wheat, rye, colza, rape, tobacco and sunflower in the absence of fertilizer nitrogen (Ville 1850, 1853). This disagreement with Boussingault led to a public confrontation at the Academie des Sciences and to a demand by Ville that a committee of the academy should examine his claim. The sequel had the aura of the Comédie Francaise in that everything went wrong: the committee's plants were poisoned by fumes from nearby paintwork, water was by accident grossly contaminated by ammonia and the chemist

Table 6. Summary of Boussingault's experiments on the utilization of atmospheric nitrogen by plants

		plant	percentage gain or loss of nitrogen
in open pots			
	1837	clover	+20
		wheat (2 experiments)	none
	1838	peas	+50
		clover	+4
		oats	small loss
in 35 l carboys without air flow[1]			
	1851	haricot	slight loss
		oats	slight loss
	1852	haricot	slight loss
		oats	slight loss
in 70–80 l containers without air flow[1]			
	1853	white lupins (5 experiments)	loss
		dwarf haricots (2 expts)	loss
		garden cress	loss
in 124 l glass case in current of washed air[2]			
	1854	lupins	loss
		dwarf haricots	loss
		lupins	loss
		garden cress	small gain

[1] In calcined pumice, manured with plant ash, watered with distilled water.
[2] As above, but fed carbon dioxide.

in charge had suddenly to decamp for urgent family reasons. The ensuing report was inconclusive (Chevreul 1855), but it did stimulate others to continue experiments on enclosed systems with a flow-through of gas and sterile growth medium; in general, these continued to show that neither cereals nor legumes fixed nitrogen.

Ville also conducted research, as did Boussingault, on the ability of plants to assimilate ammonia and nitrate, and on the release of these radicals and of molecular nitrogen from plants and from soil, as well on the possible oxidation or reduction of nitrogen by physical or chemical agents. This confusing scenario was confounded by claims by Cloez (1855), de Luca (1855) and others that porous alkaline substrates, such as calcined brick and possibly plant material, can oxidize nitrogen to nitrate, and that ozone that may be formed by plants might likewise combine directly with nitrogen.

(c) *John Bennet Lawes and Joseph Henry Gilbert*

Meanwhile Lawes & Gilbert at Rothamsted had become interested in the same problem, stimulated, as was Boussingault, by their studies on crop rotations and the long-term effects of fertilizing wheat with mineral salts that contained or did not contain combined nitrogen.

Unlike the other great men in our Pantheon, Lawes was a scion of the landed gentry, growing up in a sheltered country environment. He was educated at Eton and Oxford, leaving without taking a degree, though while at Brasenose he attended lectures on chemistry by Professor Daubeny and later came under the influence of Professor Thomson at University College.

He was only six when his father died but his mother, like Boussingault's, strongly encouraged his interest in chemistry, even allowing the conversion of a bedroom at Rothamsted Manor into a laboratory. His first unsuccessful experiments were attempts to extract the active principles of drugs such as belladonna. This was followed by pioneering work on treating insoluble sources of phosphorus, such as animal charcoal (a waste product) with sulphuric acid to render them more available as manures. This developed into a successful business that financed his widening studies in agriculture and provided a Trust Fund that continued his work after his death (Warington 1905*a*).

Because of his lack of formal training in science, Lawes in 1843 appointed the chemist Dr J. H. Gilbert, who in 1840 had taken his doctorate under Liebig at Giessen, to work with him; a noteworthy collaboration that lasted until Lawes's death in 1900. When at Giessen, Gilbert was a colleague of J. C. A. Voelker who in 1877 became associated with Rothamsted as the superintendent of the Royal Agricultural Society of England experimental farm at Woburn, where in 1884 his son became Director.

Gilbert was the son of a Congregational Minister in Hull. He was educated at Glasgow and London Universities and his appointment at Rothamsted was on the recommendation of Professor Thomson. As a young man Gilbert lost the sight of one eye and severely impaired the other in a shooting accident, but this did not so much interfere with his work because he was able to rely throughout his long life on his wife's constant help in reading and in observation (Warington 1905*b*). In contrast, Lawes enjoyed robust health without physical impairment and repaired annually to the Scottish moors for deer stalking and salmon fishing.

Lawes & Gilbert were phenomenally active and productive in many areas of agricultural research, though their chief fame rests on their manurial experiments. These were started in 1843 and already provided the most compelling evidence yet available for the contrasted responses of cereals and legumes to fertilizer nitrogen. Some of these so-called classical field experiments (Lawes & Gilbert 1895), now maintained for 143 years, continue to afford the most striking demonstration anywhere of the paramount importance of nitrogen for plant growth.

(i) *The Lawes–Liebig quarrel*

The field trials of the 1850s served to spark off, and indeed to inflame, the dispute between Rothamsted and Liebig on the mineral nutrition of plants (Liebig 1855), that was soon to be settled in Lawes & Gilbert's favour in spite of the towering reputation of their adversary.

Today it is difficult to appreciate the enormous influence of Liebig in chemistry. Both Professor Daubeny, who inspired Lawes as his pupil at Oxford, and Gilbert were disciples of Liebig, whose laboratory at Giessen attracted scientists, and even socialites, from all over Europe and America. Liebig, who studied under Gay Lussac in Paris, made many outstanding discoveries in chemistry. Almost single-handedly he founded the science of organic chemistry and before becoming involved in the dispute with Lawes he demolished the humus theory of plant nutrition. He formulated the 'law of the minimum' for growth and was among the first to warn us of the dire consequences of taking more from the soil than we put in. He was a great proselytizer for science at all levels; his '*Familiar letters in chemistry*' was averred by one commentator to be found in almost every English household, and he was instrumental in establishing Germany's first agricultural experimental station at Mockein, near Leipzig, in 1851–2 (Gilbert 1880). The journal named after him is still published (*Liebig's Annalen der Chemie*). He is also remembered less importantly as the popularizer, but not the inventor, of the

Liebig condenser and having a patent manure, a meat-extract baby food and a strong beef sausage named after him, the last perhaps not inappropriately for a man of choleric temper. From humble origins, the son of a dry-salter, he rose eventually to be elevated into the aristocracy of the German Empire as Baron Justus von Liebig (Shenstone 1895).

Lawes & Gilbert's side in their famous controversy was conducted at length in papers heavy with abundant data and argument, as in their 1851 paper and their '*Reply to Baron Liebig's principles of agricultural chemistry*' published by the Royal Agricultural Society of England (1855). Liebig's response was less measured, resorting at the end to vilification and slander as in his confidential letter to Mr Mechi, in rather good colloquial English: 'How ignorant and stupid and devoid of all good sense must be the great mass of agricultural people to allow such a set of swindlers to lead them in all these questions. If you ask any scientific man about the theoretical and practical value of their papers...it is all humbug, most impudent humbug'. Or again, 'Lawes and Gilbert hitch on to me like a vile vermin and I must get rid of them by all means. There is a cowardice among the scientific men in England which I am unable to understand and it is an offence against the public welfare that they have not the courage to take a public stand in that most degrading controversy between science and ignorance'. No correspondence between Lawes and Liebig survives, if indeed any was written. It is pleasanter to report that Liebig's son H. F. Liebig sought Lawes' advice in 1869 on setting up field experiments in Germany, expressing the hope that Lawes would not extend to him the personal feelings he held against his father. Nevertheless, even here the spirit of antagonism shows through in his gratuitous comment that in his opinion it is aimless to undertake long-term manurial trials, which Gilbert rebuts in reply.

Time heals all and in 1893 Lawes & Gilbert were jointly awarded the Liebig Silver Medal for agriculture.

(ii) *Evidence from the Rothamsted field experiments*

Table 7 gives the information available to Lawes & Gilbert in the 1850s from their experiments on continuous wheat grown on Broadbalk field at Rothamsted. Also shown is a

TABLE 7. GRAIN YIELDS OF WHEAT ON BROADBALK FIELD, ROTHAMSTED

(Yields for 1844–1856 in bushels per acre, yields for 1970–1978 in tonnes per hectare.)

	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)
1844	15	—	—	—	—	22
1845	23	—	—	32	—	32
1846	18	—	—	28	—	27
1847	17	—	—	26	—	30
1848	15	—	—	19	—	26
1849	19	—	—	33	—	31
1850	16	—	—	27	—	28
1851	16	—	—	29	—	30
1852	14	17	21	27	28	28
1853	6	10	18	24	23	19
1854	21	24	34	45	49	14
1855	17	18	28	33	31	35
1856	15	19	28	37	39	36
1970–1978	1.7	2.2	3.5	4.8	5.1	5.9

References: Lawes & Gilbert (1985); Garner & Dyke (1969); Dyke *et al.* (1983).

Column headings: (*a*), no fertilizer; (*b*), minerals only (P, K, Na, Mg); (*c*), minerals with 43 lb N per acre (as ammonium sulphate); (*d*), minerals with 86 lb N per acre (as ammonium sulphate); (*e*), minerals with 129 lb N per acre (as ammonium sulphate); (*f*), farmyard manure.

very abbreviated summary of later data, the 1970–1978 yields being given in tonnes per hectare, the yield without fertilizer, of 1.7 t ha^{-1}, being equivalent to 26 bushels† per acre.

From the earliest years of this experiment Lawes & Gilbert recorded large responses to farmyard manure (FYM) and to ammonium sulphate, initially at 86 lb N per acre and later at half and one-and-a-half times this rate; yields with the highest rate equalled that obtained with FYM. More importantly for their increasing concern about the sources of nitrogen for crops, the yields without fertilizer only declined very slowly during this period, and indeed until the end of the century and beyond, as the table 7 shows. Lawes & Gilbert were therefore well aware that there were very large reserves of nitrogen in the soil that could be tapped over long periods of time. Similar extensive trials were made during these years with different crops grown continuously on the same land and with rotations which showed that legumes were either able to trap nitrogen reserves in the soil unavailable to other plants or could use different as yet unidentified sources, possibly including the atmosphere. Table 8 summarizes some of these trials in terms of the quantity of nitrogen harvested in the crops.

The first section of table 8 gives average annual yields of nitrogen (lb N per acre) for two cereals and meadow hay and beans grown in the same field over a number of years without manure of any kind (exhaustion land) and with minerals and nitrogenous fertilizer. The meadow hay (containing legumes) and beans yielded more than 50% as much nitrogen as barley or wheat. A parallel trial with field beans, lasting eight years, showed that the nitrogen yields were increased by minerals (P, K etc.) and further raised by fertilizer nitrogen. The last section of table 8 shows the larger nitrogen yields of red clover compared with wheat when grown in sequence, except when the clover failed, as in 1851.

TABLE 8 *a*. YIELDS OF NITROGEN IN CROPS GROWN CONSECUTIVELY ON THE SAME FIELD WITHOUT MANURE AND WITH MINERALS AND FERTILIZER NITROGEN, HOOSFIELD (EXHAUSTED LAND), 1844–1859

crop	number of years cropped	average annual yield of nitrogen/(lb per acre)
wheat	16	24.4
barley	8	24.7
meadow hay, containing legumes	4	39.4
field beans, no manure	12	47.8
field beans, no manure	8	48.4
field beans, with minerals	8	60.2
field beans, with mineral and nitrogen fertilizer	8	69.0

TABLE 8 *b*. YIELDS OF NITROGEN IN A CLOVER–WHEAT SEQUENCE

crop	year	nitrogen/(lb per acre)
red clover	1849	207
wheat	1850	45
red clover	1851	29
red clover	1852	112

Table 9 refers to the well-known ‘Garden clover plot’ and provides evidence of the exceptional nitrogen-gathering value of red clover that Lawes & Gilbert noted in the introduction to their work on nitrogen assimilation in the early 1860s. Substantial amounts of

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† 1 bushel = 36.37 litres.

Table 9. Garden clover, Rothamsted: nitrogen yields (lb N per acre per year)

year	N yield
1854	125
1855	435
1856–1863	257
1864–1873	133
1874–1883	122
1954–1956	41[a]
1980–1982	615[a,b]

[a] Estimated as 4.1 % (by mass) of dry matter.

[b] C.v. Hungaropoly; K deficiency corrected and *Sclerotinia trifoliorum and Heterodera trifolii* controlled with Benomyl and Aldicarb.

nitrogen were harvested from this plot year by year, but at a declining rate over the next three decades. This decline was shown much later to be caused by other nutritional and disease problems, which when corrected in 1980–1982 allowed much larger yields of nitrogen to be obtained (McEwen *et al.* 1984).

This background and the correspondence with Boussingault as well as Liebig's onslaught and Lawes & Gilbert's experiments with nitrogen fertilizers (Liebig 1855) encouraged the Rothamsted group to undertake detailed studies on whether cereals and legumes can assimilate atmospheric nitrogen. These experiments, the most thoroughgoing so far attempted and foreshadowing those of Hellriegel & Wilfarth, call for detailed description.

(iii) *The Rothamsted experiments on growing cereals and legumes in glass enclosures*

Figure 4 shows one of Lawes, Gilbert & Pugh's plant growth units (1862) of which there were twelve in 1857 and more in 1858. In addition, two metal-framed glass cases for growing plants were sent over from France by M. Ville for their use. Their apparatus differed from ones used before in that air was forced through the equipment by positive pressure of 0.5 lbf in^{-2}† instead of being drawn through by evacuation, so that if leaks should occur there would be no ingress of air, possibly containing ammonia, from outside. The glass bells (9 × 40 in‡ or 16 × 28 in), in which the plants were grown, rested in grooves cut into base-plates of slate (lutes) made gas-tight by filling the grooves with mercury. The grooves were deepened at four places to allow the insertion of tubes for watering. The flow of air, changed $2\frac{1}{2}$ times daily, was washed by passing through concentrated sulphuric acid in Wolff bottles and in horizontal tubes packed with pumice, and then through saturated bicarbonate solution (made from the ignited salt). Condensed water from the inside of the bells was collected and returned to the pots. Additional water, required about once weekly, was prepared by distillation: the first $\frac{1}{5}$ of the distillate was discarded, the remainder being redistilled from a copper still after treating with phosphoric acid. All parts of the apparatus were thoroughly cleaned, washed in distilled water and rinsed in double distilled water before assembly. Carbon dioxide was provided at about 4 % (by volume) concentration, as recommended by Boussingault, by dropping measured amounts of acid on to marble chips.

The growth medium, contained in glazed earthenware pots of a special design, was either Rothamsted heavy clay soil with its stones removed, or pumice, previously heated to redness

† 1 lbf in^{-2} ≈ 6895 Pa.

‡ 1 in = 2.54 cm.

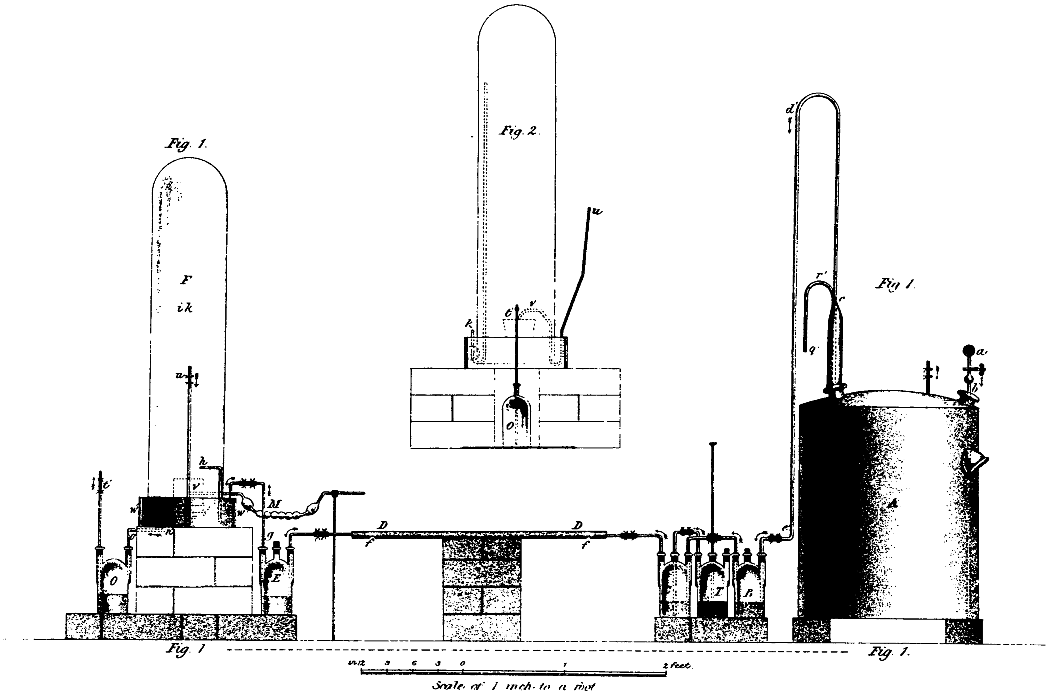

Figure 4. The equipment used by Lawes, Gilbert & Pugh in their 1857 and 1858 experiments on the growing of plants in glass enclosures. Air was passed through the apparatus from the reservoir on the right by displacement with water, being scrubbed in sulphuric acid and bicarbonate before entering the bell jar in which the experimental plants were grown. (From Lawes *et al.* 1862.)

in a muffle, then washed over 8–10 days in water, dried and re-ignited before use. The basic nutrient supply was the ash of the type of plant to be grown; in some tests ammonium sulphate was also added. Control pots containing untreated garden soil gave abundant growth, showing that the physical conditions were suitable for plant culture. Control pots without plants were included to test for possible absorption of nitrogen.

Every possible source of combined nitrogen was either eliminated or assessed by meticulous analysis. For example, the rubber tubing connecting the wash bottles etc. was shown to contain 0.1–0.15% N but this was observed not to perish or release combined nitrogen during a test.

Other processes that could possibly affect estimates of nitrogen fixation were the oxidation of nitrogen to nitrate by ozone, or its reduction to ammonia by nascent hydrogen, as suggested by Mulder (1844), and the loss of nitrogen from organic matter during decay. Before embarking upon their main experiments Lawes *et al.* assayed various substrates for ozone, oxygen, hydrogen, nitrogen and carbon dioxide by direct collection of gases and by analysis of losses. Collection was either made by immersing the material in water and boiling off the gases found, or by taking them off in a Torricellian vacuum, extending the work of Boussingault, Ville and others by using a wider range of substrates: cereal and bean meals, clover leaves and flower heads, germinating seeds and growing plants. These were incubated for different times in air or under anaerobic conditions, in shade and in the light.

Small amounts of ammonia were sometimes found in fermenting meals. Nitrate was found only in rich garden soil, where it was present originally. The losses of nitrogen were inexplicably very variable (0–40 %). Under anaerobiosis, plant intercellular gas was mostly carbon dioxide with hydrogen and traces of nitrogen; no ammonia was found. Oxygen production by plants in air was promoted by light, even by as short an exposure as 20 min; but ozone was not detected in any experiment. The authors remarked 'there is manifestly great reducing power under the influence of the sun'. They also prudently added that, although their experiments provided no support for the view that nitrogen combined either with ozone or hydrogen in their experiments, the possibility remains that they may do so under other conditions.

They also experimented on the effect of ozonized air (obtained by passing air over sticks of phosphorus) on porous and alkaline substrates thought to be suitable for oxidation of nitrogen. These included fresh and ignited soils, slaked lime, starch and bean meal in various combinations; nitric acid was not found. Further trials using germinating cereals and beans which were then allowed to decay under different moisture régimes showed losses of organic matter and total nitrogen, the authors again making the comment that nitrogen losses varied considerably and inexplicably between experiments and did not appear as nitrogen gas.

Having cleared the ground in this exemplary matter, Lawes, Gilbert and Pugh now turned to their growth chamber experiments, less concerned that their results might be questioned on the above grounds. Table 10 gives the total dry matter yields and gains or losses of combined nitrogen per plant, compared with that provided in the seed and medium in the 1857 and 1858 seasons, both for plants given no combined nitrogen and for duplicate sets given ammonium sulphate at the levels indicated.

The lutes used in 1857 were made of slate and in spite of all efforts to free them from combined nitrogen, some evidently remained. This was revealed by the growth of algae on the slate and on the tops of the pots, watered with condensate from the slate. It is to this that the small gains in nitrogen were attributed. The 1858 experiments with glazed earthenware lutes showed no algal growth and the nitrogen analyses generally revealed small losses of nitrogen. Three of nineteen comparisons showed small gains, all within the limits of experimental error.

These results were obtained with their own equipment and that provided by M. Ville and independently of the addition of ammonium sulphate. They therefore concluded that neither cereals, buckwheat nor leguminous crops have the capacity to assimilate atmospheric nitrogen and that the reason for their contrasted nutrition in the field must be sought elsewhere. They did, however, enter one small caveat by noting that everywhere the growth of the plant tops was poor, and that if conditions could be devised to permit better growth beyond that provided by the small additions of ammonium sulphate in the duplicate set of plants, then nitrogen might be fixed. In contrast to the above-ground parts of the plants, root development was prolific, prompting them to comment on the remarkable capacity of plants 'to perform the mysterious offices of the formation of new cells' whatever the circumstances, their analyses showing that this development required 'almost incredibly small amounts of nitrogen'

Had Lawes, Gilbert and Pugh's experimental procedures been less rigorous, contamination would probably have taken place and nodules would have appeared. In this event the discovery of nitrogen fixation would have been anticipated by thirty years, perhaps not altogether to the benefit of agricultural science. Instead of continuing for the next half century with their epoch-making researches on fertilizers, Lawes & Gilbert might have been diverted, with less profit, into soil microbiology, then still a long way from developing its own techniques and philosophy. It is of historical interest and not without poignancy that the only letter from

TABLE 10*a*. YIELDS AND GAINS OR LOSSES OF NITROGEN OF PLANTS GROWN BY LAWES *ET AL.* (1862) IN SEALED GLASS ENCLOSURES AND SUPPLIED WITH ASH MINERALS, N-FREE WATER, AIR AND CARBON DIOXIDE, WITHOUT FERTILIZER NITROGEN

		total dry matter yield per plant/g	gain or loss of combined N[c]/mg	percentage gain or loss of nitrogen
cereals and buckwheat				
1857[a]	wheat	1.412	−0.8	−11.1
	barley	0.867	+2.1	+26.9
1858	wheat	1.740	+0.3	+3.7
	barley	0.560	+0.1	+1.7
	oats	1.148	−0.7	−1.3
1858[b]	wheat	1.060	0	0
	oats	0.690	−0.1	−1.6
1858	buckwheat	0.450	−1.8	−9.9
leguminous crops				
1857	field bean	7.028	−0.5	−0.6
1858	field bean	4.875	+0.7	+0.9
	peas	0.970	−2.1	−12.6

TABLE 10*b*. YIELDS AND GAINS OR LOSSES OF NITROGEN OF PLANTS GROWN SIMILARLY AND SUPPLIED WITH ASH MINERALS, N-FREE WATER, AIR, CARBON DIOXIDE AND AMMONIUM SULPHATE

		total dry matter yield per plant/g	nitrogen added/mg	gain or loss of nitrogen[c]/mg	percentage gain or loss of nitrogen
cereals or buckwheat					
1857[a]	wheat	5.33	28.9	+2.8	8.5
	barley	3.72	26.0	+3.5	13.1
1858	wheat	7.31	50.8	+1.2	2.2
	barley	5.47	46.8	+3.2	6.4
	oats	1.20	28.0	+9.6	3.1
1858[b]	wheat	3.82	22.8	+0.6	2.2
	barley	2.98	22.8	+1.5	5.8
	oats	1.28	22.8	+6.2	23.9
1858	buckwheat	1.97	40.2	+1.6	5.1
leguminous crops					
1858	peas	1.01	4.0[d]	+1.6	7.0
	clover	4.20	42.8	+4.7	6.6
1858[b]	field bean	4.30	18.8	+5.1	7.9

[a] Slate lutes.
[b] In glass case provided by M. Georges Ville.
[c] Above that provided in seed, medium and ammonium sulphate.
[d] As given by Lawes *et al.* (1862); probably a misprint for 40.0.

Boussingault to Gilbert that has survived stated that he had so much confidence in the Rothamsted work that should this demonstrate nitrogen fixation he would be happy to change his mind. This letter, in an elegant flowing hand, is in contrast to the rude and blunt missives of Liebig.

Dr Pugh returned to America in 1859 to become President of the State Agricultural College of Pennsylvania and did no further work on nitrogen fixation. He was a man of great ability and strong character who from humble beginnings attained a position of great influence. Evan Pugh was orphaned at the age of 12 and after attending the Manual Labour Academy at Whitestown took up farming and blacksmithing, for which, at six feet two inches and physically powerful, he was well endowed. His father's estate provided him with a modest inheritance of \$2800 which he used for study at Leipzig, Heidleberg and Göttingen, and from there went to work at Rothamsted. He was still only 31 when he took up his onerous post in Pennsylvania, transforming in a few short years a run-down institution on the point of closure into a well-administered college with a growing reputation, and where he taught several branches of agricultural science. He died in 1864 while still a young man (Dunaway 1946).

(*d*) *The intervening years*

The period between 1862 and 1886 provided numerous reports on the high nitrogen content of leguminous crops without advancing our understanding of how they had acquired this status (see, for example, Schultz-Lupitz 1881; Atwater 1886; Deherain 1873). From time to time new ideas were floated and data presented to support one or other of the explanations so far offered, such as the influence of electricity and soil microbes (Berthelot 1877, 1885). Thinking at Rothamsted continued to be influenced by their experience of fertile soils containing large reserves of combined nitrogen, the availability of which might provide the key for the solution of the problem, a point that was again stressed by Gilbert in his Berlin address. Lawes & Gilbert visited Boussingault at Liebfrauenberg in 1883 and recorded that they continued to believe that plants could not use the nitrogen of the air; reaffirming their correspondence on this question in 1876.

During this intervening period Lawes & Gilbert continued to amass much information on the nitrogen content of leguminous crops and of the soils beneath them. Table 11 gives examples of this later work, comparing five different legumes with wheat after fallow, all grown on unfertilized, exhausted land (Hoosfield). The legume yields of nitrogen ranged from 22–160 lb N per acre, and very large differences were found in the N content of the underlying soils. Soil nitrogen appeared to be most exhausted beneath lucerne, which had the highest yield of nitrogen, thus reinforcing their long-maintained view that the major source of a legume's nitrogen lay within the soil.

TABLE 11. YIELDS OF NITROGEN (LB PER ACRE) IN WHEAT ALTERNATING WITH FALLOW AND IN VARIOUS LEGUMINOUS CROPS WITHOUT NITROGENOUS FERTILIZER; AND THE AMOUNTS OF NITRATE (LB PER ACRE) DETERMINED IN THE SOIL AT DIFFERENT DEPTHS BENEATH THESE CROPS (LAWES & GILBERT 1895)

	fallow–wheat	*Trifolium pratense*	*Trifolium repens*	*Vicia sativa*	*Melilotus leucantha*	*Medicago sativa*
average N yield	12	22	47	75	64	160
nitrogen content of soil in						
0–27 inch layer	30	—	67	18	—	11
28–54 inch	8	—	25	13	—	3
55–81 inch	8	—	25	18	—	21
82–108 inch	7	—	29	16	—	1

At Bernburg poor soils provided exactly the opposite experience, which increased the opportunity for detecting the effects of nodulation. Nevertheless, even if the work at Bernburg had not been successful, the discovery of nitrogen fixation could not have been long delayed; the time for it was overripe. That the crucial connection between nodulation and fixation was missed for so long is surprising when it is recalled that Boussingault noted in 1859 that the best-grown legumes were the ones with root tubercles and made the comment that 'mycodermic vegetation' might be the agents of fixation. Lachman (1856, 1858), who first described bacteria-like organisms in nodules, also made the suggestion that the nodules fixed nitrogen; pregnant speculations then as yet unsupported by facts.

That the discovery was at last made at Bernburg also owes something to the influence of Frederick of Prussia, who was strongly persuaded by Liebig to promote agricultural education and science. He was responsible for founding the institutes at Dahme and Bernburg, and by 1886 there were in Prussia four higher colleges of agriculture, staffed by eighty professors, forty-one agricultural schools of a lower status, and experimental farms specializing in crop and pasture work, irrigation, industrial agriculture, horse management, silk production, bee-keeping, fisheries, viticulture and gardening (Adams 1886). Although Bachelbronn, Rothamsted and Pulawy in Poland were the pioneers in agricultural research they were soon joined by these developments in Germany and by the founding of the State Colleges in America.

4. THE FIRST REACTIONS TO HELLRIEGEL AND WILFARTH'S DISCOVERY

As we have already noted, Professor Frank seemed to be the only doubter at the Berlin meeting (Frank 1888, 1889, 1890, 1892). Soon confirmatory results were widely reported (Marshall-Ward 1888; Bréal 1889; Atwater & Woods 1890; Peterman 1892; Schloesing & Laurent 1892*a*, *b*; Nobbe 1896; Buckhout 1889, 1890), those at Rothamsted in the three years after the discovery being the most extensive (Gilbert 1890; Lawes & Gilbert 1890). In a postscript to their first paper after Berlin (Lawes & Gilbert 1889) they reported benefit from inoculation (microbe seeding) with peas but not lupins, and although conceding Hellriegel & Wilfarth's case they still thought that the bulk of a legume's nitrogen came from the soil. Then followed intensive work with annual and perennial species of legumes, namely peas, vetch, clover, lucerne, sainfoin and yellow and blue lupins (being advised by Hellriegel after their earlier failure that lupins only grow well in open-textured media). In his 1889 paper Gilbert quaintly refers to crimson clover, *Trifolium incarnatum*, as 'incarnate clover'. Plants were grown in sterilized soil or in sand in specially made tall glazed earthenware pots or in slate-lined pits so as to be able to examine nodulation in sequential sampling. Sterilization procedures were good, only occasional plants forming a few late nodules. Microbe seeding in nearly all instances induced nodulation and strikingly improved growth; the nodulated plants were rich in nitrogen but no increases in nitrogen were recorded in the sand.

Lawes & Gilbert speculated on the mechanism of nitrogen fixation, and having measured the reaction of fresh nodules which were 'red and glistening' thought that their alkaline reaction was important for the fixing process (Gilbert 1891).

Just as Hellriegel's work depended upon the special analytical skill of Wilfarth, so Lawes & Gilbert's later work relied upon the sensitive methods that Way (1903), Warington (1883) and later Miller were developing at Rothamsted for determining nitrate in soil, rain and drainage waters (Lawes & Gilbert 1881). It was Warington also (1878–1891) who, extending the studies of Schloesing & Müntz (1877, 1878) showed that nitrification was a two-stage process mediated by different microorganisms. Warington was only narrowly beaten by Winogradsky (1890) in the isolation of *Nitrosomonas*. Indeed Winogradsky later admitted that his culture contained ammonia as well as nitrite oxidizers (Macdonald 1986).

Dr N. H. J. Miller and Mr J. J. Willis were in charge of the last set of experiments done at Rothamsted on nitrogen fixation by Lawes & Gilbert. Edwin Grey, the field superintendent at the time, gives a nice account of them in his 'Reminiscences of Rothamsted' (1922). Because no glasshouse space was then available, Gilbert took over an old glasshouse that Lawes had provided earlier for the allotment-holders on the estate and which had fallen into disrepair. This was refurbished and provided with benches, perforated zinc screens and sunblinds. It was also securely locked against the curious, the key being hung on a nail by Edwin Grey's large and famous balance. Each morning Sir Henry, with Mr Willis and Mr Grey in attendance, would take the key and proceed to the allotment greenhouse to water the plants and take notes. 'As they grew we soon saw a remarkable difference between those in the microbe-seeded pots and those in the pots where no extract had been applied...'. Edwin Grey concluded his reminiscences as follows: 'The newer and up-to-date Laboratories that have arisen, together with the host of young people, research workers and others who have passed through right up to the present time, have formed most happy associations. All these pleasant memories, I trust will abide with me until my life's end'. This is a sentiment that today's microbiologists at Rothamsted are no longer able to echo.

However, not all commentators were so sanguine. The report of the Agricultural Association (Jamieson 1905) concluded that 'the legume tubercle theory is untenable...the nitrogen of the air is directly utilizable by plants', and Moeller (1892*a*, *b*) was quite unpersuaded that the bacteria in the nodules fixed nitrogen.

5. Into the twentieth century

At about the time of Hellriegel's discovery, or shortly thereafter, the nodule bacteria were isolated (Beijerinck 1888), as were other soil organisms involved in the nitrogen cycle: free-living nitrogen fixers (Bertholet 1885; Winogradsky 1893, 1894; Beijerinck 1888*a–e*, 1890, 1891, 1901), nitrifiers (Winogradsky 1890, 1892; Warington 1891) and denitrifiers (Weissenberg 1897). Earlier ideas on nodule aetiology (Lachmann 1858; Frank 1890; Marshall-Ward 1895) had to be revised and a start was made on basic studies on the structure and physiology of the symbiosis and on applying the results to practical agriculture (Beijerinck & van Delden 1903; Bottomley 1907; Remy 1907; Nobbe & Hiltner 1896; Nobbe *et al.* 1891; Morck 1891; Pruching & Harding 1906; Süchting 1904*a–c*).

It would be impossible to present an adequate conspectus of work on nitrogen fixation over the past 100 years, even if space and time were not limiting. Nor is it wise, in the face of this gathering, to follow a 'Great Man' theory of historical presentation by picking out only those who might be thought to be responsible for the more momentous advances. Instead I shall endeavour to trace the spread of ideas worldwide from those centres caught up in the original discovery and its aftermath; the topology of nitrogen fixation. The turn of the century was unpropitious for agricultural research. Then, as now, purblind accountants were chiefly concerned with cost-effectiveness (though this silly term had not yet been coined) and short-term objectives. Cheap food was to be had from overseas, and the Board of Agiculture for Great Britain stated 'Agriculture is dead and the business of the Board is to bury it'.

Lawes & Gilbert had recently died, full of years and showered with honours, and had not their farm and laboratories been endowed with a trust fund and by a public supporting organization founded in 1904 (The Society for Extending the Rothamsted Experiments), the farm would have been sold and the remarkable achievement of sixty years would have come to an end. It was also fortunate at this juncture, partly as a result of the recent work on nitrogen fixation, that Mr J. F. Mason provided funds to build a new bacteriology laboratory at Rothamsted, completed in 1906. Before this benefaction he conducted, in association with Rothamsted, large-scale experiments with legumes on his farm at Eynsham Hall, Oxfordshire, and as Member of Parliament for Windsor he often spoke in the House on scientific agriculture.

After confirming Hellriegel & Wilfarth's results, Rothamsted's contribution to nitrogen fixation research lapsed until its first bacteriologist, H. B. Hutchinson, who trained with Koch at Göttingen, was appointed in 1909. Miller continued at Rothamsted until 1916 but did no further work on nitrogen fixation, working instead on fertilizer chemistry under the close supervision of Gilbert and later his successor Sir Daniel Hall, but his continuing interest in nitrogen fixation was shown in his review article on soil inoculation (1896) and by the large collection of reprints he amassed on the subject. These he had bound and left to the Bacteriology Department. Hutchinson and his colleague Richards (1917, 1918) were more interested in free-living than symbiotic fixation, the extensive study of which had to await the appointment of the Oxford zoologist H. G. Thornton in 1919 to lead the Rothamsted group.

From 1910 Winifred Brenchley worked in the Botany Department on the toxic effects of copper, arsenic, etc., on crops and weeds and discovered the stimulating activities of manganese and boron on plant growth, particularly of legumes (1914), later to be followed in collaboration with Thornton (1925) by demonstrating the requirement of the nodule's vascular system for boron. The department's first visiting worker from India to study nodulation came in 1923, to be followed by others from that continent and from China, Portugal, Poland and various centres in Africa, Australia and the Americas.

Although the discovery of symbiotic nitrogen fixation occurred towards the end of the working lives of Hellriegel & Wilfarth (and of Lawes & Gilbert) its effects on further work could hardly have been greater. Certainly until 1914 most of this work was done in Europe and the U.S.A. at centres having no connection with Bernburg or Rothamsted.

Table 12 shows the quinquennial distribution of papers on legume nitrogen fixation from 1830 to 1940 (as listed by MacDougal 1894; Fred *et al.* 1932 and supplement; Wilson 1940); (*a*) by source and (*b*) by major topic. After the small peak in publication in the middle years of the past century, interest lapsed until 1886 when there followed a sharp increase in papers, mainly from workers in France and Germany with fewer contributions from the U.K., U.S.A., Scandinavia and Italy, and at the very end of the century by two from Australia. By this time nitrogen fixation research was well established in America and by 1918 this became dominant and has remained so in terms of the numbers of reports published. During the last decade under review, papers on nitrogen fixation appeared in twenty countries. Until about 1890 most studies were descriptive or physiological–nutritional but after this date these were superseded by papers covering many aspects of the bacteriology of *Rhizobium* and of agricultural applications. After some vacillation the taxonomy of the nodule bacteria was elucidated, being assigned successively to *Bacillus*, *Bacterium*, *Pseudomonas*, *Schinzia* and *Rhizobium*, with species tentatively defined by host relationships; the type species, somewhat ironically, was designated *Rhizobium leguminosarum* Frank (Smith 1911, Waxman 1927). Progress was made in tracing the routes of infection in the major hosts and much further descriptive work was done on nodule structure and development.

Table 12. Quinquennial distribution of papers relating to nitrogen fixation between 1830 and 1940

(Abbreviations: F, French; G, German; E, English; Am, North American; O, Other; M, morphology, structure, etc.; P, nutrition and physiology; Ba, bacteriology; Ag, agronomy; I, inoculation; Ec, ecology.)

	(*a*) distribution by location					(*b*) distribution by main topic (%)					
	F	G	E	Am	O	M	P	Be	Ag	I	Ec
1836–1840	6	—	—	—	—	—	100	—	—	—	—
41–45	—	1	—	—	—	—	100	—	—	—	—
46–50	3	—	—	—	—	100	—	—	—	—	—
51–55	16	1	2	—	1	10	90	—	—	—	—
56–60	3	2	1	—	1	43	57	—	—	—	—
61–65	—	—	5	—	—	—	100	—	—	—	—
66–70	1	—	—	1		50	50	—	—	—	—
71–75	2	—	—	—	2	25	75	—	—	—	—
76–80	2	7	—	—	—	66	22	11	—	—	—
81–85	—	5	—	2	—	57	43	—	—	—	—
86–90	26	46	11	9	13	34	51	7	4	4	—
91–95	14	55	5	13	5	22	51	9	3	12	3
96–00	6	33	8	18	4	4	16	16	10	54	—
1901–05	5	20	2	17	6	8	14	34	6	34	4
06–10	2	17	4	32	8	5	8	38	10	32	6
11–15	3	22	1	29	8	9	9	23	2	50	6
16–20	—	4	2	42	10	5	20	29	8	27	7
21–25	3	11	5	63	9	7	12	34	5	36	7
26–30	8	16	15	106	26	9	16	30	10	24	10
31–35	12	23	22	114	64	7	23	32	21	13	5
36–40	12	20	32	126	92	3	50	29	11	6	1

Shortly after the recommended use of soil as an inoculant to promote nodulation (Salfeld 1896), legume inoculants containing pure cultures of nodule bacteria were produced, firstly in Germany under the name 'Nitragin'. They were quickly developed in America (Atwater & Woods 1889; Duggar 1897; Moore 1905) and later at Rothamsted, supported by basic research in the Mason Laboratory, which led to commercial production by an outside firm (Thornton & Gangulee 1924; Thornton 1929). Recently, under the new dispensation that supports things that make money, this activity has returned to Rothamsted. Other speakers in this symposium discuss the principles and practices of inoculation; all I need to add in the historical context is to recall that problems were met from the outset (see, for example, Harrison & Barlow 1906); inoculants were a fruitful area for premature, ill-prepared and sometimes exploitive development and gross overcharging; Ferguson (1906) complains of a rate of U.S. $6 an acre.

Only a residuum of work continued in Europe during the war and into the postwar period, when three centres of research on symbiosis became pre-eminent: those at Helsinki, Rothamsted and Wisconsin. The Wisconsin group had early connections with both Rothamsted, where Elizabeth McCoy (1932) was a visiting worker, and Helsinki, where P. W. Wilson was involved in joint experiments with A. I. Virtanen on the question of the excretion of fixed nitrogen from the root (Virtanen 1937; Virtanen & Laine 1939; Wilson & Burton 1938). The Wisconsin group prepared the first comprehensive monograph on nitrogen fixation, '*Root nodule bacteria and leguminous plants*' (Fred *et al.* 1932), and its sequel '*Biochemistry of symbiotic nitrogen fixation*' (Wilson 1940). Wilson & Fred (1938) were already concerned as to how to handle the imminent literature explosion. By now the rate of increase in the number of papers on nitrogen fixation was intermittently exponential as each discovery triggered a spate of new work. They thought that this could not continue indefinitely, forecasting a levelling off at about 100 papers per year in the 1970–1980 decade. In the event this has been much exceeded because of the stimulating effects of new developments, many unimaginable fifty years ago. The sheer volume of this work is intimidating. Papers on nitrogen fixation have appeared mainly in biological and agricultural journals, and increasingly in miscellaneous reports, symposia and edited compilations (e.g. Hardy *et al.* 1977; Broughton 1981–1983). These record the inception and flowering of the many growing points of our subject.

The significance of a discovery (unlike Hellriegel & Wilfarth's) is not always immediately apparent, only becoming so when other advances make their further development feasible. Thus haem and cytochrome chemistry had to be sufficiently well known to point the significance of the very early observations on nodule pigments. Similarly the discovery of the legume's particular requirements for minor elements first shown sixty years ago for boron at Rothamsted, and for molybdenum by Anderson at Adelaide in 1942 were only of agronomic interest until their roles in enzyme function were elucidated, and until nitrogenase was isolated and characterized. Again, early work on *Rhizobium* variation, bacteriophage and plasmid curing did not much advance our understanding until the modern techniques of microbial genetics were available; many more examples will come to mind.

The current trend towards increasingly detailed analysis of the genetics, structure, metabolism and biochemistry of nitrogen-fixing systems will continue because in spite of all the advances that have been made, many of the fundamental questions relating to symbiosis and its control remain unanswered.

The oil-price rise in the 1970s also stimulated work on how best to use legumes in agriculture and promoted a large increase in internationally funded research, especially to improve the food pulses of the tropics. These programmes covered *Phaseolus* beans in the Centro Internacional de Agricultura Tropical (C.I.A.T.) at Cali, Colombia; *Vigna unguiculata*, African soybean and winged bean at the International Institute for Tropical Agriculture (I.I.T.A.) at Ibadan, Nigeria; *Cicer arietinum* (Kabuli type), *Vicia faba*, and lentils at the International Center for Agricultural Research in the Dry Areas (I.C.A.R.D.A.) at Aleppo, Syria; *Cajanus cajan*, *Arachis hypogea* and *Cicer arietinum* (large seed type) at the International Crops Research Institute for the Semi-arid Tropics (I.C.R.I.S.A.T.) at Patancheru, India; and soybean and mung bean at the Asian Vegetable Research Development Centre (A.V.R.D.C.) at Shanhua, Taiwan. Each of these centres holds large collections of germ plasm, in excess of 10000 accessions for each species, in addition to the national collections, such as the soybean collection at the University of Illinois and the *Pisum* collections at Weibullsholm, Sweden, and at the Institute for Plant Industry, Leningrad, USSR. Collections of *Rhizobium* are held at some of these centres and at NifTAL, Hawaii, U.S.A., and elsewhere (Summerfield & Roberts 1985; Davies *et al.* 1985; Smartt & Hymowitz 1985; Skinner *et al.* 1983). Forage legume collections listed by Ellis Davies (1984) under the auspices of the International Board for Plant Genetic Resources (IBPGR) contain 162 species of legume in sixty collections. These are mostly wild ecotypes, the Australian and Israeli collections being the most comprehensive. However, as the annual report of IBPGR for 1984 points out, there is significant genetic erosion of tropical forage legume resources. Many minor grains and other legumes are not covered by these organizations and unless measures for their conservation are put in hand valuable resources will be irretrievably lost. Botanically the Leguminosae is one of the largest families of flowering plants, comprising about 20000 known species of extraordinary diversity, including many actual and potential sources of food, fodder, fibre, drugs, timber and other products (Allen & Allen 1981). In the drier regions and on poor soils wild legumes are good survivors but their diversity is also great in tropical rain forests, currently most under threat from clearances for cultivation and from large-scale commercial logging (Fosberg 1973; ICUN 1980). Because they destroy the soil's integrity these processes render the cleared tropical rain forest, unlike its temperate counterpart, a non-renewable ecosystem (Gomez-Pompa *et al.* 1972). Legumes, in common with other crop plants, also suffer from drastic reductions of genetic diversity because of more intensive farming methods (Harris 1969); as Frankel & Soulé (1981) urge, land-race populations are a precious heritage that must be conserved.

6. THE TOPOLOGY OF RECENT NITROGEN FIXATION RESEARCH

The interactions between researchers, their workplaces and subject matter are extensive and complex and to focus attention upon those of interest today I will restrict myself to examining only the evidence from material published in international Congresses on nitrogen fixation edited by: Lie & Mulder (1971), Newton & Nyman (1975), Stewart (1975), Nutman (1976), Newton & Orme-Johnson (1979, 1980), Newton *et al.* (1977), Lyons *et al.* (1980), Stewart & Gallon (1980), Gibson & Newton (1981), Veeger & Newton (1984) and Evans *et al.* (1985).

Evidence of a connection between laboratories will be shared authorship. (Other important influences sometimes appear in the acknowledgement section of a paper, but unless they follow from or lead to joint publication they will have to be left on one side.)

To illustrate these links, laboratories are set out on the circumference of a circle, and where two or more laboratories have collaborated in a study they are joined by a line. The size of the disc representing each laboratory indicates the number of papers attributable to it.

Figure 6 summarizes these relationships for the 1971 symposium, which like the succeeding ones covered free-living as well as symbiotic fixation, and relevant studies on the chemistry of the reduction of nitrogen. Thus Kyoto and Dacca presented a joint report, and Rothamsted had links with Sofia, Prague and Luton Technical College, whereas C.S.I.R.O. Canberra, which on this occasion contributed most papers, had no links with other institutions. The symposium volume contained 48 papers attributable to 90 authors from 34 centres, 11 of which were linked.

These relationships are summarized in table 13, which lists the number of centres contributing papers to each symposium, showing those most productive in this respect and those most active in collaborative work. Forty-seven papers (103 authors) were published in the symposium held at Pullman, Washington in 1975. The Corvallis, Kettering, du Pont and Wisconsin laboratories submitted most papers, none of which, however, was published jointly with other centres. Sixteen centres were linked in pairs, no laboratory being associated with more than one other. The I.B.P. symposium of 1975 contained 69 papers from 53 centres, Rothamsted and Wisconsin contributing most papers. Twenty-seven papers were linked by joint authorship, Dundee, Rio de Janeiro, Rothamsted and Wageningen having two links each, the remainder being single. The 1978 conference at Wisconsin was of similar size, Kettering, Wisconsin and Brighton again contributing most reports; the actual percentage of joint work was smaller than heretofore. The 1977 conference at Salamanca was also smaller with 37

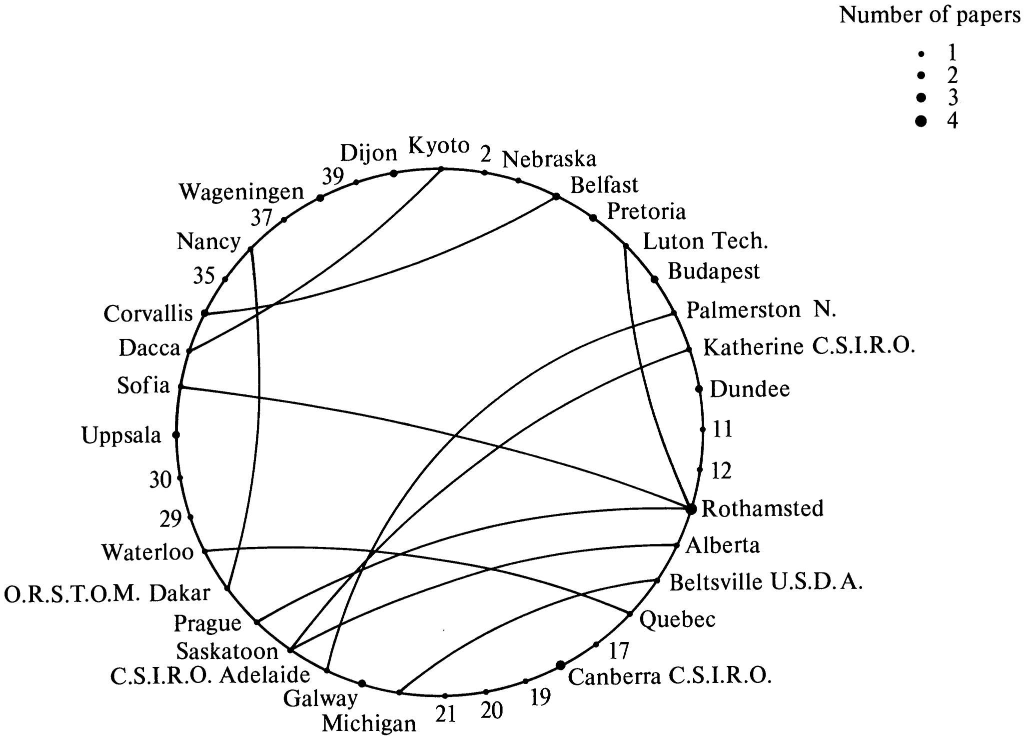

Figure 6. Diagram to show the number and sources of the contributions to the 1971 symposium on nitrogen fixation and their interrelationships. The size of the disc representing each laboratory is proportional to the number of papers attributable to it; joint publications are joined by curved lines.

papers contributed from 32 centres. Wisconsin and Brighton, with 3 and 4 papers respectively, had links with three other centres; the remaining eight links were between different pairs of institutes and 18 laboratories had no outside authors. The proceedings of the Phytochemical Society meeting on nitrogen fixation at Brighton in 1979 appeared as 19 invited chapters from 14 different laboratories. Brighton contributed 5 papers and Wageningen 2; there were no links.

The 1980 Australian conference was large, having contributions from 134 centres (240 contributions). Canberra (C.S.I.R.O. and A.N.U.), Brighton, Kettering, Harvard and Michigan contributed most papers and the Massachusetts Institute of Technology (M.I.T.) Canberra (C.S.I.R.O. and A.N.U.), Brighton, Kettering and Sydney collaborated with the largest number of other institutes, although it is of interest that seven of the eight linkages for M.I.T. relate to a single paper on the prosthetic groups of nitrogenase. Exactly half of the centres were not associated with other laboratories; these included Corvallis, Dundee and Rothamsted. The Netherlands meeting in 1984 was even larger, with papers from 179 laboratories, half being linked. Most linkages were given by Brighton (12 links). Rothamsted (7) and Canberra C.S.I.R.O. and Wageningen (5 each). All the 15 papers from A.N.U. Canberra were unlinked, and only one of the 14 papers from Leiden had a co-author from another institution.

At the Corvallis meeting, also in 1985, work was presented from 160 centres. Most papers and posters came from Brighton (with 25), Canberra A.N.U. (19), Wisconsin (15) and Corvallis (14). Brighton had joint reports with 15 other laboratories, Canberra A.N.U. with

Table 13. The contributions to international symposia on nitrogen fixation, 1971–1985, classified by location and by occurrence of joint publications, etc.

year and place of conference	no. of contributing laboratories	no. of papers and posters	no. of authors per paper	percentage of joint papers	centres contributing most papers (no. of papers and posters)	most linked centres (no. of linkages)
1971 Prague, Wageningen	34	48	1.98	50.0	Rothamsted (4), Canberra C.S.I.R.O. (3)	Rothamsted (3), Adelaide C.S.I.R.O. (2)
1975 Pullman	40	47	2.19	40.0	Dupont (4), Brighton (3), Kettering (3), Wisconsin (3)	All linkages single
1976 Edinburgh	53	69	1.92	50.1	Rothamsted (4), Wisconsin (4)	Dundee (2), Rio de Janeiro (2), Rothamsted (2), Wageningen (2)
1977 Salamanca	32	37	2.59	46.8	Brighton (4), Kettering (4), Wisconsin (3), Wageningen (3)	Brighton (3), Wisconsin (3)
1978 Wisconsin	40	41	3.00	25.0	Kettering (4), Wisconsin (3), Brighton (3)	Harvard (2), Ontario (2)
1979 Brighton	14	19	3.30	0	Brighton (5), Wageningen (5)	no joint papers
1980 Lake Tahoe	23	37	2.75	43.5	Davis (17), Beltsville, etc. (2)	Davis (9), Florida (2), Beltsville (2)
1980 Canberra	134	240	2.92	50.3	Canberra C.S.I.R.O. (13), Brighton (11), Canberra A.N.U. (13), Harvard (7)	M.I.T. (8), Canberra A.N.U. (5), Canberra C.S.I.R.O. (6)
1984 Noordwigkerhout	179	389	3.65	50.3	Brighton (19), Wageningen (18), Canberra A.N.U. (15), Leiden (14)	Brighton (12), Rothamsted (7), Canberra C.S.I.R.O. (5), Wageningen (5)
1985 Corvallis	160	363	3.30	59.6	Brighton (25), Canberra A.N.U. (19), Wisconsin (15), Corvallis (14)	Brighton (15), Canberra A.N.U. (8), Rothamsted (6), Wisconsin (5)

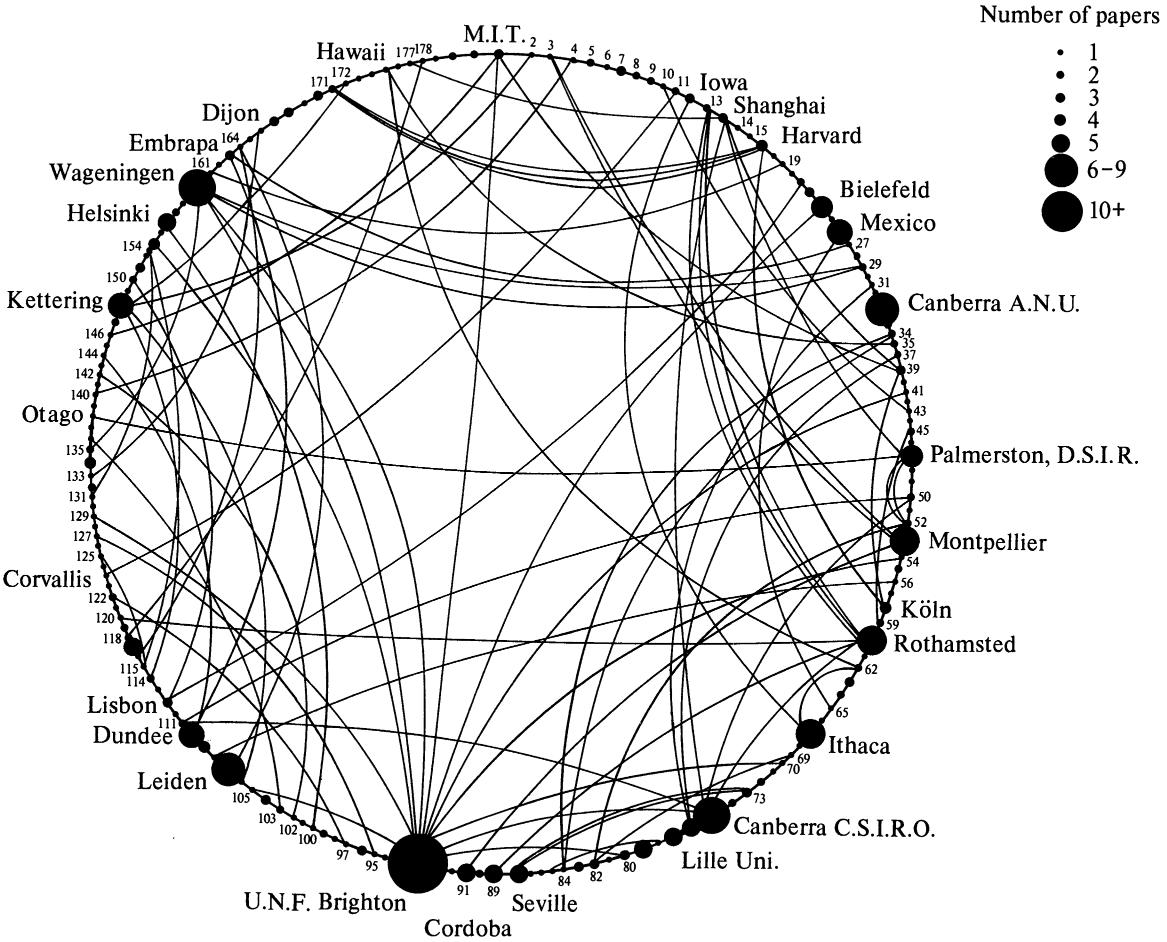

Figure 7. Diagrammatic representation of the 1985 symposium on nitrogen fixation.

8, Rothamsted with 6 and Wisconsin with 5. Figure 7 represents graphically the results of the survey of the Corvallis meeting, showing the phenomenal increase in nitrogen fixation research since 1971 (figure 6) and the very complex patterns of interactions between research centres and their staffs. The sharp rise in numbers of reports since 1980 is attributable in part to the inclusion of 'posters' in the published proceedings. The number of authors per paper increased over the period, in individual instances sometimes dramatically as when a four page report required the collaboration of 14 specialists. However, except for the Wisconsin and Brighton meetings, the proportion of papers submitted from more than one laboratory remained almost constant.

Discounting the quirks of geography and the problems of funding people to attend far-off conferences, it is clear that changes take place in the influence of the major research centres. Rothamsted appeared to be the most influential in 1971, Rio de Janeiro, Rothamsted, Wageningen and Dundee in 1975–6, M.I.T., Canberra (C.S.I.R.O. and A.N.U.), Kettering, Brighton and Sydney in 1980, Brighton, Rothamsted, Canberra (C.S.I.R.O.), Wageningen, Palmerston North, Kettering and Harvard in 1984 and Brighton, Canberra (A.N.U.), Rothamsted, Wisconsin, Canberra (C.S.I.R.O.) and Michigan in 1985.

Knowing the special interests of the major centres these diagrams show at a glance how the focal areas of interest have changed radically over even so short a period as 14 years, perhaps most dramatically shown by the results of the combined chemical and genetic onslaught on the structure and function of nitrogenase. In spite of its almost unsurpassed complexity, nitrogenase is now better characterized than most enzymes, even to its genetic coding and DNA sequencing.

The front runners in these endeavours change places from time to time but tend to keep ahead of the rest of the field. This may reflect the time taken for an idea to be translated into a research programme, and for an individual or research school to become influential and attract collaboration. Once established, such reputations have their own momentum, but eventually they decline and are replaced by others. The new ideas or novel techniques that will initiate future growing points may or may not arise in the large research establishments; one hopes that the lone researcher will not disappear altogether.

These changes are natural and proper processes of birth, growth, maturity and supersession. However, it has to be said that, certainly in this country, there are also unnatural and improper influences at work that affect research on nitrogen fixation and much else besides. This makes it unprofitable to speculate on future trends. The nitrogen fixation unit at Brighton, the Canberra groups, Wisconsin, Corvallis and other identifiable candidates on our diagram look to have assured and exciting futures, but the same can no longer be said of Rothamsted. As ruefully remarked in the recently published 'The Future with Rothamsted' its full-time research staff on nitrogen fixation is now reduced to one person, recalling the first nine stanzas of the nursery rhyme 'Ten Little Nigger Boys'. About the Rothschild contractual arrangements that have led to this sorry situation, Anthony Tucker has written 'Arguably they (the responsible Research Councils) are presiding over the interment of British scientific integrity, and for this they may never be forgiven'.

Today we commemorate and celebrate the achievements of the past and it is therefore inappropriate to animadvert upon present ills. Hellriegel had his own difficulties. In 1897 he wrote a short article '*Eine Plauderei über Forschungs-Methoden*' (which freely translates as 'A fireside chat on doing research') in which he remarks, more in sorrow than in anger, on his own difficulties in obtaining support for his staff, quoting the tag:

'Wer't mag, die mag't
Un wer't nich nag,
Die mag't so woll nich maegen'.

This old North German vernacular is not easily translated. Perhaps it is best rendered by our own saying 'Like it, or lump it'; in the context of today, this is *not* good advice.

I thank former colleagues at Rothamsted for their critical reading of this study, especially Miss Blanche Benzian for her invaluable help with the German literature; and George Dyke for guidance through Rothamsted's extensive nineteenth-century archives. The librarians at Rothamsted, Oxford and the Royal Society have also greatly helped in locating some of the older and more obscure literature.

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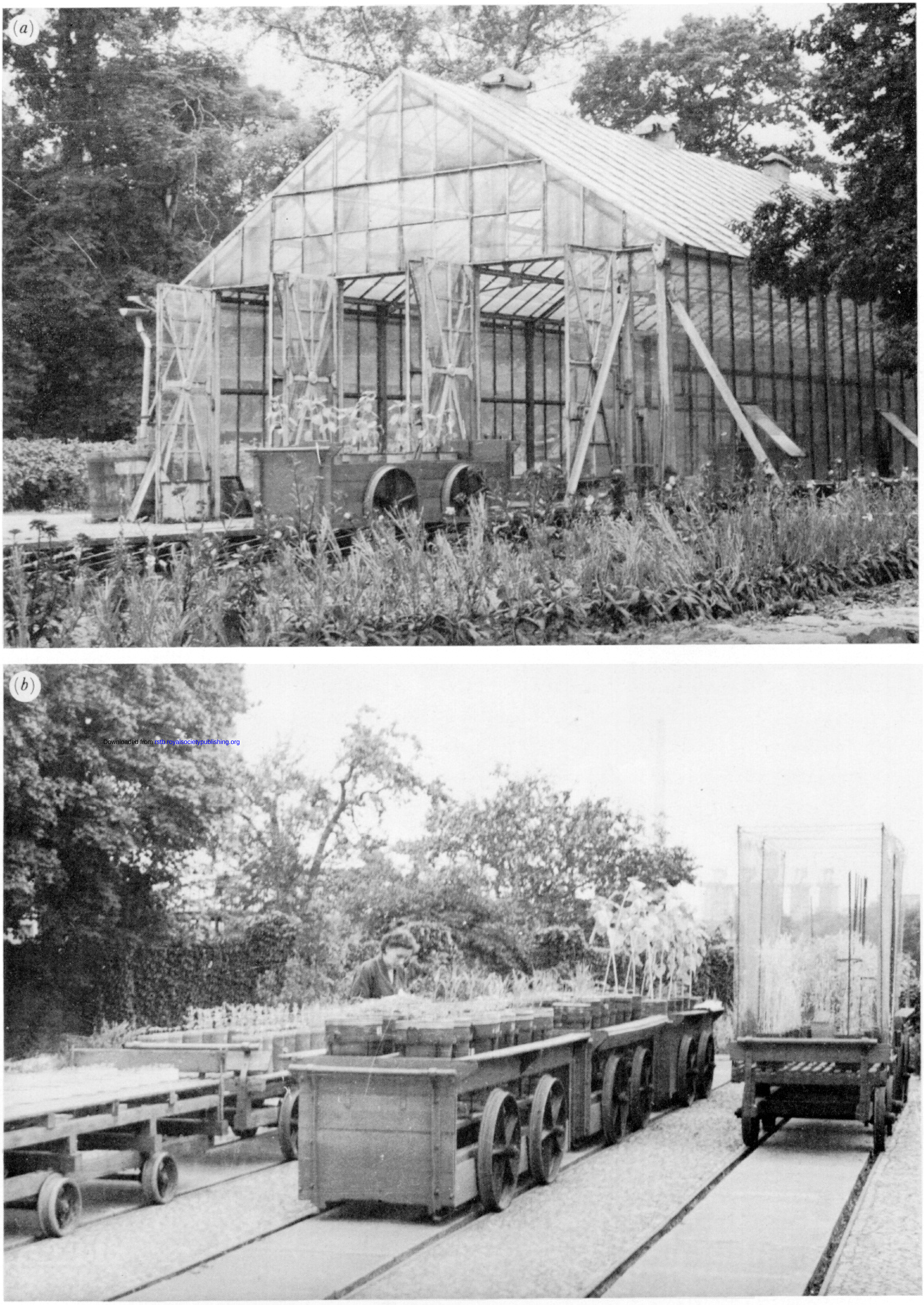

IGURE 3. (*a*) The glasshouse at Bernburg where Hellriegel and Wilfarth conducted their historic experiments; (*b*) the glasshouse trollies at Bernburg.

IGURE 5. Portraits of the major workers on nitrogen fixation in the nineteenth century: (*a*), Professor Hermann Hellriegel (1831–1895); (*b*), Bust of Hellriegel at the Institut für Getreideforschung, Bernburg-Hadmersleben; (*c*), Professor Hermann Wilfarth (1853–1904); (*d*), Jean Baptiste Boussingault (1802–1887); (*e*), Georges Ville (1824?–1897); (*f*), Baron Justus von Liebig (1803–1873); (*g*), Sir John Bennet Lawes (1814–1900); (*h*), Sir Joseph Henry Gilbert (1817–1901); (*i*), Dr Evan Pugh (1828–1864).