# Nitrogen Fixation by Grain Legumes in the U.K.

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# Nitrogen fixation by grain legumes in the U.K.

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About 150 kha of grain legumes are grown annually in the U.K. Of these, 62% are planted with *Pisum sativum*, 30% with *Vicia faba* and 8% with *Phaseolus* spp. *Pisum* and *Vicia* are temperate in origin, nodulate freely in the U.K. without inoculation and fix sufficient nitrogen to support high yields. In *Vicia*, nodules formed on autumn sown plants can overwinter and begin $N_2$ fixation at the time that spring sowings are made. Yield in *Vicia* and *Pisum* is likely to be limited by factors other than $N_2$ fixation.

*Phaseolus vulgaris* nodulates erratically in the U.K., and even when well nodulated needs the addition of some nitrogenous fertilizer. Its mean temperature for growth and its nodule physiology reflect its tropical or subtropical origin. However, there are good prospects for selecting cool-tolerant host cultivars and for crossing with the more cold-tolerant *Phaseolus coccineus*. *Rhizobium phaseoli*, which nodulates *Phaseolus*, has considerable homology with *R. leguminosarum*, which nodulates *Vicia* and *Pisum*, and the two can be crossed. Breeding suitable rhizobia for the cold-tolerant host selections is a real possibility. *Glycine max*, which is closely related to *Phaseolus*, is much less well adapted to cool conditions; its endophyte (*R. japonicum*) does not occur in soils in the U.K. and has little homology with temperate rhizobia.

## Introduction

In temperate regions at least, $N_2$ fixation by *Rhizobium* spp. in symbiotic association with members of the Leguminosae provides the major input to the nitrogen cycle. In agricultural economic terms, the legumes of significance can be broadly divided into grain and forage types. This paper considers grain legumes, i.e. those whose seeds, sometimes together with the enclosing pericarp (shell), are harvested. Grain legumes in the U.K. are grown principally for use as vegetables, but a significant quantity is grown for fodder (table 1).

Table 1. Area and gross yield of grain legumes grown in the U.K.: averages for 1976–9

(Data from M.A.F.F. statistics.)

| crop | area grown kha | gross yield $t\ ha^{-1}$ | product |
|---|---|---|---|
| vegetable | | | |
| peas | | | |
| dry | 36.6 | 3.2 | dry seed |
| green | 4.1 | 8.0 | pods and seed |
| for processing† | 52.7 | 4.2 | shelled green seed |
| broad beans | 5.6 | 10.1 | young pods and seed |
| runner and French beans | 12.0 | 8.8 | young pods and seed |
| fodder | | | |
| field beans | 39.0 | 3.1 | dry seed |

† Freezing, canning and freeze-drying.

Over half the total area of vegetables grown in the U.K. (excluding potatoes) consists of grain legumes, representing an important part of the economy. The extent to which they can fix their own N, thus saving on expensive fertilizer, is clearly an important attribute. The biological and climatic constraints upon this process will be considered here, together with some indications of how $N_2$ fixation by grain legumes may be extended and improved.

TABLE 2. MEAN TEMPERATURES FOR GROWTH OF GRAIN LEGUMES OF ACTUAL OR POTENTIAL SIGNIFICANCE IN THE U.K.

(Data from Duke (1981).)

| tribe | species | common name(s) | mean growth temperature/°C |
|---|---|---|---|
| Vicieae | *Pisum sativum* L.† | garden pea | 12.9 |
| | *Vicia faba* | broad bean, horse bean, tic bean, field bean‡ | 12.1 |
| Phaseoleae | *Phaseolus vulgaris* L. | French bean, navy bean, etc.§ | 19.3 |
| | *Phaseolus coccineus* L. (= *P. multiflorus*) | runner bean | 13.2 |
| | *Glycine max* (L.) Merr | soybean | 18.2 |

† For present purposes this is taken to include *P. arvense*, also known as the field pea.
‡ The term 'field bean' is used for *P. vulgaris* in the U.S.A. and some other countries.
§ Many variants of this species are grown in different countries, with regional common names.

## SPECIES OF GRAIN LEGUME GROWN IN THE U.K.

Botanically, these fall into two tribes of the subfamily Papilionoideae, as given in the classification of Polhill (1981). *Pisum* and *Vicia* are included in the temperate tribe Vicieae, and *Phaseolus* and *Glycine* in the tropical or subtropical tribe Phaseoleae (table 2). The differences in origin of these species is reflected in the mean temperatures for growth, which are generally higher for the Phaseoleae than for the Vicieae and in the growing seasons for these crops in the U.K. (figure 1).

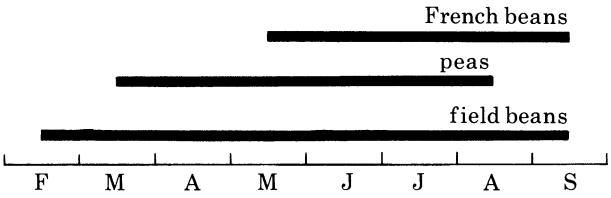

FIGURE 1. Generalized growing seasons for grain legumes in the U.K.

The root nodules of the Vicieae are indeterminate in their growth, with an apical meristem, whereas those of the Phaseoleae are generally determinate and thus shorter-lived. Some properties of these two types of nodule are summarized in table 3 and are discussed in more detail in Sprent (1980). In addition, nodulation is induced by different species of *Rhizobium*, which vary in their distribution (table 4).

In the U.K., peas and *Vicia* beans nodulate very freely and are not normally given N fertilizer. Nodulation is unpredictable in French bean and absent in soybean, unless inoculated with suitable rhizobia. Nitrogen fixation by these crops will now be considered in turn.

TABLE 3. SOME DIFFERENCES BETWEEN NODULES FORMED ON THE LEGUMINOUS TRIBES VICIEAE AND PHASEOLEAE

| | Vicieae | Phaseoleae |
|---|---|---|
| shape | elongate, often branched | more or less spherical |
| surface | with scattered air spaces | air spaces restricted to lenticels |
| growth | indeterminate | determinate |
| vascular system | open at distal end | closed at distal end |
| principal products exported | asparagine and glutamine | allantoin and allantoic acid |

TABLE 4. *RHIZOBIUM* SPP. NODULATING GRAIN LEGUMES GROWN IN THE U.K.

| *Rhizobium* sp. | host range | occurrence in U.K. |
|---|---|---|
| *leguminosarum* | peas, *Vicia* beans | widespread |
| *phaseoli* | *Phaseolus* | patchy |
| *japonicum* | *Glycine* | absent |

## *VICIA FABA*

Nitrogen fixation in this species has been studied in detail in widely spaced locations in the U.K., including Wales (El-Sherbeeny *et al.* 1977), Scotland (Sprent & Bradford 1977) and England (Day *et al.* 1979). Under field conditions nodulation is profuse, nodules are very active and, except when attacked by *Sitona* weevils, fix sufficient N to support high yields. Fixation of more than 400 kg N $ha^{-1}$ in a growing season may be achieved (Sprent & Bradford 1977). This crop is potentially the highest yielding grain legume in Britain, over 9 t $ha^{-1}$ having been obtained in small-plot experiments (Sprent & Bradford 1977). However, it is not popular with farmers because of its unreliability: strenuous efforts are currently being made to overcome problems such as flower and pod abortion. It is the most cold-tolerant of U.K. grain legumes and about one-third of the field beans grown in the U.K. are autumn sown.

Figure 2 shows data obtained with a spring (Herz Freya) and an autumn cultivar (Throws M.S.), both sown in October and March. Although the spring cultivar when sown in autumn remained chlorotic until March, it nodulated as well as the autumn cultivar and started fixing nitrogen in spring at approximately the same time, considerably in advance of spring-sown plants. The arguments for and against autumn and spring sowing have been widely discussed (e.g. in *Farmers Weekly*, 2 November 1979), but the effects on $N_2$ fixation have previously been neglected. A detailed study of the effects of cold on nodulation and $N_2$ fixation in *V. faba* has been made by Fyson (1981).

A general problem with annual grain legumes is that when pods start to fill, they form a stronger sink for carbohydrate than nodules, which senesce rapidly. Figure 3 gives data for *Vicia faba* cv. Minor, grown in the field: flowers were removed from one set of plants as soon as they were large enough to handle. In this particular experiment spring sowing was used and was delayed because of wet weather, so that flowering began later than usual.

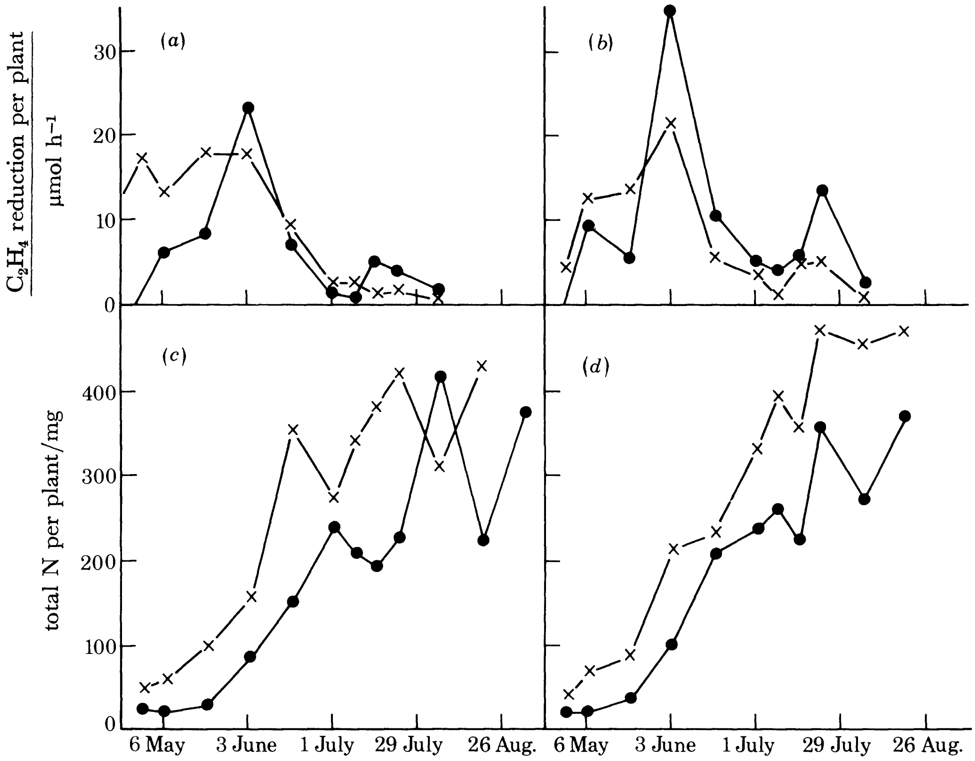

FIGURE 2. (*a*, *b*) Seasonal patterns of acetylene reduction for Throws M.S. (*a*) and Herz Freya (*b*). (*c*, *d*) Total N in plants of Throws M.S. (*c*) and Herz Freya (*d*). ×, Autumn-sown plants; ●, spring-sown plants. Unpublished data of Sprent & Bradford.

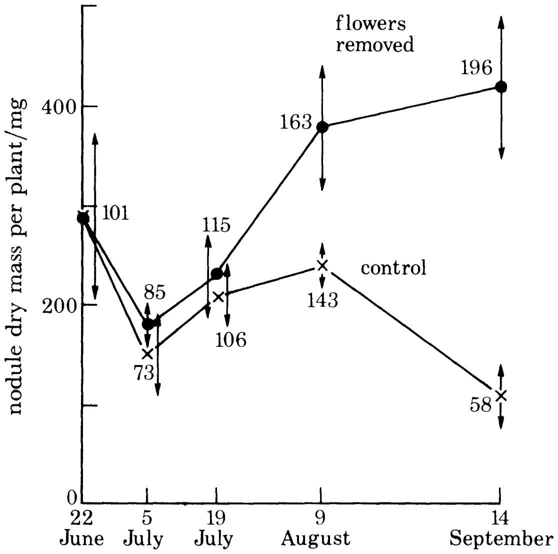

FIGURE 3. Seasonal patterns of nodule dry mass per plant for *V. faba* cv. Minor. ×, Control plants; ●, plants with flowers removed as soon as practicable. Bars indicate standard errors and numbers the average number of nodules per plant at harvest. Unpublished data of Brook & Sprent.

The initial decrease in nodule dry mass and number in both control and treated plants reflects the senescence of some of the earliest nodules formed on the tap root. Nodules developing on lateral roots were not sufficiently well developed to be scored. Subsequently nodule numbers and mass increased again, more quickly in the treated plants. During August, when pods were filling rapidly, control plants showed a rapid decrease in nodule number, mass and activity (not shown). Further details of these experiments, including growth analyses, can be found in Brook (1980).

### *PISUM SATIVUM*

Compared with *Vicia faba*, the total growing season of peas is short (figure 1). Few data are available on seasonal patterns of activity in the field in the U.K. but the general picture seems to be similar to that in other countries, with a peak between flowering and early pod-fill (Sprent 1976). As with *Vicia*, nodulation is profuse in the field and neither inoculation nor addition of N fertilizer is recommended. However, the total activity per unit area achieved in the field is generally considerably below that for *Vicia*. Table 5 shows data for three cultivars on two harvest dates. On 22 June the peas would have been near maximal $N_2$-fixing activity. A month later, pod-fill was advanced and activity declining. Comparative figures for *Vicia faba* show much higher activitives and reflect the fact that the seasonal peak of activity for spring-sown beans is generally later than for peas.

TABLE 5. ACETYLENE-REDUCING ACTIVITY (MICROMOLES PER SQUARE METRE PER HOUR) OF PEAS AND BEANS

(Unpublished data of Sprent & Thompson (1979).)

| species and cultivar | | 22 June | 24 July |
|---|---|---|---|
| *P. sativum* | Maro | 260 | 219 |
| | Rosakrone | 196 | 73 |
| | Minerva | 148 | 75 |
| *V. faba* | Maris Bead | 507 | 1079 |

As in *Vicia*, much of the N for seeds is fixed before flowering, stored in leaves and stems and subsequently transported to the fruits (Harvey 1977). In *Vicia* when pod set is poor, more N is retained in the leaves (Sprent & Bradford 1977). Similar patterns probably obtain for other legumes. When peas are grown for freezing, harvesting is carried out when seed have their highest sugar content. At this stage leaves are still green and the haulms are often used for silage. If not, they are chopped and ploughed in, to reduce the N fertilizer required for the subsequent crop. Thus the whole plant is used efficiently. In crops such as *Vicia*, where pod set is erratic, it may be more realistic when pod set is low to harvest the plants green and use them for fodder, rather than wait until seed has ripened.

### *PHASEOLUS VULGARIS*

The area of this species grown in the U.K. is small, and the crop is mainly used as a green vegetable. Nearly 100 kt of dried beans are imported into the U.K. every year, mostly to be used for canning as baked beans. For this reason there has been considerable effort, mainly at the National Vegetable Research Station, to produce hardier and more reliable cultivars for conditions in the U.K. (Hardwick *et al.* 1978; Innes 1980).

*P. vulgaris* is one of the most widely grown of grain legumes, being cultivated from the tropics to temperate regions. With few exceptions (e.g. in Idaho) (Westermann & Kolar 1978) $N_2$ fixation alone has been found to be insufficient to support maximum yield in the field. There are various possible reasons for this, including host cultivar (Westermann & Kolar 1978; Awonaike *et al.* 1980) and temperature (Graham 1979). Perhaps surprisingly, the species is tolerant of a wide range of irradiance, possibly because there is a strong negative correlation between leaf area ratio and net assimilation rate (this and other environmental aspects are discussed by Wallace (1980)). Certainly plants grown at very low irradiances (7 W $m^{-2}$) can nodulate effectively (Antoniw & Sprent 1978). In Britain the response to inoculation suggests that many soils do not contain suitable rhizobia (Taylor & Dudley 1978; Taylor *et al.* 1979, 1980). A further problem with most cultivars of *P. vulgaris* grown is that they are short-lived annuals and may not have a sufficiently long vegetative phase to fix enough $N_2$ before reproductive growth. Once the latter has started, nitrate reduction may provide the major source of N (Franco *et al.* 1979). Graham & Rosas (1977) found that climbing cultivars had more active nodules than bush cultivars, even though nodule production was similar in the two types. Their data show that leaf dry mass and N content are maintained at a higher level after 60 days in climbing than bush cultivars. No data for pod development were given, but the growth and N data suggest that climbing cultivars (which are the more primitive) may be able to support both nodules and fruits, while bush cultivars concentrate on the latter. As the authors imply, this may reflect subconscious selection by breeders of bush beans against $N_2$ fixation, since most varietal trials are carried out on well fertilized plots.

As far as the U.K. is concerned, there may be a further constraint on $N_2$ fixation in *P. vulgaris*, namely that the plant exports ureides (allantoin and allantoic acid) rather than amides, a strategy associated with species of tropical origin (Sprent 1980, 1981). The major advantage of exporting ureides is commonly supposed to be a saving of C (see Pate *et al.* (1981) for a detailed costing). However, as Israel & Jackson (1981) have shown for soybean, $N_2$-fixing plants transport considerable quantities of organic acids to maintain a suitable cation–anion balance. These acids bring the C:N ratio of xylem sap to over 2, even though ureides have a C:N ratio of 1. Thus the advantages of ureide transport are still uncertain. A possible disadvantage at low temperatures is their low solubility: an order of magnitude below the amides (asparagine and glutamine) (Sprent 1980).

The nitrate assimilated by *P. vulgaris* roots is transported chiefly as asparagine. We are currently examining the relative temperature sensitivity of $N_2$ fixation and nitrate reduction in *Phaseolus* and how temperature affects the balance of compounds exported.

### *Can* $N_2$ *fixation in* P. vulgaris *be improved?*

There are encouraging indications that both the host and rhizobial partners of *P. vulgaris* nodules can be better adapted for cool conditions and that greatly increased seed yields are feasible. Already, cold-tolerant selections are being developed (Hardwick *et al.* 1978), some of which can nodulate and fix $N_2$ at low temperatures although they grow better with combined nitrogen (Hardwick *et al.* 1981). Improved rhizobia are becoming available, and a doubling of yields on unfertilized plots has been obtained as a result of inoculation (Taylor *et al.* 1980), although some fertilizer is still recommended.

*P. coccineus* has a much lower mean temperature for growth than *P. vulgaris* (table 2), it nodulates much more freely in $N_2$ soils and may export over half of its N as amides and amino acids, principally glutamine (Sprent, unpublished). Crosses between these two species are simpler when *P. vulgaris* is the female parent (Evans 1980), but even then the $F_2$ tends to segregate into forms more typical of one or other of the original parents than hybrids (R. C. Hardwick, personal communication). If this problem can be solved, it may help considerably in producing cold-tolerant beans. Another feature of *P. coccineus*, namely that it is a tuberous perennial when grown in mild climates, may be significant. Legumes with a marked annual growth strategy (soybean, *P. vulgaris*) tend to be of the self-destructing type, in the sense of Sinclair & de Wit (1975). It may be worth investigating whether plants with photosynthetic capacity to direct materials to storage tissues can support $N_2$ fixation longer into the pod-fill period.

Although *Phaseolus* is a genus of tropical or subtropical origin, its endophyte, *Rhizobium phaseoli*, is much more closely related to the endophytes of temperate legumes. It sometimes nodulates peas (as *R. leguminosarum* sometimes nodulates *Phaseolus*) and there is a degree of cross-reaction between *R. leguminosarum* and *R. phaseoli* in agglutination tests. These facts, which have been known for some time (see Vincent 1970, tables 2.2 and 2.3) have recently been reinforced by genetic crosses. The genetic analysis of *Rhizobium* is a rapidly expanding field and has recently been reviewed by Beringer *et al.* (1980). It is possible to transfer genetic material between *R. phaseoli* and *R. leguminosarum*. Both are 'fast growing' as opposed to the slow growth characteristic of many tropical rhizobia (this distinction is becoming more blurred; see Trinick (1980)). In the infected cell, however, *R. phaseoli* bacteroids are similar in size and appearance to those in related hosts such as soybean (nodulated by *R. japonicum*) rather than the enlarged pleomorphic bacteroids in nodules of *Pisum*, *Vicia* and related genera (Sprent 1980, 1981). There are several interactions between host and bacteria, the nature of which is still largely unknown (Sprent 1982). One facet of interest is that bacteroids in all members of the Phaseoleae so far tested result in the export of ureides (synthesized by host cells) from nodules. Although ureides may be produced in small amounts by non-symbiotic plants of *P. vulgaris* (Thomas *et al.* 1980), the amounts are insignificant compared with those of $N_2$-fixing plants. How the bacteroids affect the host cell physiology remains obscure but we now have the possibility of modifying the effect by putting *R. leguminosarum* or other temperate rhizobial DNA into *R. phaseoli*.

Thus there is considerable scope for modifying the genotypes of both *P. vulgaris* and *R. phaseoli* to develop a symbiotic system more suited to cool temperate conditions.

## *Glycine max*

Because of its high oil content, in addition to protein, soybean is an economically valuable grain legume. It is being introduced as a crop in various parts of Europe, for example France and Czechoslovakia. Cultivars suitable for growth in Sweden are available, and these have stimulated interest in soybean as a possible crop in the U.K. However, soybeans are sensitive to low irradiance as well as low temperature and there are no obvious relatives that can be used to introduce tolerance to cold and low light, apart from selecting within the existing gene pool. In addition, *R. japonicum* is not native to Europe. This could be considered an advantage, in that it is possible to inoculate seed with the most suitable *Rhizobium* and not have to worry about competition with native rhizobia. However, *R. japonicum* has all the characteristics typical of tropical species; it has resisted most attempts at genetic analysis and certainly shows little

DNA homology with temperate rhizobia (Beringer *et al.* 1980). Thus the physiology of the symbiosis may be inherently unsuited to U.K. conditions. Prospects for improvement are considerably less bright than for *P. vulgaris*. As far as protein feedstuffs for animals (including humans) are concerned it may well be better to concentrate efforts in the crops covered above, which already have many of the required criteria.

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