

## NODULATION STUDIES IN LEGUMES

### II. THE INFLUENCE OF VARIOUS ENVIRONMENTAL FACTORS ON SYMBIOTIC EXPRESSION IN THE VETCH (*VICIA SATIVA* L.) AND OTHER LEGUMES

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#### Summary

The major sequences of symbiosis in field-grown vetch plants (*Vicia sativa* L.) are similar to those described in Part I of this series (Pate 1958) for the field pea (*Pisum arvense* L.). In both species symbiotic development is found to be characteristically synchronized with host plant leaf production and nitrogen accumulation. A generalized scheme for the symbiosis and nitrogen economy of the annual legume is derived from nodulation studies carried out in Australia and Northern Ireland.

Symbiotic expression of vetch as a winter annual is essentially similar to that of the same plant grown as a summer annual. There is no evidence of impairment of nodule growth and efficiency, or curtailment of nodule span in vetch plants overwintering in Northern Ireland.

Plant flowering induces marked decreases in nodule number and total nodule weight in the annual legume. Nodule losses in vetch may be temporarily arrested by regular removal of flower buds. Continued root growth on plants with artificially extended vegetative phase permits development of a larger nodule complement on minor roots than on similarly aged flowering plants. The significance of this "flowering factor" in annual legume symbiosis is discussed.

Qualitative studies of the seasonal nodulation of various Irish legumes depict successful overwintering of nodules as a common event in all winter annual, biennial, and perennial species. Biennial nodules are described for the woody perennial genera *Ulex* and *Sarothamnus*. The nodulation sequences of all legume species studied are found to be determined largely by changes associated with general host plant development, e.g. periods of root expansion and rapid plant growth, the onset and completion of reproductive phases of the life cycle, etc. Examples are described where climatic and biotic effects are superimposed on this inherent symbiotic pattern of the legume.

#### I. INTRODUCTION

Remarkably little information exists on the normal life span and turnover of nodules on wild or cultivated legumes in field conditions. Even less is known of the effect and action of host plant and environment factors on nodule functional life.

It is generally agreed that in temperate climates the normal life of an effective nodule is for only one season or growing period. According to Russell (1952) biennial and perennial herbaceous legumes generally conform to the plan of an annual renewal of nodules. Jones (1943) points out that nodules on tap-rooted legumes are frequently developed on annual investments of non-cambial roots and hence are unlikely to

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survive into a second season. For annual species, Fred, Baldwin, and McCoy (1932) describe host-induced nodule shedding in reproductive stages of the plant life cycle. They state that a sudden disappearance of nodules occurs in flowering and fruiting of large-seeded legumes, while nodule shedding extends over a longer period in the host plant cycle of small-seeded species.

Certain tropical tree and shrub legumes are exceptional in possessing perennial nodules which exhibit continued growth activity in the form of annual or seasonal increments of bacterial tissue. Such lobed nodules have been described for the genera *Sophora* and *Acacia* (Spratt 1919), *Wistaria* (Jimbo 1927), *Sesbania* (Harris, Allen, and Allen 1949), and *Caragana* (Allen, Gregory, and Allen 1955). The above authors associate various anatomical features of the perennial nodule with its prolonged symbiotic activity. Viermann (1929) provides an interesting example of perennial nodules in the herbaceous legume *Lupinus*. In perennial species grown in Germany nodule meristems were shown to be preserved from year to year to give peripheral additions of bacterial tissue during each growing season.

Several environmental factors have been described as influencing nodule longevity. In each case physiological shocks to the host plant are reflected by adverse effects on nodule populations. Drought-induced shedding of large portions of nodule populations has been noted by Wilson (1942). Under Wisconsin climatic conditions he found that several cycles of alternate shedding and renewal of nodules on field-grown legumes followed the onset and alleviation of soil moisture stresses during the growing season. Extensive damage to nodules by larvae of the weevil *Sitonia lineatus* has been described by Mulder (1948) and Masfield (1952) for European legumes. Clipping of leaves (Eaton 1931; Wilson 1931) or shading (Thornton 1930; Strong and Trumble 1939) are stated to induce premature nodule losses, the latter generally attributed to carbohydrate deficiencies in host tissues. It is surprising to find that there is no detailed information available on nodule destruction in relation to the periodic grazing activity of animals.

The purpose of the present paper is to present quantitative observations on climatic, biotic, and host plant influences on nodule longevity in field-grown legumes. Special study is made of nodule survival in overwintering legumes and of the host plant flowering factor in relation to nodule emptying. The quantitative studies on *Vicia sativa* L. also provide an example of synchronized host and symbiotic development remarkably similar to that described for *Pisum arvense* L. in Part I of this series (Pate 1958). An interesting sideline to the *V. sativa* studies is found in the account of effective and ineffective strain behaviour in multiple infection of host plants in field conditions.

## II. MATERIALS AND METHODS

### (a) Plant Material

Growth and nodulation of vetch (*Vicia sativa* L.) was studied by periodic sampling from three series of field-grown material:

*Series 1.*—Autumn sowing; growing period September 1955–July 1956.

*Series 2.*—Spring sowing; growing period April–July, 1956.

*Series 3.*—Summer sowing; growing period June–October, 1956.

Series 1 and 2 sowings were made in the same soil environment to compare symbiotic performance of vetch as a winter or summer annual. Series 3 sowing was designed to examine the effect of plant flowering on nodule initiation and longevity. It comprised control plants which were allowed to reproduce normally, and a treated series where all flower buds were removed to maintain plants in an artificially prolonged vegetative condition through the declining season.

For each sowing some 600–800 sq. ft of ground were cultivated, plots being chosen from nitrogen-deficient soil of sandy character at Belfast, Northern Ireland. Series 1 and 2 constituted one randomized block experiment. Series 3 had a similar randomization of treated and untreated portions of the plot. Adequate nitrogen-free mineral fertilizer was applied to all plots before the sowings. In the overwintering series cloche protection was provided from December to April. Seeds of series 2 and 3 were sown 1–1½ in. deep; a deep (3–4 in.) sowing was employed in series 1 to favour autumnal growth and winter hardiness.

None of the sowings was artificially inoculated. In each series multiple infection by indigenous rhizobia resulted in the production of two contrasting nodule types. These proved of ineffective and effective character as gauged by their haemoglobin-pigmented life, nodule meristem activity, and nitrogen fixation potential in pure culture with vetch as host plant (see Section III).

#### (b) *Sampling Procedure*

Sampling methods were similar to those used for field-grown material of *P. arvense* (see Pate 1958), and reference should be made to this paper for details of technique and the relevance and accuracy of the various characters recorded.

A 50-plant sample unit was selected from the plots at 3–7 day intervals in host plant life cycles. Sampling involved a 0·5–3 per cent. standard error in recorded host symbiotic quantities.

#### (c) *Recordings from Samples*

The following plant and nodule characters were recorded for each 50-plant sample in the series:

##### (i) *Series 1, 2, and 3*

(1) *Leaf Age (expanded leaves/plant)*.—As a winter annual, *V. sativa* developed a compact autumn rosette of three to six shoots of limited growth bearing a total of some 10–20 leaves/plant. The following spring, the short shoots died, total leaf number showed a temporary decrease, and then a flowering axis of some 15–25 leaves developed. Similar development occurred in spring or summer sowings but fewer preflowering shoots formed and there was less marked leaf abscission in late vegetative development (see leaf-age scales on Figs. 1–9).

(2) *Nodule Number and Colour*.—Three colour classes were recognized in nodule counts, white (*W*-type) young nodules, red (*R*-type) haemoglobin-pigmented nodules, and green (*G*-type) senescent nodules. Counts were taken of the various nodule colour classes on primary and secondary root systems. The few nodules forming on tertiary roots were classed as secondary root nodules in counts and

weight determinations. The autumn series showed considerable infection of the rootlet-invested epicotyl in addition to normal nodulation of primary and lateral roots. This supracotyledonary infection was undoubtedly related to the deep sowing of the overwintering series.

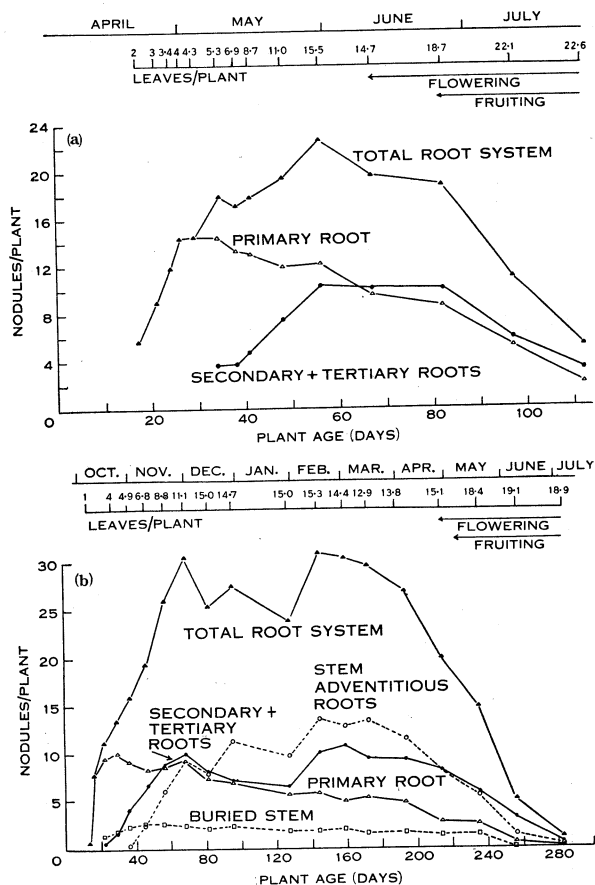


Fig. 1.—*V. sativa* nodulation: nodule numbers on various parts of the root system. (a) As a summer annual (1956 series); (b) as a winter annual (1955-56 series). Each point on the various curves of Figures 1-9 represents a mean value derived from a sample of 50 plants.

## (ii) Additional Recording Series 1 and 2

Fresh weight determination and nitrogen analyses were made of host plants organs and nodule colour samples. Techniques were similar to those used in study of the nitrogen economy of the field pea (Pate 1958).

## (d) Qualitative Observations on Nodulation of Native Legumes in Northern Ireland

Several plant communities containing a total of 29 legume species were kept under observation over the period 1953-1956. At monthly intervals sample plants of the various species were extracted and notes made on rooting characteristics and

size and condition of nodule populations. Nodule distribution patterns and nodule longevity cycles were constructed from the data.

### III. RESULTS

#### (a) Comparison of Nodulation in Vetch as a Summer and Winter Annual

##### (i) Nodule Number (Fig. 1)

Winter annual development is characterized by autumnal and vernal maxima in total nodule numbers (Fig. 1(b)). The autumn maximum represents the combined initial infection of all four root systems in the sequence of their expansion—

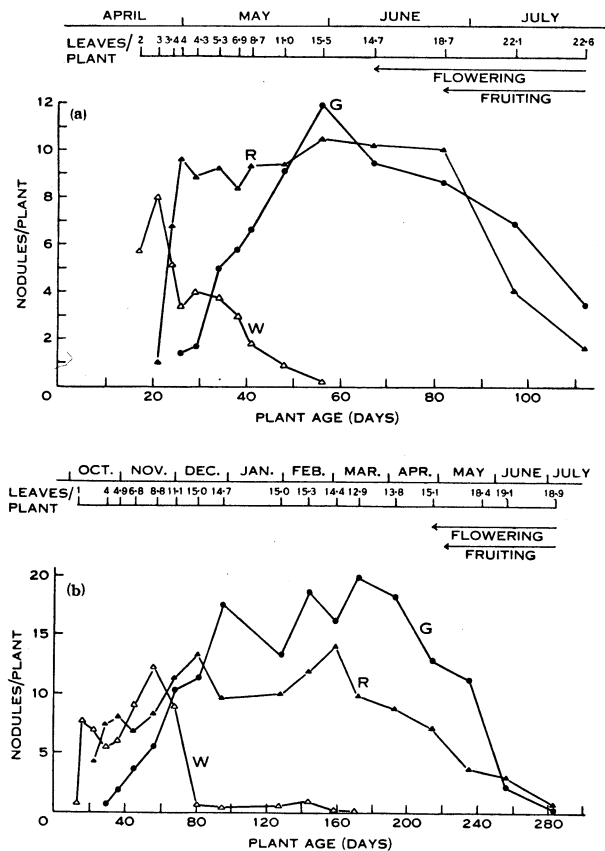


Fig. 2.—*V. sativa* nodulation: nodule colours for complete root system. W, young white nodules; R, red actively fixing nodules; G, green senescent nodules. (a) As a summer annual (1956); (b) as a winter annual (1955-56).

primary root system, buried epicotyl, secondary roots, epicotyl adventitious roots. The vernal maximum comprises the additional infection of minor roots following resumption of root growth in early February. The spring sowing shows a single peak in nodule numbers in late vegetative development (Fig. 1(a)).

All three vetch series show marked declines in nodule numbers in late vegetative development, nodule disappearance being most noticeable on flowering and fruiting plants.

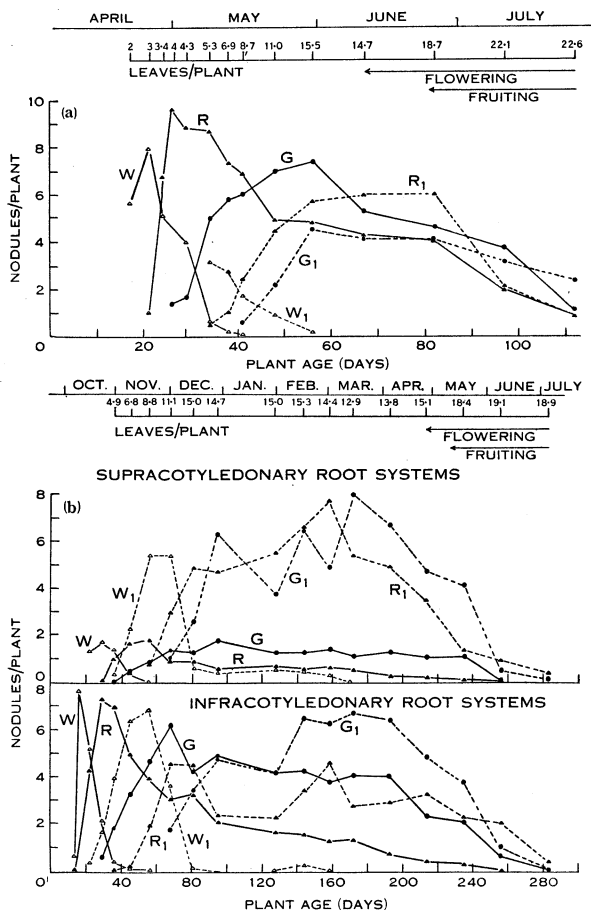


Fig. 3.—*V. sativa* nodulation: nodule colour for various parts of the root system. *W*, *R*, and *G* symbols as in Figure 2. (a) As a summer annual (1956): *W*, *R*, and *G* curves for primary root; *W*<sub>1</sub>, *R*<sub>1</sub>, and *G*<sub>1</sub> curves for secondary + tertiary roots. (b) As a winter annual (1955-56): supracotyledonary root systems, *W*, *R*, and *G* curves for epicotyl nodules and *W*<sub>1</sub>, *R*<sub>1</sub>, and *G*<sub>1</sub> curves for adventitious roots from buried epicotyl; infracotyledonary root systems, *W*, *R*, and *G* curves for primary root and *W*<sub>1</sub>, *R*<sub>1</sub>, and *G*<sub>1</sub> curves for secondary + tertiary roots.

## (ii) Nodule Colour (Figs. 2 and 3)

On complete plants or individual root systems of either series, nodule populations exhibit the same progressive colour changes from white to red to green as those observed in the life of any effective or ineffective nodule on the roots. This effect is well illustrated in the winter vetch sowing where successive maxima in the *W*, *R*, and *G* curves are attained in the same sequence as the four root systems were expanded in early seedling growth (Fig. 3(b)).

(1) *Root Infection (W Curves).*—*W* curves for complete root systems are bimodal, the two peaks corresponding to periods of nodule formation and accumulation of young nodules on primary and secondary roots (Fig. 2(a)). A third minor peak in the overwintering series in February marks nodulation of minor root systems

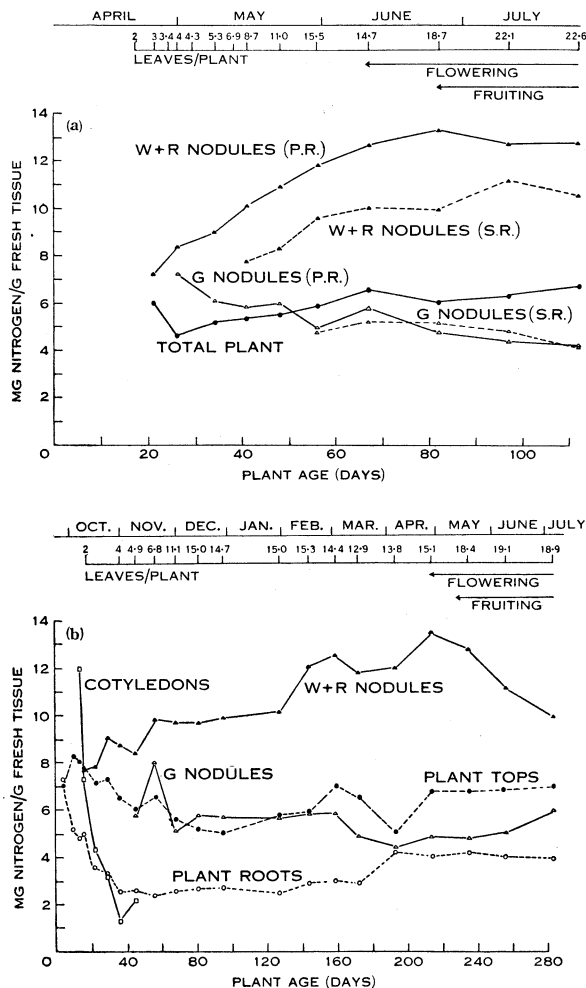


Fig. 4.—*V. sativa* nodulation: nitrogen concentration in nodules and host plant tissues. P.R., primary root nodules; S.R., secondary + tertiary root nodules; W, R, and G symbols as in Figure 2. (a) As a summer annual (1956); (b) as a winter annual (1955-56).

expanded or added to in spring growth (Fig. 2(b)). Root development and nodule initiation is completed in both series well before flowering commences.

(2) *Nodule Activity and Senescence in Effective and Ineffective Nodules (R and G curves, Figs. 2(b), 3(b)).*—Nodule pigmentation rates may be determined by comparing ascending portions of the *W* and *R* curves of the various root systems. In *spring* and *summer* sowings, pigmentation of the first few nodules on a root

system took place some 4–8 days after their appearance on the primary root. As in the summer sowings of field pea (Pate 1958), later-formed nodules on root systems were pigmented more quickly (3–5 days) than their earlier-formed neighbours (5–9 days). In the *winter* sowing, adverse effects of decreasing light and temperature on host plant growth produced an opposite effect to the above. Thus, nodules on the primary axis were pigmented in 7–19 days in October, while those which developed 6 weeks later on lateral root systems remained white for 18–30 days before acquiring visible pink coloration. It may be concluded that haemoglobin production is closely related to host plant growth rate as influenced by current climatic conditions.

The two series showed invasion by native soil rhizobia of two major and contrasting strain types giving highly effective and highly ineffective symbiosis with *V. sativa*. Approximately equal proportions of the two nodule types appeared on the roots of the series. The following observations were made on the biology of the two strain reactions both in the conditions of the experiment and in sterile cultures of vetch in association with the two classes of rhizobial variants:

*Ineffective Nodules.*—These were small, hemispherical structures with meagre and transient meristematic activity and an active (red) life of 5–8 days in summer conditions. Mature fresh weight of the ineffective nodule was about 0.5–1.0 mg, less than 5 per cent. of the size of mature effective nodules. Anatomical investigations revealed an early disorganization of the nodule meristem as a primary cause of ineffectiveness. Less than 10 per cent. of the bacterial tissue of the nodule was comprised of bacteroid-filled cells, despite the presence of numerous infection threads. The latter remained filled with rod-shaped bacteria for periods of up to 10 weeks after haemoglobin destruction had been completed in the nodule.

Once-green ineffective nodules persisted on root systems for long periods, many remaining intact until root decay on fruiting plants. It is not known why these ineffective nodules were so highly resistant to the internal and external decay agencies which quickly disintegrated green effective nodules on vetch and other legumes in field conditions.

Sterile cultures of vetch with the ineffective *Rhizobium* gave no evidence of nitrogen fixation activity when compared with nitrogen-free uninoculated controls. Indeed, in plants exhibiting multiple infection by effective and ineffective rhizobia, a load of green, persistent nodules might deplete host carbohydrate reserves and thereby detract from the full benefit of effective nodule activity. Study is now being made of this latter aspect of symbiosis.

The progressive accumulation of ineffective members of nodule populations accounts for the very high numbers of green nodules in many samples of the three series (cf. the rapid elimination of senescent members of the effective nodule populations of the field pea series described in Part I).

*Effective Nodules.*—These were large cylindrical structures with a mature average weight of 20–30 mg. They showed an active life of from 20 days to the death of host plants (i.e. as long as 200 days in overwintering primary root nodules). More than 90 per cent. of nodule bacterial tissues was comprised of swollen, bacteroid-filled cells.



In aging effective nodules, haemoglobin destruction commenced in proximal tissues and proceeded towards the nodule meristem. Completely green nodules persisted for only a few days on root systems before decaying and being sloughed off into the surrounding soil.

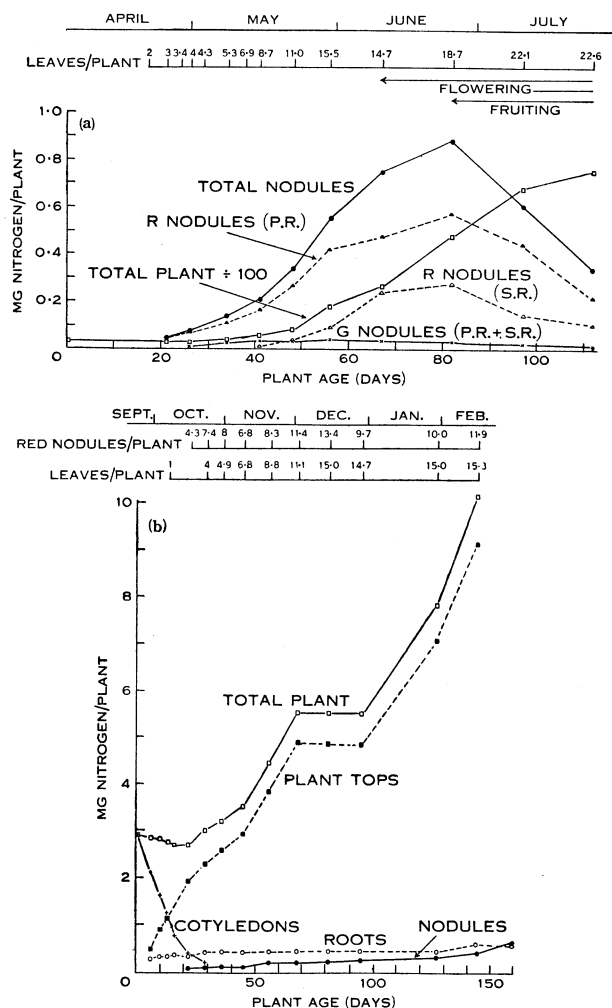


Fig. 5.—*V. sativa* nodulation: nitrogen content of nodule and host plant tissues. (a) As a summer annual (1956); (b) seedling nitrogen economy in the overwintering series (1955-56).

### (iii) Nitrogen Contents of Plant Tissues (Figs. 4-6)

Many of the salient features of the nitrogen economy of the two vetch sowings are similar to those described for field pea nodulation. To avoid undue repetition only the more important results summarized in Figures 4 and 5 will be mentioned here.

(1) *Nitrogen Accumulation in Nodules*.—On a fresh weight basis, nitrogen levels in red nodules rise as nodule populations age. Nitrogen concentrations in

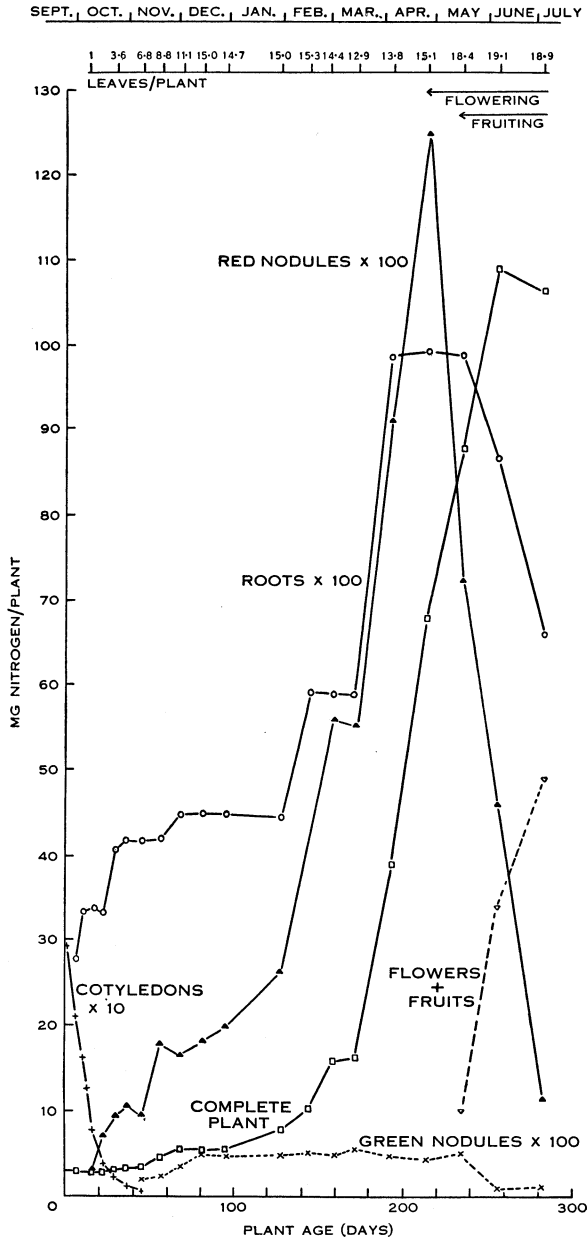


Fig. 6.—*V. sativa* nodulation: nitrogen content of host plant and nodule tissues over complete life cycle of the winter annual series (1955–56).

nodules on minor roots (series 2, Fig. 4(a)) are consistently lower than in nodules on the main root axis. At all stages of plant growth, *G* nodule nitrogen levels are

some 40–60 per cent. of those in *R* nodule samples of similar age. Total red-nodule nitrogen increases to a maximum in early flowering, some time before a maximum in plant nitrogen is established (Figs. 5(a) and 6).

In Part I it was suggested that the red-nodule nitrogen content of the plant might serve as a useful approximation to the current fixation potential of the symbiotic organs. The potential fixation activities of secondary root, and primary root nodule populations over the life cycle of series 2 may be compared with reference to the areas under the red-nodule nitrogen curves for these root systems (Fig. 5(a)). Such a comparison would appropriate a possible fixation contribution from primary

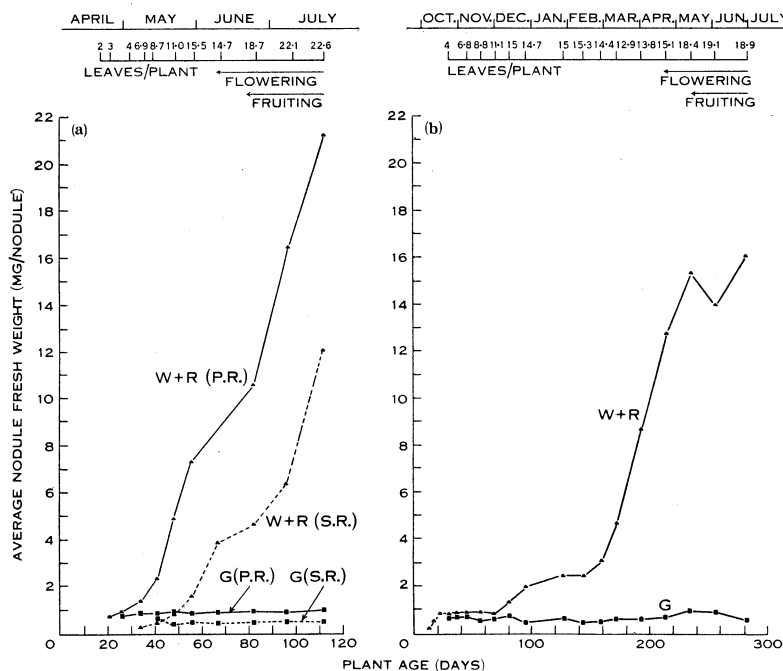


Fig. 7.—*V. sativa* nodulation: average nodule fresh weight (mg/nodule). (a) As a summer annual (1956); (b) as a winter annual (1955–56); W + R, white + red nodule sample; G, green nodule sample; P.R., primary root; S.R., secondary + tertiary roots.

roots some three times greater than that of secondary roots. However, there is no evidence of equal fixation efficiency in secondary and primary root red nodules on a weight or nitrogen basis. Experiments are being designed to cover this aspect of symbiosis.

(2) *Nitrogen Accumulation in Host Tissues*.—Seedling nitrogen nutrition is outlined for the overwintering series in Figure 5(b). Total plant nitrogen commences to rise in 25-day-old seedlings as nodules colour and commence fixation. Root nitrogen remains fairly constant over the 30–150-day period, although nitrogen in aerial portions is increased five times during this period. In later stages of growth nitrogen is again preferentially sequestered by the shoot system until at plant

maturity more than 99 per cent. of total plant nitrogen is concentrated in plant tops (Fig. 6). Root nitrogen concentrations are consistently lower than in other plant organs (Fig. 4(b)).

There is thus abundant evidence of an immediate transfer of fixation products to plant tops at all stages of vetch development, with neither roots nor nodules functioning as nitrogen storage organs.

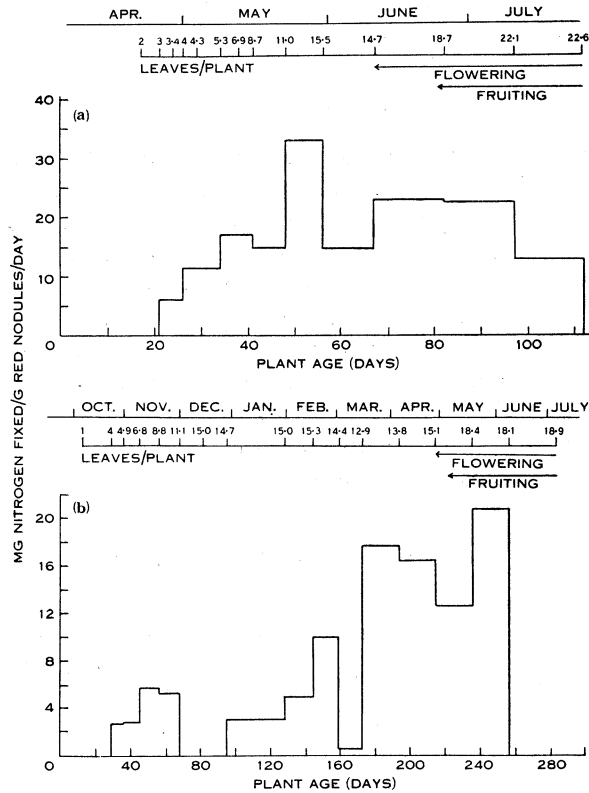


Fig. 8.—*V. sativa* nodulation: fluctuations in apparent nodule fixation efficiency. (a) As a summer annual (1956); (b) as a winter annual (1955-56).

Both series show sigmoid curves for increase in total plant nitrogen with time. Nitrogen redistribution to flowering parts in series 1 occurs largely at the expense of nitrogen stored in plant tops (Fig. 6). The general nitrogen economy patterns described here for vetch and field pea are essentially similar to those described for non-legume annual species by Williams (1955).

#### (iv) Average Nodule Weight (Fig. 7)

**W + R Nodules.**—Average nodule size (fresh weight) in both series increases from less than 0.5 mg to 15-20 mg. Nodules continued to grow through the winter period in series 1 with a sudden increase in growth rate following resumption of plant growth in March. Average nodule sizes of primary and secondary root nodules

are recorded in series 2 (Fig. 7(a)). Sigmoid growth curves are depicted, with nodules consistently smaller on the finer lateral roots.

*G Nodules.*—It has been mentioned already that *G* samples in both series were largely composed of persisting ineffective nodules. Hence the average weight of *G* nodules approximates to the mature size of an ineffective nodule and, since the latter does not continue to grow once green, average size remains fairly constant over the life cycle at a value just less than 1 mg/nodule. In series 2 (Fig. 7(a)) *G* nodules on secondary and tertiary roots are seen to be consistently smaller than on the primary root.

(v) *Apparent Fixation Intensity (Fig. 8)*

The histograms of Figure 8 depict fluctuations in the apparent efficiency of red nodules over the life cycles of the two series. Results are expressed as milligrams nitrogen fixed/gram fresh weight of red nodules/day, assuming that all nitrogen accumulated above cotyledon level is derived from nodule fixation. This is considered a reasonable approximation for the extreme conditions of nitrogen deficiency encountered in the plots. In both series it can be seen that the tempo of nodule activity increases as plant flowering is approached (cf. similar results for *P. arvense* (Pate 1958)). In the overwintering series, fixation ceased temporarily in cold weather in December and early January. A second apparent depression of nodule activity over the 160–170-day period coincided with a period of leaf loss following “scorching” under cloches in early spring.

(b) *The Effect of Flower Bud Removal on Nodulation of Vetch as a Summer Annual (Series 3)*

Fluctuations in nodule numbers and colours are recorded in the three graphs of Figure 9. The vertical line through the figure marks the point where flowering commenced and where flowers were first removed in the treated series. The leaf-age scales for the series show greater leaf production in the treated series.

(i) *Nodule Number (Fig. 9(a))*

Twenty-five days after flower removal commenced, plants of artificially extended vegetative phase showed significantly greater nodule numbers than control flowering plants. Three weeks later more than three times as many nodules remained on secondary roots of plants of the treated series.

(ii) *Nodule Colour*

The graphs in Figures 9(b) and 9(c) express nodule colour classes as percentages of the total nodules present on primary and secondary+tertiary roots respectively. No significant differences are evident in colour or number changes in the primary root system indicating that flower removal has no visible effect on the fate of existing nodules on the primary axis.

A significantly higher proportion of red nodules is recorded for secondary roots of plants of the treated series. Also white nodules were seen to form on tertiary roots of this series right to the end of the experiment, indicative of con-

tinued root expansion in the prolonged vegetative phase. By contrast, nodule initiation continued in the control series only until 30 days after flowering.

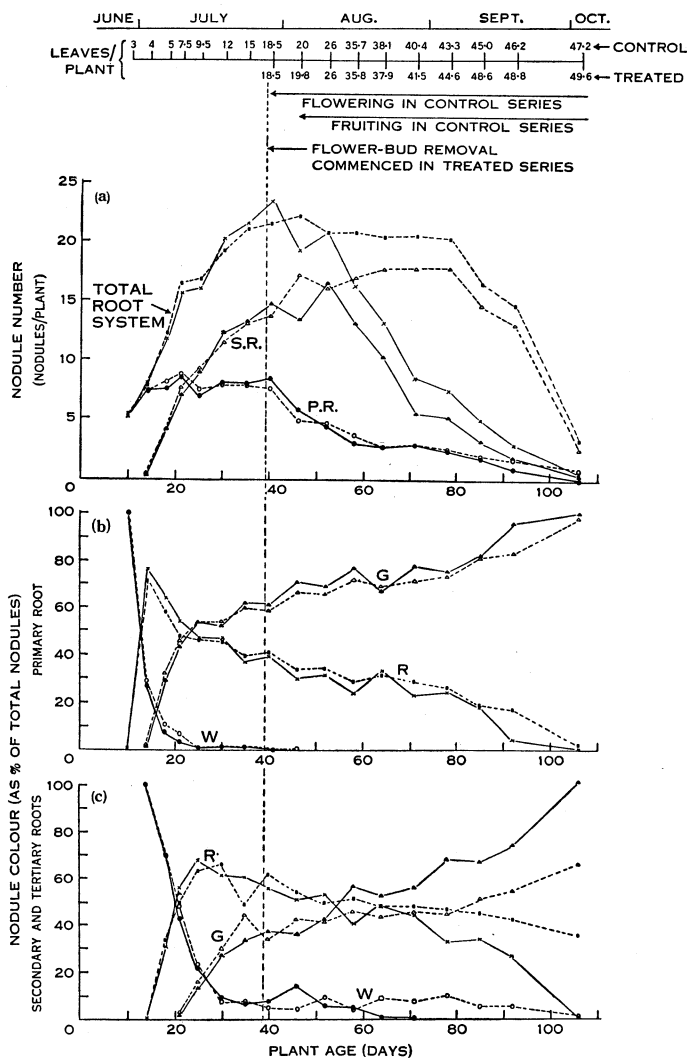


Fig. 9.—*V. sativa* nodulation: the effects of flower-bud removal on nodule production and nodule destruction in a summer sowing of vetch (1954). (a) Effects on nodule number. (b), (c) Effects on nodule colour on primary root, and secondary + tertiary roots respectively. W, young white nodules; R, red actively fixing nodules; G, green senescent nodules; P.R., primary root; S.R. secondary + tertiary roots. ----- Treated series (all flower buds removed). — Control flowering series.

The greater number of nodules on minor roots of the treated series may thus be analysed as being due to both a prolongation of nodule initiation on tertiary roots and an increase in the life span of nodules already existing on secondary roots.

(c) *Nodule Longevity Cycles in Native Legumes in Northern Ireland*

The 29 species of legumes examined conform to one of six major root growth types. Four distinct nodule longevity cycles are found to operate in normal seasons in Northern Ireland, three of these involving a regular overwintering of nodules. Results of examinations for four seasons are summarized below:

(i) *Rooting Patterns*

*Type 1: Fibrous-rooted Annual.*—Characteristic of summer and winter annuals where nodulation is similar to that described for *V. sativa* and *P. arvense*. Nodules, if effective, usually remain on the primary and secondary root until the fruiting of the host plant. Autumnal and vernal root growth, with attendant nodule initiation, was observed in all overwintering species (cf. Section III(b)). Examples: *Trifolium procumbens* L., *T. dubium* Sibth., *T. arvense* L.; *Medicago arabica* Huds., *M. lupulina* L.; *Vicia hirsuta* Gray, *V. sativa* L., *V. angustifolia* L., *V. lathyroides* L.

*Type 2: Tap-rooted Perennial or Biennial.*—In seedling growth a sparsely nodulated fibrous root system is developed. The primary axis of this system commences to store starch. In subsequent seasons new growth regions of secondary roots are infected, and an investment of nodulated roots is developed annually from latent root primordia on the tap root. Lower portions of extensive root systems may not develop nodules especially where roots are in poorly aerated soil. Examples: *Anthyllis vulneraria* L.; *Melilotus officinalis* Willd.; *Onobrychis sativa* L.; *Trifolium pratense* L., *T. hybridum* L.

*Type 3: Tap-rooted Perennial with Suckers.*—Similar nodulation to type 2, but with a few nodules developed on adventitious roots on the sucker shoots arising from the crown, e.g. *Lotus corniculatus* L., *L. uliginosus* Schkuhr.

*Type 4: Tap-rooted Perennial with Runners.*—Both the original root system and nodal adventitious roots are nodulated. Runners may become isolated from the parent shoot stock and develop into independent, nodulated plants, e.g. *Trifolium repens* L., *T. fragiferum* L.

*Type 5: Woody Perennial.*—The expanding root system is progressively nodulated. In the second and later years of growth older nodules are sloughed off the root system until, in mature plants, nodules are to be found only on minor fibrous roots. In water-logged or poorly aerated soils nodules are restricted to roots exploring the upper soil horizons. Examples: *Sarothamnus scoparius* Koch; *Ulex europaeus* L.; *U. galli* Planch.

*Type 6: Rhizomatous Perennial.*—A short-lived, fibrous system is developed and nodulated in the first few months of seedling growth. This is replaced by an extensive system of starch-storing rhizomes, each with its own adventitious roots. Portions of the rhizome system may become separated from the parent plant. Nodules predominate on adventitious roots from the current season's rhizomes. Examples: *Lathyrus montanus* Bernh., *L. pratensis* L., *L. palustris* L.; *Vicia cracca* L., *V. sylvatica* L., *V. sepium* L.; *Ononis repens* L.; *Trifolium medium* L.

(ii) *Nodule Longevity*

*Cycle 1: Summer Annual, Root Pattern Type 1.*—The cycle is essentially similar to that described for summer vetch and field pea, and gives a maximum nodule

life of 6–8 months. White nodules are present only while root systems are expanding; green nodules tend to accumulate in large numbers in the closing stages of the plant life cycle.

*Cycle 2: Winter Annual, Root Pattern Type 1.*—Nodulation follows periods of root growth in autumn and spring. Survival of the autumn nodule set varies with the season. For example some 80 per cent. of nodules on *Vicia angustifolia* and *Trifolium dubium* failed to survive the 1953 winter, while in the next two years most nodules overwintered successfully. Maximum nodule life for this cycle is 10–11 months.

*Cycle 3: Biennial and Perennial Species, Root Pattern Types 2–6.*—The season's nodule complement is completed in early spring. Nodules may remain on a root system for 12–14 months and hence two sets of nodules may coexist for a short period on root systems in early spring, the older set still haemoglobin-pigmented and presumably active in fixation. It is possible that one aging set of nodules may provide the necessary growth materials for juvenile members on the same root system. A large proportion of each season's nodule set survive the normal Irish winter.

*Cycle 4: Woody Perennial Species, Root Pattern Type 5.*—In several gorse (*Ulex europaeus*) and broom (*Sarothamnus scoparius*) populations near Belfast, nodules at least 20 months old were noted on several plants in their third and later years of growth. The life cycle of these "biennial" nodules was further studied by "tagging" individual nodules in pot-grown plants. It was found that nodules which developed on young roots in spring or early summer remained active in fixation until the following autumn when their lower halves turned green. In those few nodules destined to resume growth, the nodule meristem was preserved and allowed a second season's increment of bacterial tissue to be made to the existing bacterial tissue. At the end of its second season the nodule had become an elongate structure, lower half brown and shrivelled, upper half composed of healthy, haemoglobin-pigmented tissue. There was no evidence that any nodules persisted into the spring of a third season, yet this 20-month longevity cycle is reminiscent of the perennial nodules observed on many tropical woody genera.

#### IV. DISCUSSION

In Part I (Pate 1958) evidence was given of a characteristic integration of host and symbiotic development in the annual legume *Pisum arvense*. The results presented here describe similar host-*Rhizobium* relationships in further legumes and in addition demonstrate that symbiotic expression is sensitive to biotic and climatic influences. The contributions of the sections of the experimental results are discussed below.

##### (a) Quantitative Studies of *V. sativa* Nodulation as a Summer or Winter Annual

Figure 10 compares the synchronization patterns for leaf production, plant nitrogen accumulation, and symbiotic development in the winter annual and summer annual sowings. The two series show remarkably similar sequences of attainment of various symbiotic maxima despite marked differences in host plant growth and



final yield. The major differences between the series relate to the climatic effects on symbiosis following exposure to frost and host plant quiescence in the winter annual. The processes of nodule initiation, nodule growth, and nitrogen fixation are clearly interrupted by winter conditions, but there is no evidence of any curtailment of nodule life, or reduction in final nodule size and efficiency in the subsequent development of overwintering nodules.

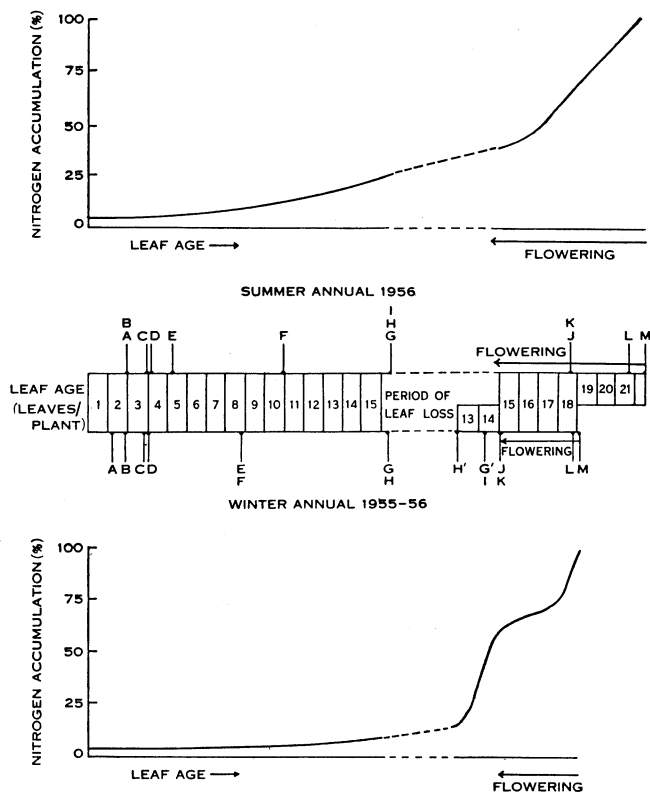


Fig. 10.—Diagram summarizing the synchronization patterns for host and symbiotic development in *V. sativa* grown as a summer or a winter annual. Host plant nitrogen accumulation (expressed as a percentage of total nitrogen present at plant maturity) and various features of the nodulation cycles in the two series are compared on a common leaf-age scale. A, maximum primary root white nodules; B, first nodule turns red; C, maximum primary root red nodules; D, 90 per cent. of cotyledon nitrogen exhausted; E, maximum secondary roots white nodules; F, maximum in nodules/plant fresh weight ratio; G, maximum nodules on total root system (G', vernal maximum in overwintering series); H, maximum red nodules on total root system (H', vernal maximum in overwintering series); I, nodule initiation ceases; J, maximum nitrogen concentration in red nodules; K, maximum in red nodule nitrogen/plant; L, 50 per cent. of red nodules destroyed; M, maximum in total plant nitrogen.

Multiple infection in the two series illustrates the rhizobial specificity of symbiotic expression. Annual legume symbiosis involving an ineffective or poorly effective bacterial relationship has the obvious disadvantage of a single setting of nodules which can contribute only an early and meagre fixation return to the host

plant. The ineffective relationship described here for *V. sativa* is peculiar in showing persistent, inactive nodules of life span equal to that of companion effective nodules. Thus the short functional life and limited growth capacity of nodules which characterize this type of ineffectiveness are not accompanied by the early necrosis of nodule tissues specified in many other descriptions of symbiotic maladjustment (e.g. see Thornton 1954 and the recent appraisal of ineffectiveness by Bergersen 1957).

Similar proportions of ineffective and effective nodules developed in the two sowings although different climatic conditions prevailed in the respective periods of infection. It would be interesting to know whether nodulation from mixed soil populations of *Rhizobia* is a property little influenced by environment. Studies are now being made of this aspect of legume symbiosis.

(b) *Comparisons of the Symbiotic Patterns of Various Annual Legumes*

The detailed descriptions of symbiosis in field pea and vetch and the observations on other annual species in Northern Ireland and Australia (unpublished work; see also Section III(c)) permit the following generalizations to be made for annual legume nodulation studied by the author:

- (i) Root growth and attendant nodule initiation are precocious. Maximum numbers of nodules are present on roots by mid- or late-vegetative development, and nodule initiation usually ceases well before flowering.
- (ii) In effective symbiosis, nodules acquire haemoglobin pigmentation in ordered sequence on the various portions of the host plant root system. The extent and rate of pigmentation is related to the current growth rate of the host plant.
- (iii) Average nodule size and efficiency increase as nodule populations age. There is some evidence of a progressive elimination of the smaller members of effective nodule populations throughout plant growth.
- (iv) The commencement of nitrogen fixation in the seedling coincides with the pigmentation of the first few nodules on the primary root. The former event usually anticipates the exhaustion of available cotyledon reserves.
- (v) Nitrogen accumulation in host tissues and symbiotic organs proceed such that the maximum in total nodule nitrogen is attained some time before the maximum in total plant nitrogen. This observation reflects the general increase in fixation efficiency in aging nodule populations (see (iii) above).
- (vi) There is evidence of an immediate transfer of fixation products from nodules to host plant tissues. At no time in annual legume development do roots or nodules act as substantial nitrogen storage organs on the plant.
- (vii) Haemoglobin destruction in senescent nodules progresses from base to apex. Approximately one-third of the nitrogen in the nodule is removed in early senility. It is estimated that in effective symbiosis of the annual legumes field pea and vetch less than 3 per cent. of the nitrogen fixed in the symbiotic cycle is returned to the soil in the form of dead nodule tissue (see Pate 1958 for further discussion).

- (viii) Dramatic decreases in nodule numbers and total weight of nodules occur in flowering and fruiting stages of the life cycle. Minor differences are noted in this symbiotic feature for the nodulation performances of vetch and field pea. Root development, nodule initiation, nodule disappearance, and several attendant symbiotic features are earlier in field pea than in vetch when compared on a host plant nitrogen accumulation basis. These differences may constitute host species nodulation characters or may merely reflect compatibility differences between host and bacterial partners of the two particular associations examined in this series of investigations.

(c) *Analysis of the "Flowering Factor" in Nodule Destruction in V. sativa*

In the experimental series, where nodulation of vetch plants maintained in prolonged vegetative condition by flower-bud removal was compared with the nodulation of similarly aged untreated plants, it is seen that plant flowering inhibits both root growth and nodule initiation while concurrently hastening the destruction of existing nodules. This analysis does not necessarily imply action of two separate physiological effects of flowering. The nutritional demands of flowering may promote withdrawal of protein and carbohydrate from vegetative organs and the consequent starvation of underground portions of the plant may then both arrest root growth and promote rapid nodule emptying. Thus, for the present, the flowering factor in the nodulation cycle may be conveniently relegated to the many little-understood physiological effects associated with annual plant flowering. More detailed analyses of the carbohydrate and nitrogen nutrition of nodules on flowering plants might well lead to a greater appreciation of the actual physiological processes involved in nodule destruction.

The practical implications of delaying or preventing flowering in an annual legume are obvious. For instance, greater final yield with longer growing season could be obtained from the symbiosis of a species grown in climatic conditions unsuited to flowering or seed setting, provided that the symbiotic machinery permitted continued nitrogen fixation throughout the extended vegetative phase. Similarly, cutting of plant tops or light grazing might retard flowering and its accompanying nodule degeneration.

(d) *Nodule Longevity in Various Irish Legumes*

The most surprising result of the seasonal nodulation studies in Northern Ireland is that the normal life span of the nodule is not moulded by climatic influences to the extent depicted by previous authors. Successful overwintering of nodules in Ireland is recorded as a common event in each of the legume life forms studied, and it would be interesting to compare these effects with the overwintering of legumes in geographical regions experiencing more prolonged and intense cold in the autumn and winter months. The biennial nodules described for the woody genera *Ulex* and *Sarothamnus* provide further evidence of restricted climatic interference with nodule longevity patterns. In the case of these woody perennials in temperate climates it is suggested that nodules may be classed as "potentially

perennial", i.e. their anatomical and physiological constitution will allow for perennation if suitable climatic conditions persist throughout their life span.

Frost destruction of nodules, where occurring, is clearly incidental and superimposed on the normal nodulation pattern of the host plant. Thus, the autumn set of young nodules in the winter annual may be partially or completely destroyed by winter conditions, while mature nodules may be destroyed in the overwintering biennial or perennial. One might speculate that other influences such as high soil temperatures, drought, and grazing effects would promote a similar interruption of the normal sequences of nodulation. Study of the effects of these factors would be particularly interesting in the Australian environment.

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