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A COMPARISON OF THE EFFECT OF COMBINED NITROGEN ON NODULATION IN NON-LEGUMES AND LEGUMES

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As pointed out previously [4] evidence is accumulating that the non-legume root nodule plants play a significant part in connection with the biogeochemistry of nitrogen *. Their habitat requirements and thus their distribution tend to be completely different from those of legumes. For example *Myrica gale* inhabits peat bogs, *Alnus glutinosa* likewise favours swampy situations, *Shepherdia canadensis* is a scrub-plant extending to sub-arctic regions, while *Casuarina equisetifolia* occurs as a strand plant in the Indian and Pacific Ocean regions. In view of the nitrogen-fixing properties which have been demonstrated for the nodules of most of the genera, it cannot be doubted that these plants play a part in the maintenance of soil nitrogen. Thus the study of factors liable to affect the development or the activity of the nodules is of interest.

In the present instance a study has been made of the effect of the presence of combined nitrogen in the rooting medium on the development of nodules in *Myrica* and *Alnus*. It is well known that like free-living fixers such as *Azotobacter*, the nodulated legume uses combined nitrogen in preference to free nitrogen, and that this is accompanied by a reduced development of nodules. Large amounts of combined nitrogen, in excess of those likely to be present naturally in soils, are required to produce complete suppression of nodules. Thus it is a matter of common observation that broad beans and peas show abundant nodulation in the garden even though the soil has been well manured or fertilised.

* Apart from the legumes the following Angiospermous genera also form root nodules inhabited by a symbiotic micro-organism: *Myrica*, *Alnus*, *Hippophaë*, *Elaeagnus*, *Shepherdia*, *Casuarina*, *Coriaria*, and *Ceanothus*.

Some previous observations on the effect of this factor in non-legumes are available. Bjorkman[1] grew alders in a humus-sand mixture and observed that in the presence of 100 mg ammonium-nitrate nitrogen per litre of culture solution the volume of nodules formed was reduced from 33 cu.mm per plant (the value with no added nitrogen) to 15 cu.mm. Nodulation was almost completely suppressed in the presence of 200 or 400 mg nitrogen per litre. Bond, Fletcher and Ferguson[3] reported on the effect of up to 100 mg ammonium nitrogen per litre on nodulation chiefly in *Alnus* and *Hippophaë*. In *Alnus* after 12 weeks' growth the absolute weight of nodules per plant was greater at all nitrogen levels than with zero nitrogen, though relative to the increased weight of the plants the nodule development was smaller.

Nodulation in *Hippophaë* was found to be much more sensitive. Quispel[5] reported rather different findings for *Alnus*. After 7 weeks' growth a level of approximately 5 mg ammonium nitrogen per litre was found to have reduced the absolute weight of nodules per plant to half, while 50 mg practically suppressed nodulation. These are much greater effects than those observed by the previous authors.

In the present work the case of *Alnus* has been further investigated, particularly in that the effect of feeding the plants with combined nitrogen prior to inoculation has been tested, thus approaching more nearly conditions in the field. *Myrica gale* also has been studied, and for purposes of comparison a similar experiment with a legume (*Ulex europaeus*) has been included.

METHODS

Seedlings were raised in peat (*Myrica gale*), sand (*Alnus glutinosa*) or on mineral salt agar (*Ulex europaeus*), the seed of the last species being surface-sterilised prior to sowing. The seedlings were transferred into water culture in Crone's nitrogen-free solution adjusted to a favourable pH, the containers being 2-litre glazed earthenware jars each with 5 or 6 plants supported in teak tops. Combined nitrogen was added as desired in the form of ammonium sulphate. To inoculate the non-legume the appropriate nodules were ground up in water (3.5 g for *Myrica* and 20 g for *Alnus* per 100 ml water), the suspension so obtained being applied to the roots of the young plants. With *Ulex* a similar suspension was prepared from a pure culture of an effective strain of rhizobium. In certain instances the plants were allowed to grow for a period in the presence of combined nitrogen prior to inoculation.

In experiments of this type involving the utilisation of ammonium nitrogen it is of critical importance that the pH of the culture solution, which of course tends to fall, should be frequently restored to its proper level. The pH was tested every second day, and when necessary was restored to the correct value by the addition of an appropriate amount of sodium hydroxyde, determined by titration of a small sample of the culture solution. The ammonium-nitrogen content of the culture solution was also determined periodically and further amounts added as necessary to maintain the desired level. The culture solution was completely renewed every 3 to 4 weeks.

DATA

Myrica gale

Young plants with 3 or 4 leaves were set up in water culture on 22nd May at pH 4.5. Four levels of combined nitrogen were established in different jars on the same day, viz, 0, 10, 50, and 100 mg nitrogen per litre, and inoculation was effected 2 days later The first nodules appeared 2 weeks later. Considerable losses of plants occurred, which in subsequent unpublished work Miss I. C. Gardner has shown can be entirely obviated by using a more dilute form of Crone's solution. Thus the numbers of plants available for harvest on 28th August were rather small. Typical plants are shown in Plate IA and the harvest data are provided in Table I. Plant growth was much stronger in the presence of combined nitrogen and it will be observed that nodule weight was also greatly increased, though relative to the dry weight of the plant as a whole (see right-hand column) nodule weight was depressed. An analysis of variance shows that the fall from 12.2 to 5.4 is highly significant, but that the subsequent further falls are not significant at $P = 0.05$.

TABLE I

Effect of added combined nitrogen on nodulation and growth of *Myrica* plants *

Mg NH_4-N added per litre of culture soln.	Mean height of shoot in cm	Mean dry weight per plant in mg		Mean value for nodule weight as percentage of whole plant weight
		Nodules	Whole plant	
0	3	4	33	12.2
10	8	17	304	5.4
50	12	24	561	4.2
100	10	19	488	3.6

* The numbers of plants harvested at the different nitrogen levels, starting with zero nitrogen, were: 9, 12, 6, and 5 respectively.

Alnus glutinosa

The experiment comprised two parts. With one series of plants the differential dosage with combined nitrogen was commenced directly after transplanting into water culture, the plants being at the 1 to 2 leaf stage with an over-all height of 3 to 4 cm. Inoculation was effected the following day. With the other series the plants were grown on in water culture in the presence of 50 mg ammonium nitrogen per litre for a period of 5 weeks prior to the commencement of the differential treatment with nitrogen and to inoculation. By this time the plants had 4 to 5 leaves and an over-all height of 7 to 9 cm. The procedure was so timed that the plants of both series were all inoculated on the same day (30th July) with the same inoculum. Analysis of similar plants showed that the percentage nitrogen content (dry matter basis) at the time of inoculation was 1.3 for the plants of the first part of the experiment and 3.4 for those of the second. The first nodules appeared only 10 days after inoculation. Growth was allowed to continue for approximately 10 weeks, the pH of the culture solution being maintained near to 6.3.

Typical plants of the first series are shown in Plate IB, while the harvest data are given in the upper part of Table II. It will be observed that as in *Myrica* the growth of the plants was again much benefited by the presence of combined nitrogen. The dry weight of nodules is higher in the presence of 10 mg nitrogen per litre than in zero nitrogen, but is smaller when 100 mg is present. Relative to the whole plant weight, nodule weight falls sharply between the 0 and 10 mg nitrogen levels (see right-hand column), and thereafter tends to fall more slowly.

Data for the second series of plants, given pre-treatment with nitrogen prior to inoculation, are shown in the lower part of Table II. These plants were naturally much larger than those of the first series. It will be noted that the dry weight of nodules is approximately doubled by the presence of 10 mg nitrogen per litre, but at higher nitrogen levels is much reduced, so that with 100 mg nitrogen present the dry weight is only one quarter of that in nitrogen-free solution. Thus in this respect the effect of combined nitrogen is considerably more marked than in the first series. The right-hand column shows that pre-treatment with nitrogen reduced the subsequent nodulation in nitrogen-free solution, since the value of 3.4 per cent is to be compared with 5.3 per cent in the upper part of the Table. The presence of combined nitrogen after inoculation depressed this value further.

TABLE II

Experiment with *Alnus* *				
Procedure	Mg NH_4-N added per litre of culture solution **	Mean dry weight per plant in mg		Mean value for nodule weight as percentage of whole plant weight †
		Nodules †	Whole plant	
First Series				
Differential treatment with nitrogen and inoculation applied directly after transplanting into water culture	0	3.3	65	5.3
	10	5.5	394	1.7
	50	3.6	436	1.0
	100	2.6	403	0.7
Second Series				
Plants all supplied with uniform combined nitrogen for 5 weeks prior to start of differential treatment and inoculation	0	28	878	3.4
	10	54	2275	2.4
	50	20	2363	0.8
	100	7	2116	0.4

* 15 plants were harvested at each nitrogen level in the first series and 12 in the second series.

** The maximum deviations from the desired levels of NH_4-N during the growth period were as follows: — 10 mg fell to 6 mg, 50 to 47 mg, 100 to 95 mg.

† Differences required for significance ($P = 0.05$) are as follows; — Nodule Dry Weight, 0.9 mg (first series) and 10 mg (second series); Nodule Weight as percentage of Whole Plant Weight, 0.8 (first series) and 0.3 (second series).

In the above experiments no counts were made of the numbers of nodule clusters formed in the different treatments, but it was obvious from inspection of the plants that the beneficial effect of the lower levels of combined nitrogen on nodule weight was due more to increase in size of the nodule clusters than to increase in their total number.

A

B

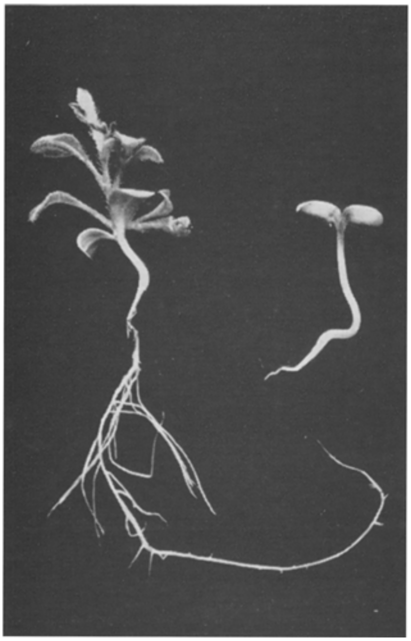
Plate I.

A. Nodulated plants of *Myrica gale* after 14 weeks' growth in water culture, supplied with (left to right) 0, 10, 50 and 100 mg NH_4-N per litre. The upward-growing roots spring from the nodule lobes.* (× 1/3).

* Note that while the photograph suggests that plants supplied with 100 mg N per litre were superior to those supplied with 50 mg, the harvest data subsequently obtained (Table I) revealed no significant difference in the mean growth of these plants.

B. Nodulated plants of *Alnus glutinosa*, first series, after 10 weeks' growth in water culture, supplied with (left to right) 0, 10, 50 and 100 mg

A



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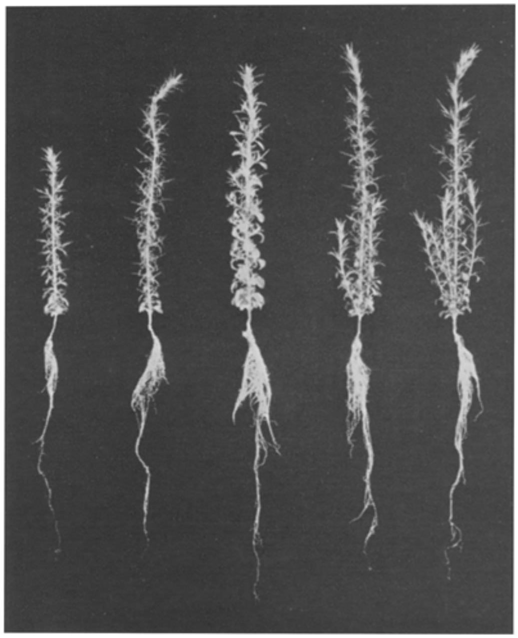

C

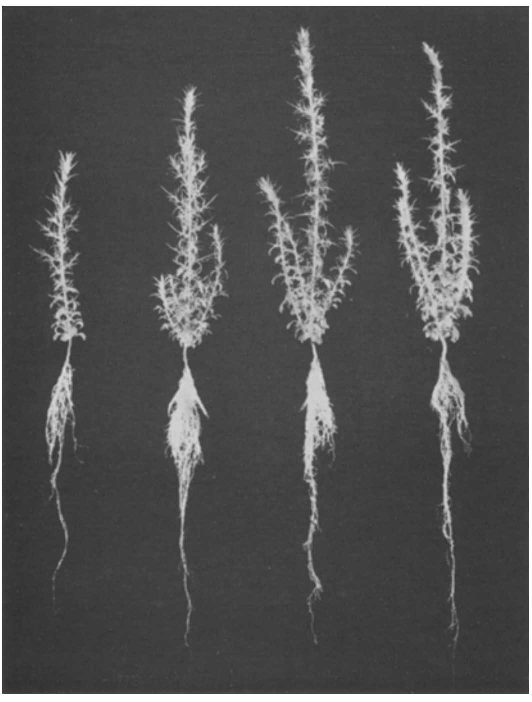

Plate II.

A. Young plants of *Ulex europaeus* at the time of inoculation, plant from first series on right, from second on left. (× 1).

B. Nodulated plants of *Ulex* (first series) after 10 weeks' growth in water culture, supplied with (left to right) 0, 10, 50, 100 and 150 mg NH_4-N per litre. (× 1/7).

C. Nodulated plants of *Ulex* (second series) after 10 weeks' growth in water culture, supplied with (left to right) 0, 10, 50 and 100 mg NH_4-N per litre. (× 1/6).

Ulex europaeus

The general plan of the experiment was as in *Alnus*. The two series of plants, the first newly transplanted into water culture, the second grown for 3 weeks in the presence of 50 mg combined nitrogen per litre, were provided with different levels of combined nitrogen on 22nd May and inoculated the following day. Typical plants at this stage are shown in Plate IIA. Analysis of similar plants showed percentage nitrogen contents on the dry matter basis of 7.4 for plants of the first series and 4.1 for those of the second. All plants were nodulated within a fortnight. Growth was allowed to continue for about 9 weeks at pH 6.3.

TABLE III

Experiment with *Ulex* *					
Procedure	Mg NH_2-N added per litre of culture solution **	Mean number of nodules per plant †	Mean dry weight per plant in mg		Mean value for nodule weight as percentage of whole plant weight †
			Nodules †	Whole plant	
First Series					
Differential treatment with	0	38	11	166	6.6
nitrogen and inoculation	10	82	10	304	3.4
applied directly after	50	69	9	342	2.8
transplanting into water	100	95	8	409	2.1
culture	150	79	8	355	2.3
Second Series					
Plants all supplied with	0	97	12	200	6.1
uniform combined nitrogen for 3	10	148	16	468	3.6
weeks prior to start of	50	76	8	600	1.3
differential treatment and inoculation	100	60	6	544	1.1

* The number of plants harvested varied from 17 to 21 at each treatment.

** The maximum deviations from the desired levels of NH_4-N during the growth period were as follows; — 10 mg fell to 5 mg, 50 to 40 mg, 100 to 87 mg, 150 to 140 mg.

† Differences required for significance ($P = 0.05$) are as follows: — Nodule Number, 34 (first series) and 22 (second series); Nodule Dry Weight, 2 mg in both series; Nodule Weight as percentage of Whole Plant Weight, 0.7 (first series) and 0.6 (second series).

Typical plants at harvest of the first series are shown in Plate IIB, and the corresponding data in the upper part of Table III. It will be noted that plant dry weight was about doubled in the presence of combined nitrogen, but this was not accompanied by an increase in nodule weight. The latter declined in the presence of 50 mg or more of nitrogen. Nodule number was doubled by the change from zero nitrogen to 10 mg per litre, and remained at this high level with further increase in nitrogen. Obviously the nodules were smaller in weight and size in the presence of nitrogen. Calculation from the data presented shows that the mean dry weight per nodule diminished from 0.29 mg in zero nitrogen to 0.10 mg in the 150 mg level of nitrogen. Nodule weight as a percentage of whole plant weight (right-hand column) shows a marked fall in the presence of 10 mg nitrogen, and thereafter falls more slowly.

Plants of the second series are illustrated in Plate IIC, and their data presented in the lower part of Table III. In most respects combined nitrogen now had a more adverse effect on nodulation than in the first series. Thus nodule weight and number, and nodule weight as a percentage of whole plant weight, were all more drastically reduced in the presence of 50 or 100 mg nitrogen per litre than in the first series. However with 10 mg nitrogen present nodule weight per plant was significantly increased, and mean dry weight per nodule fell only from 0.12 mg in zero nitrogen to 0.10 mg in 100 mg nitrogen per litre. These findings will be further discussed below.

DISCUSSION

The results of the *Alnus* experiment (first series) may be compared with those of the similar experiment of Bond, Fletcher and Ferguson [3]. Both agree in showing that as the amount of combined nitrogen supplied is increased, nodule weight falls continuously relative to the weight of the plant as a whole, but that at low levels of nitrogen the absolute weight of nodules is substantially increased. In the experiment of Bond *et al.*, with 100 mg nitrogen supplied nodule weight was still greater than in zero nitrogen, while in the present experiment this was only true up to some nitrogen level between 10 and 50 mg per litre. Other experience shows that the reason for this difference is that the present experiment was started a month later in the growing season, when conditions are less favourable to nodule development.

The increases in absolute weight of nodules noted in the above and other experiments are obviously related to the increased growth of the plant as a whole in the presence of combined nitrogen. The nodules share in this greater growth, but not to a proportionate extent, *i.e.* the combined nitrogen is already exercising a differential effect on nodule growth on one hand and on root and shoot growth on the other. The enhanced growth of the plant in the presence of combined nitrogen arises because of the time required for the nodules to develop and become functional, the growth of plants in nitrogen-free solution being greatly handicapped during this period. In longer-term experiments the growth of the latter plants would probably gradually overtake that of those supplied with combined nitrogen and as this happened the superiority in absolute nodule weight would diminish. Thus the effect is probably a temporary one.

As noted already, when the commencement of differential treatment with nitrogen and inoculation were delayed until the *Alnus* plants had grown to a considerable size with the help of combined nitrogen, as in the second series, then the unfavourable effect of the higher levels of nitrogen on nodule development were more pronounced. The procedure in this part of the experiment was generally similar to that employed by Quispel [5], but there are marked differences in result since, as noted earlier, in his experience ammonium nitrogen at only 5 mg per litre had an entirely depressing effect on nodule formation. Although it is clear that in most respects Quispel's experiments were carefully carried out, it is not quite certain that his technique excluded the possibility of the pH falling to levels harmful to nodulation. Thus he used a solution of inferior buffering power to Crone's, a high ratio of number of plants per unit volume of solution, and corrected the pH only once weekly.

Myrica gale obviously resembles *Alnus glutinosa* closely as regards the effect of combined nitrogen on nodule development. Thus as the nitrogen level is increased the weight of nodules per plant at first rises and subsequently falls, while relative to whole plant weight the value for nodules is continuously depressed.

Besides their immediate value in providing a basis of comparison for the non-legumes, the *Ulex* data are of wider interest in that, unlike many previous data for legumes, they were obtained by a technique permitting close control of the level of combined nitrogen and of pH. At the levels used, combined nitrogen did not exert a particularly drastic effect on nodulation. Thus in the first series, levels of nitrogen clearly in excess of the requirements of the plants (under favourable growth conditions) resulted in increased nodule numbers, a rather slight reduction in nodule weight per plant, and reductions to about one third in the weight of nodules relative to that of the whole plant and in the mean weight per nodule. Adverse effects were somewhat stronger after pre-treatment with combined nitrogen. It is important to restate that in this experiment the first series of plants showed a higher percentage of nitrogen (7.4) at the time of inoculation than those of the second series (4.1). It has been shown by previous workers that in legumes the carbohydrate-nitrogen ratio within the tissues is an important factor in the control of nodulation. A high ratio usually tends to favour nodulation. This is undoubtedly the reason why in zero nitrogen the percentage nodule weight was not considerably lower in the second series than in the first, as was the case in *Alnus*.

For the comparison of *Alnus* and *Ulex* it will be best to confine attention chiefly to the second series of plants of each experiment in view of the large disparity in the nitrogen balance of the plants at the time of inoculation in the first series, for as noted already the percentage nitrogen in *Alnus* was 1.3, but in *Ulex* was 7.4. This is due chiefly to differences in seed composition. By the time of inoculation in the second series of plants the difference had largely disappeared, the percentage nitrogen having increased to 3.4 in *Alnus* and decreased in *Ulex* to 4.1. Reference to the second series of Tables II and III reveals many similarities in response to combined nitrogen. With 10 mg nitrogen present nodule weight per plant is increased in both species, though the increase is smaller in *Ulex* than in *Alnus*. At higher levels of nitrogen nodule weight falls. In both species nodule weight relative to plant weight falls continuously. Thus *Alnus* resembles *Ulex* closely in its responses to combined nitrogen, and it is clear that the same is true of *Myrica*, although the data for it are less extensive.

This article is intended to be essentially factual in type and the possible explanation of the observed effects of combined nitrogen on nodule development will not be considered in detail. A full discussion of suggested explanations for legumes is provided by Wilson [6]. It is reasonable to suppose that the mechanism of the combined nitrogen effect in non-legumes is similar to that in legumes.

The data reported for *Alnus* indicate that the combined nitrogen exerts its effect internally in the plant tissues. Thus the effect was greater when the tissues were already relatively rich in nitrogen as a result of pre-treatment (second series).

Indirectly the present data confirm that *Hippophaë* is much more sensitive to combined nitrogen than *Alnus* or *Myrica*. Bond, Fletcher and Ferguson [3] reported that in the first genus the presence of only 5 mg nitrogen per litre greatly decreased nodulation, but they pointed out that this referred to plants which had been grown for a period in the presence of combined nitrogen to inoculation. The present experiments show that *Alnus* similarly treated is still much less sensitive to combined nitrogen than is *Hippophaë*.

This article is not concerned with the question of how active in fixation are the nodulus formed in the presence of combined nitrogen. On the basis of isotopic tests with *Alnus* and *Myrica*, Bond [2] concluded that such nodules fix appreciable quantities of free nitrogen.

The data presented indicate that the nodulation of *Alnus* and *Myrica* in the field will not be seriously reduced by the levels of combined nitrogen likely to be present in the normal habitats of the plants.

SUMMARY

1. The effect of the presence of combined (ammonium) nitrogen in the rooting medium on nodule development has been investigated in *Alnus glutinosa* and *Myrica gale*, the plants being grown by a water culture technique with provision for the control of the level of combined nitrogen and of pH. For purposes of comparison a similar experiment with a legume (*Ulex europaeus*) has been included.

2. In some instances the differential treatment with combined nitrogen was commenced and inoculation effected at an early stage in plant development, while in others the plants were grown on to a larger size before these treatments were applied.

3. In the two non-legumes the presence of combined nitrogen led to an increase in the weight of nodulus formed per plant, at least at the lower levels of nitrogen. Relative to the enhanced growth of the plant as a whole, nodule development was continuously depressed as the level of combined nitrogen was increased.

4. In *Ulex* when the nitrogen status of the plants at the time of inoculation was similar to that in the corresponding non-legume experiment, the above responses were also shown by the legume. It is concluded that *Alnus* and *Myrica* resemble legumes closely in their responses to combined nitrogen.

5. The data show that in *Alnus* the effect of a given level of combined nitrogen on nodulation depends on the nitrogen status of the plant at the time of inoculation, the adverse effect tending to be stronger if the plant is initially relatively high in nitrogen. This suggests that as in legumes the effect of the combined nitrogen is exerted internally in the plant tissues.

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