Netherlands Journal of Agricultural Science

VOLUME 10 NO. 1 FEBRUARY 1962

The effect of soil-pH, stable manure and fertilizer nitrogen on the growth of red clover and of red clover associations with perennial ryegrass

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Summary

An investigation was made into the effect of soil-pH, fertilizer nitrogen and stable manure on the growth of red clover and of red clover-perennial ryegrass associations. The experiments with clover-grass mixtures were performed on a series of garden plots of different pH during the years 1957 and 1958. In 1959, red clover and grass were grown separately on these garden plots. In each year the clover and the grass were sown on part of the plots where no clover had been grown for at least 35 years; the seeds or the soil were not inoculated with *Rhizobium trifolii*. Investigations comparable with those in the field were carried out in a number of pot experiments.

On the garden plots, soil-pH was found to be of major importance in the response of the clover-grass association to fertilizer nitrogen. On acid soil (pH below 5,3) where nodulation, nitrogen fixation and development of the legumes were poor, the yield of herbage, which consisted mainly of grass, was wholly dependent on fertilizer-nitrogen supply. On slightly acid and neutral soil (pH 6 and higher), however, the response to added nitrogen was slight, due to the profuse nodulation, nitrogen fixation and development of the red clover with little or no fertilizer nitrogen. With increasing amounts of fertilizer nitrogen the amount of grass produced increased considerably but that of clover decreased sharply, being suppressed by the faster growth of the grass.

When grown alone, the response of red clover on the acid soils to fertilizer nitrogen was similar to that of the ryegrass, demonstrating that it was competition by the grass which suppressed the clover when grown together with high nitrogen dressings.

Nodulation of the red-clover plants on the uninoculated garden plots was absent or poor at a soil-pH of 5,2 or lower, moderate at pH 5,3 to 5,8, and profuse at higher pH. Inoculation of the soil with an effective strain of *Rhizobium trifolii* gave normal nodulation even at pH 5,0. With increasing supply of nitrogenous fertilizer, nodulation was increasingly delayed. Under certain conditions (pot experiment with inoculated acid soil) this led to nitrogen-deficiency in plants having received fertilizer nitrogen since no effective nodules had formed by the time the fertilizer nitrogen was used up. Control plants without fertilizer nitrogen appeared normal because they had formed effective nodules.

Stable manure, particularly when residual in the soil, promoted nodulation and nitrogen fixation of clover plants on uninoculated acid soil (pH 5,0); on inoculated acid soil this effect was still clearly perceptible, but weaker.

Received for publication 16th August, 1961.

1. Introduction

The importance of leguminous plants, particularly clovers, in supplying nitrogen for the vegetation of grassland is generally accepted; in many countries they provide the only supply of nitrogen (WALKER, 1956; SEARS, 1960). The transfer of the fixed nitrogen from legumes to grass may have more than one cause, viz. excretion by the nodules or the roots of the legumes, decay of the root systems of the legumes, and distribution of the legume nitrogen after its consumption by grazing animals (BUTLER and BATHURST, 1956).

Although clover plants may fix large amounts of nitrogen (up to 300 kg of N per ha per year under the moderate climatic conditions of Western Europe), it is highly probable that in a sward consisting of legumes for only 25—30 %, this nitrogen supply would be inadequate for maximal yields of herbage. Otherwise, it would mean that only 25—30 % of the vegetation must fix up to 300 kg N per ha (the amount required for the production of 10.000 kg of dry matter per ha). This lack of nitrogen available to the grasses is of particular importance in spring when their demand for nitrogen starts earlier than legume nitrogen becomes available to them.

The latter considerations may provide the reason why, in countries with intensive grassland cultivation like the Netherlands, large amounts of fertilizer nitrogen must be applied to grassland when high yields are required. Under the conditions prevailing in large parts of this country (permanent pastures with a relatively low content of clover on moist soils of relatively low pH), this procedure is presumably economically preferable to a system of short-term leys with a much higher proportion of clover in the sward. On many wet peaty soils and heavy clay soils of poor structure, tillage is almost impossible. It was found that, on such permanent pastures, increasing amounts of fertilizer nitrogen up to several hundred kg N per ha lead to an almost linear response of grass growth (MULDER, 1949, 1952).

A drawback to the use of fertilizer nitrogen on pastures is the suppression of legumes by grasses which are more encouraged by added nitrogen than are the legumes. This is an indirect effect mainly resulting from competition for light, plant nutrients (WALKER, 1956) and perhaps water (ROBINSON and SPRAGUE, 1947). Although this effect may be largely prevented by grazing or close clipping (FRANKENA, 1937), decrease of legumes in a sward amply dressed with fertilizer nitrogen is virtually inevitable. This means that a natural and economical source of nitrogen may be lost and that on grass-clover swards a large proportion of the applied fertilizer nitrogen merely substitutes for the legume nitrogen (see HOLMES and ALDRICH, 1957).

The role of white clover in grassland has recently been reviewed by MARTIN (1960). HOLMES and MACLUSKY (1955), GREEN and COWLING (1960) and LINEHAN and LOWE (1960) compared the effect of fertilizer nitrogen on clover mixtures and pure grass stands in their dry matter production and nitrogen yield.

The purpose of the present investigation was to study the effect of soil-pH, stable manure and added fertilizer nitrogen on the growth of clover and of clover-grass associations in short term field experiments. Because of the harvesting technique employed, red clover was used in most experiments.

Experiments similar to those in the field were conducted in Mitscherlich pots and in Neubauer glass jars containing soil from the plots used in the field experiments.

2. Experimental

2.1. Field experiments

Red clover and perennial ryegrass were grown on a number of plots with different pH-values in the garden of the Laboratory of Microbiology at Wageningen. These plots were laid down in triplicate on a poor sandy soil more than thirty years ago by applying calcium carbonate in the following amounts: 1.250, 2.500, 3.750, 5.500 and 11.000 kg per ha. As a result of this treatment the soil-pH of the six plots within each series ranged from 5 to 7. For maintaining the pH-levels, calcium carbonate was added periodically.

On these plots a primary dressing of 100 kg P_2O_5 per ha as superphosphate and 125 kg K_2O per ha as potassium sulphate was applied yearly. All plots were divided into three equal parts to enable a 3-year rotation. Each of these parts was subdivided into 5 subplots of 1,40 m square which were dressed with different amounts of ammonium nitrate limestone. These nitrogen dressings were given two or three times in spring and summer.

In 1957, red clover (var. *Gendringen*) and perennial ryegrass (var. *Mommersteeg*) were sown in separate and alternate rows 7,5 cm apart on 10 April. Nitrogen as ammonium nitrate limestone was applied in different amounts. Clover and grass were harvested and weighed separately; representative samples for dry matter and nitrogen analyses were taken from the first cuttings. The experiment consisted of the following three series of plots:

	N applied on	*Cut on*	
Series I	11 April	25 June	
	no nitrogen	19 August	
	20 August	9 October	
Series II	11 April	11 July	
	no nitrogen	19 August	
	20 August	11 October	
Series III	11 April	14 August	N-dressings: 0, 40, 80,
	20 August	12 October	120 and 200 kg N/ha.

In 1958 the lay-out of the field experiment was similar to that of the preceding year except that a different variety of red clover (C.B.) was used and the rates of nitrogen application were reduced (see below). The experimental design was as follows:

	N applied on	*Cut on*	
Series I	25 April	8 July	
	no nitrogen	6 August	
	7 August	10 October	
Series II	25 April	8 July	
	9 July	6 August	
	7 August	10 October	
Series III	25 April	8 July	
	9 July	6 August	N-dressings: 0, 40, 60,
	no nitrogen	10 October	80 and 100 kg N/ha.

The 1959 experiment differed from those of the two preceding years in that red clover (var. *Kühn*) and perennial ryegrass were grown in monoculture. Nitrogen was applied at 30, 60, 90 and 120 kg N per ha as ammonium nitrate limestone; these dressings were repeated after each cutting. Clover and grass were sown on 25 April and cut on 15 July and on 19 August. Yield data are averages of triplicate values. No clover had been grown on the plots in the years preceding the experiments and no effort was made to introduce *Rhizobium trifolii* during the 1957—'59 experiments. In 1958 and '59 the clover was grown on a part of the plot where in the preceding year no clover was grown.

2.2. Pot experiments

To investigate the combined effects of soil-pH, fertilizer nitrogen and stable manure on growth of red clover, experiments in Neubauer glass jars and in Mitscherlich pots were carried out.

1957. In this experiment the acid sandy soil (pH 5) from the garden plots was contained in Neubauer glass jars, approximately 0,6 kg of soil per jar.

The primary dressing consisted of superphosphate and potassium sulphate which had been applied to the plot from which the soil was taken. *Nitrogen* in the form of NH_4NO_3 was supplied in amounts of 21, 42 and 84 mg N per pot. For comparison, *stable manure* was added in amounts of 5, 10 and 20 g fresh material corresponding respectively with 21, 42 and 84 mg N per pot. Extract of stable manure, prepared by shaking fresh stable manure with water for 18 h and filtering (see MULDER and VAN VEEN, 1960), was also included in the experiment. Some pots were supplied with 2,5 mg *sodium molybdate* each.

Approximately twenty plants of red clover were grown per pot. Four control pots were left uninoculated, the others were inoculated with the effective K 8 strain of *Rhizobium trifolii*. The experiment was started on 28 June and concluded on 30 September 1957. The plants were kept outdoors during day time and transferred to the greenhouse overnight and in rainy weather.

1958. Soils of pH 5,0 and 7,0 taken from the above-mentioned garden plots were used. As a primary dressing 1 g KH_2PO_4 and 0,25 g $MgSO_4.7H_2O$ were added per pot. *Nitrogen* in the form of KNO_3 was applied in amounts of 150, 300, 450, 600, 800, 1.000, 1.500 and 2.000 mg N per pot in the experiment with acid soil and of 150, 450, 800 and 1.500 or 300, 600, 1.000 and 2.000 mg N per pot in the experiment with neutral soil.

The amount of *stable manure,* when used, was 50 g fresh weight per pot. In addition, one series of pots was filled with acid soil which had been treated with stable manure a few years earlier (see MULDER and VAN VEEN, 1960).

In some series the soil was mixed with a suspension of the effective K 8 strain of *Rhizobium trifolii* grown on glutamate agar. Approximately 28 plants were grown per pot; pots were kept outdoors throughout the entire experimental period.

2.3. Analytical methods

Nitrogen was determined by the Kjeldahl-Lauro method.

3. Results

3.1. Effect of ammonium nitrate limestone on the growth of red clover-grass mixtures at different pH of the soil

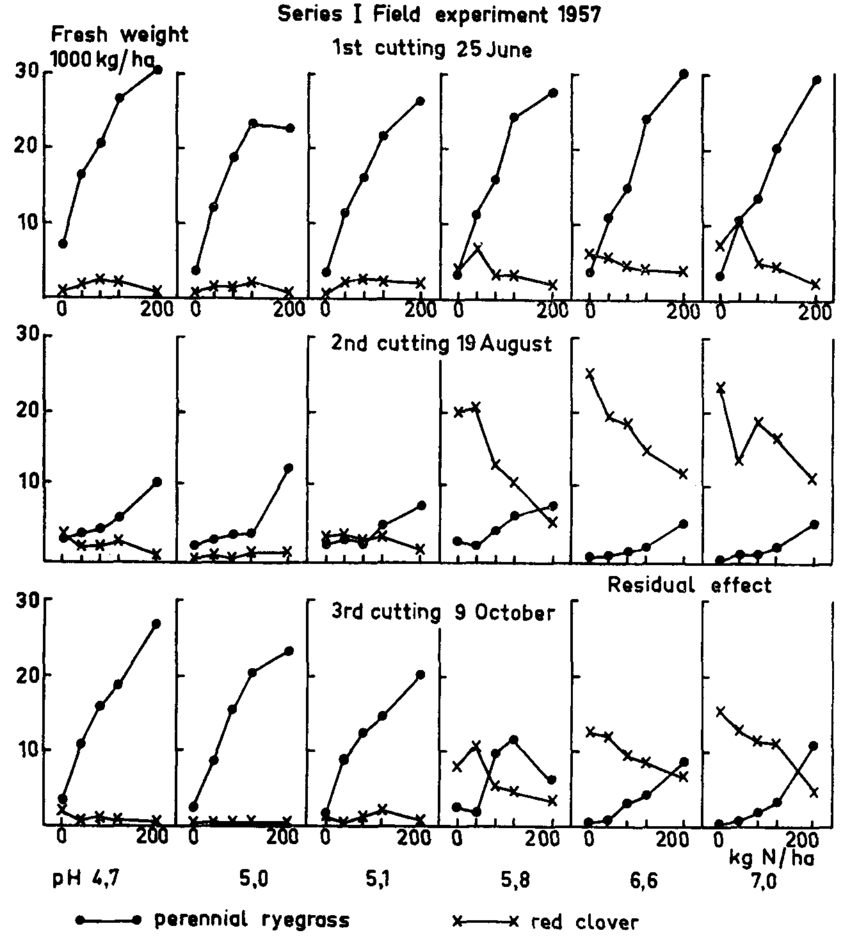
3.1.1. *Field experiment 1957*

The yield data[1] are plotted in FIGURES 1, 2 and 3. It will be seen that the first cutting of series I consisted mainly of grass, whose response to the applied nitro-

[1] See foot-note next page.

gen was hardly affected by soil-pH (see FIG. 1). When supplied with small amounts of nitrogen and in the absence of added fertilizer nitrogen, the red clover responded clearly to the soil-pH. At pH 5,1 and lower the clover plants were poor and had a yellow colour, apparently due to nitrogen deficiency. At pH 5,8, 6,6 and 7,0 nitrogen fixation was adequate so that the leaf colour of the plants was dark green and the yields were higher. With increasing supply of fertilizer nitrogen the yields

FIG. 1. Effect of soil-pH and added ammonium nitrate limestone on the yields of perennial ryegrass and red clover grown in association.



1 Since dry-matter contents of grass and clover were available for the first cuttings of the 1957 experiments only, the results of all experiments are recorded as fresh-weight values. The dry-matter contents available showed that red clover generally had somewhat higher values than perennial ryegrass. Nitrogen-deficient grass had a higher content of dry matter than that well-supplied with nitrogen. Due to the large responses to nitrogen obtained in the present investigation, the latter differences in dry-matter content do not affect the conclusions derived from the fresh weight values.

of clover decreased due mainly to the depressing effect of the grass plants on the growth of the clover and partly to the reduced number of clover plants at the highest N-dressings. The latter had caused some injury to the germinating clover.

The responses to nitrogen of the grass of the first cuttings of the second and third series were similar to those of the first series (FIG. 2 and 3). However, the delayed cutting date considerably enhanced the development of the clover on plots with little or no added nitrogen. Therefore at the late cutting date the response of total herbage to added fertilizer nitrogen was slight on plots with a soil-pH of 6 or higher compared with that on plots with a low soil-pH, where the growth of the clover was poor. The depressing effect of the higher levels of fertilizer nitrogen on development of the clover at a late cutting date was as pronounced as at an early cutting.

The yield curves of the second cuttings from series I and II of the plants

FIG. 2. Effect of soil-pH and added ammonium nitrate limestone on the yields of perennial ryegrass and red clover grown in association (s.m. = stable manure, see p. 9).

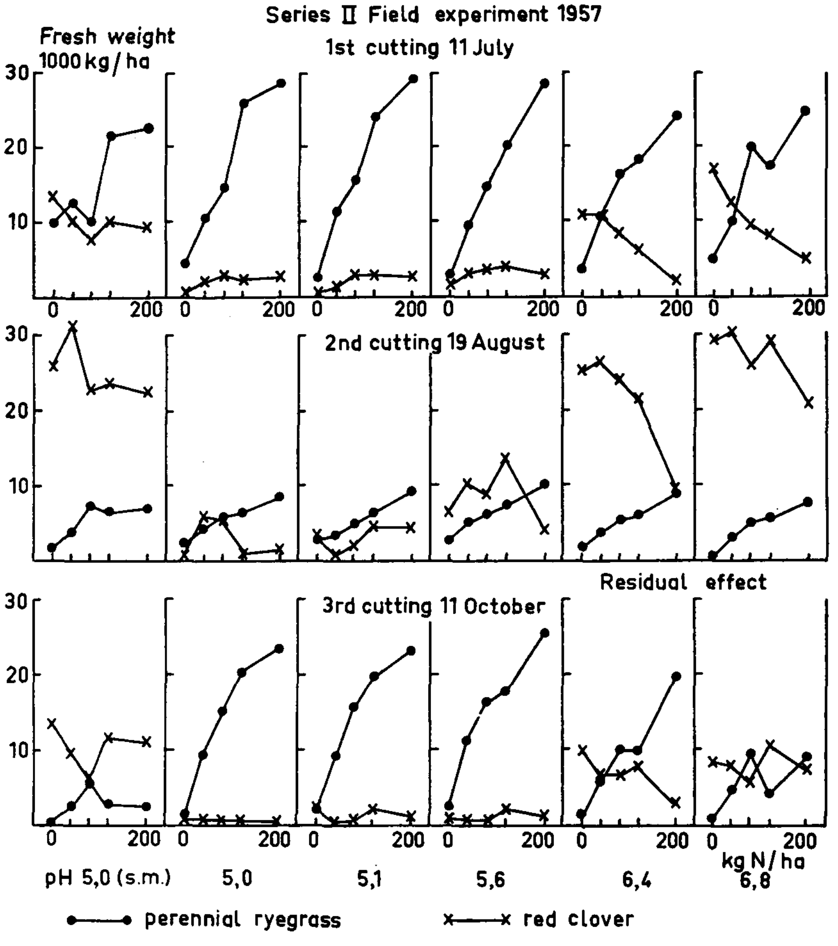
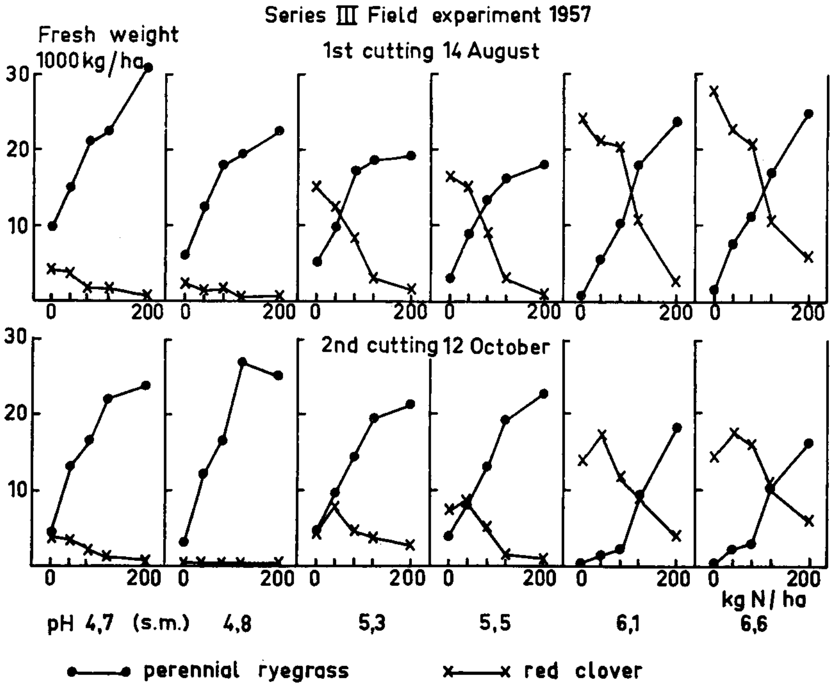


FIG. 3. Effect of soil-pH and added ammonium nitrate limestone on the yields of perennial ryegrass and red clover grown in association (s.m. = stable manure, see p. 9).



which had grown without fertilizer nitrogen showed a completely different picture. Grass yields were small, showing that the residual effect of the nitrogen supplied to the herbage of the first cutting was slight.

There was a pronounced difference in clover yield between the plots with different pH. On the acid plots (pH approximately 5) the development of the clover was very poor; at pH 6 and 7, however, the clover grew luxuriantly and high yields were obtained. Yields were inversely proportional to the amount of fertilizer nitrogen supplied to the herbage of the first cutting. This demonstrates that fertilizer nitrogen given to a grass-clover mixture suppresses the development of the clover not only at the first cutting but also at later stages.

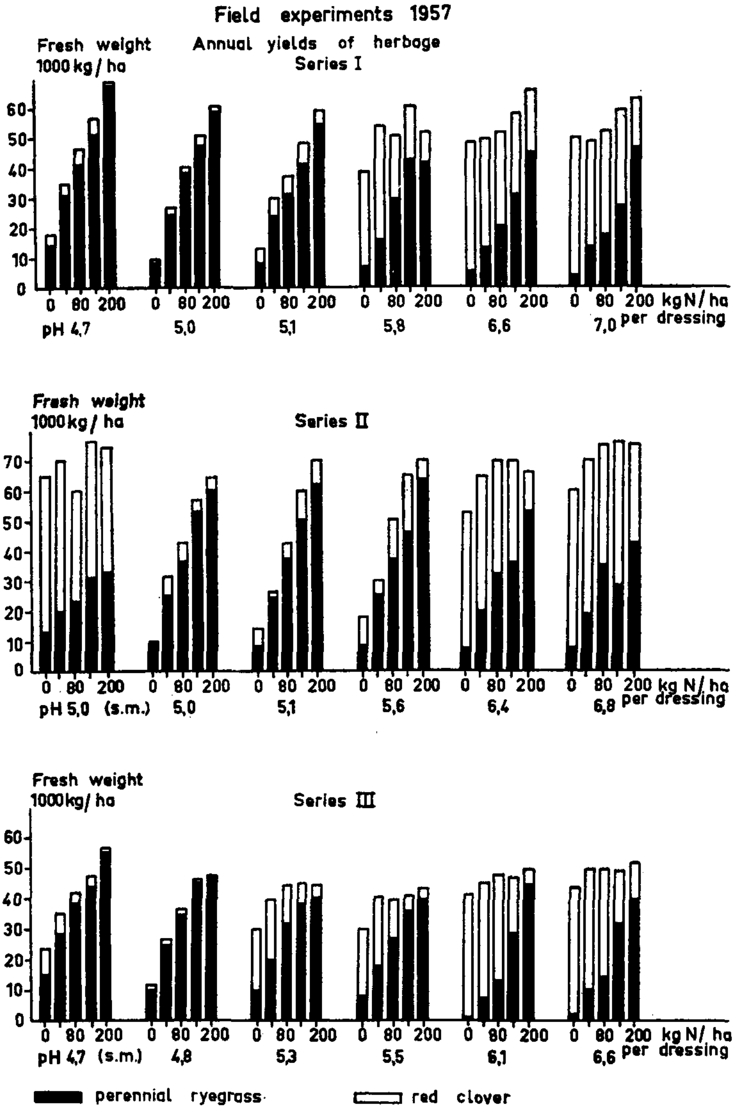
Despite their 16 days shorter growing period, clover yields of series II in general were higher than those of series I, demonstrating that the later cutting date of the first harvest had favoured the regrowth of the clover.

The herbage of the third cutting of series I and II was dressed with amounts of ammonium nitrate limestone equal to those applied at the first cutting. This resulted in a vigorous grass growth on the acid plots where the growth of the clover in the preceding period had been poor. At higher pH-values, however, the response of the grass to the added nitrogen was poor, apparently due to the depressing effect of the luxuriant clover growth during the preceding growing period.

In series III, which was cut only twice, the response of the grass of the second cutting to the added nitrogen was not so small at pH 6 and 7 as in series I and II. This was because series III, unlike series I and II, had no growth period without fertilizer nitrogen.

The annual production of both clover and grass of series I is presented in FIG. 4. It will be seen that on the acid plots, the overall harvest consisted mainly of grass, the growth of which showed a pronounced response to fertilizer nitrogen. At pH 5,8 and higher, however, clover formed a considerable proportion of the

FIG. 4. Effect of soil-pH and added ammonium nitrate limestone on the annual yields of perennial ryegrass and red clover grown in association (s.m. = stable manure, see p. 9).



overall harvest, whereas grass yield was depressed due to competition by the clover. Total yield of clover was inversely related to the amount of added fertilizer nitrogen. Thus the total yield of herbage was only slightly increased by fertilizer nitrogen.
The annual yields of clover and grass of series II, plotted in FIG. 4, responded to the combined effects of pH and nitrogen dressing similarly to those of series I; both grass and clover yields of series II were somewhat higher.
The annual yield of herbage of series III (FIG. 4), which was cut only twice, was considerably lower than that of series I and II due to the delayed cutting of the first harvest which resulted in loss of plant material by decay of the lower leaves.

Residual effect of stable manure on growth of clover and grass. In the abovementioned series two acid plots were included which had been treated with stable manure a few years before starting the clover experiments. On one of these plots, the growth of the red clover with little or no added fertilizer nitrogen was quite normal despite the lower pH of their soil than in acid plots which had not been treated with stable manure (see s.m. FIGURES 2 and 4, series II). On a second acid plot which had been treated with a smaller amount of stable manure the residual effect on the growth of the clover was less spectacular (see s.m. FIGURES 3 and 4, series III).

Nodulation of the clover plants. To study the effect of pH and nitrogen dressings on nodulation of the clover plants, root samples were collected from an area of 10 by 100 cm on each subplot after the first cutting. The root systems were dug up and washed and the nodules from those of series I were collected, dried and weighed. The results, recorded in TABLE 1, are calculated as mg dry nodules per gram of tops (dry weight), to eliminate the differences in development of red clover as affected by fertilizer nitrogen. It will be seen that nodulation was very poor in soil of pH 5,1 and lower but sharply increased at pH 5,8 and higher. Fertilizer nitrogen depressed nodulation considerably; at 200 kg N per ha, even at pH 7,0, almost no nodules were found.

TABLE 1. Effect of soil-pH and nitrogen supply on yield of root nodules (mg dry weight per gram of dry tops)

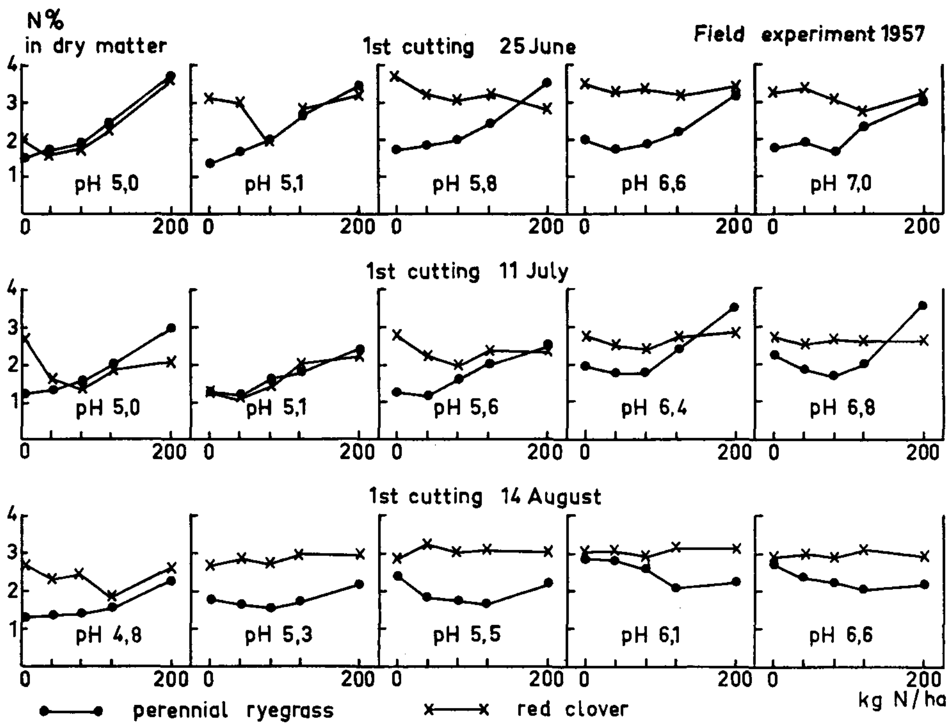
	Soil-pH					
Nitrogen added	4,7	5,0	5,1	5,8	6,6	7,0
Without N	18,8	0	68,2	41,6	44,2	41,1
40 kg N per ha	0,3	0	18,8	24,4	40,6	29,7
80 „	0	0	4,9	38,2	31,1	48,3
120 „	16,4	0	1,3	15,0	21,2	20,6
200 „	0	0	0	1,2	11,5	12,6

This depression of nodulation of the clover plants by the nitrogen dressings was less pronounced in the cuttings of 11 July (Series II) and 14 August (Series III). This was probably due to the removal of the nitrogen by the grass plants. At low nitrogen levels the nodules were distributed throughout the root system; at high levels they occurred in clusters in the top soil.
As the growing season progressed, nodulation was observed at lower pH-values. More-

over on the acid plots small areas of well nodulated and better developed plants were sometimes found. These areas expanded during the growing season, suggesting that well-developed clover plants stimulated the nodulation of neighbouring plants.

Nitrogen content of the herbage. Nitrogen analyses of both grass and clover were carried out on the herbage of the first cutting of all three series of 1957; the results are plotted in FIG. 5. From these data it will be seen that in the grass of series I (cut on 25 June), the nitrogen content of the dry matter varied from 1,5 to approximately 3,5 %, depending on the amount of nitrogen applied; the nitrogen content of the clover at a low soil-pH behaved similarly. At pH-values of 5,8 and higher, however, the nitrogen content of the clover varied between 3,0 and 3,5 % and was independent of added fertilizer nitrogen, presumably due to symbiotic nitrogen fixation.
The nitrogen-content curves for the grass of series II (cut on 11 July) are similar to those of the first series, except that at pH 6,4 and 6,8 the lowest N-content of the grass was found at a dressing of 80 kg N per ha. The higher nitrogen contents of the grass with smaller nitrogen dressings at these pH-values were apparently derived from the clover plants. At low pH-values where the growth of the clover was poor, the lowest nitrogen contents of the grass were found at the lowest levels of fertilizer nitrogen.

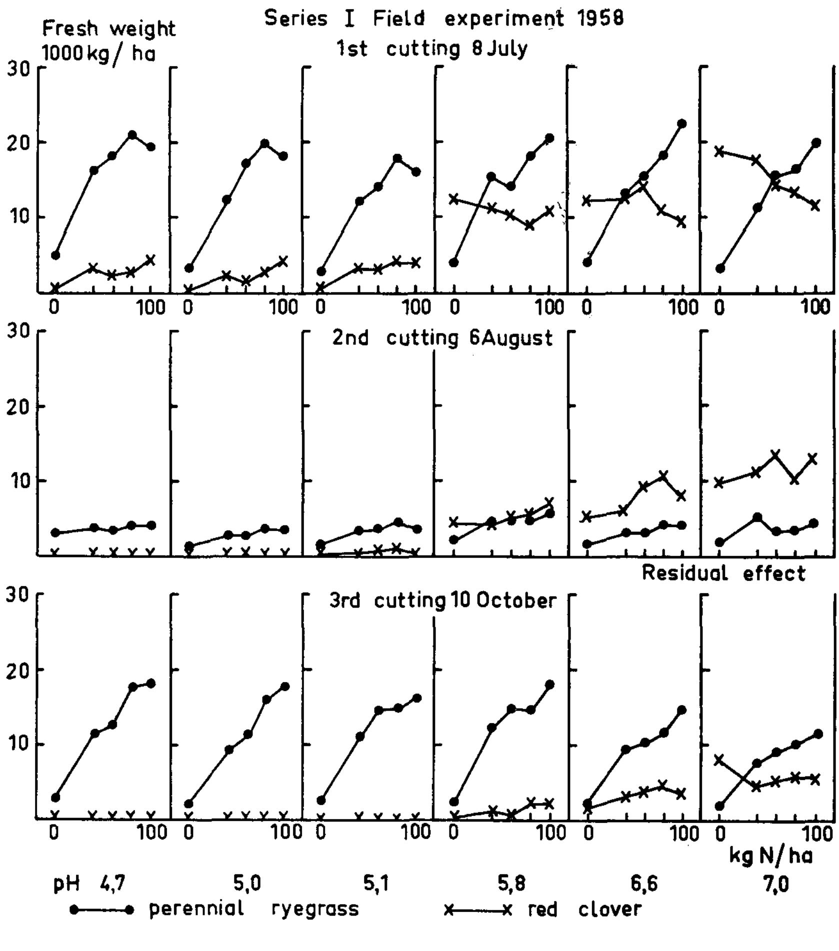
FIG. 5. Effect of soil-pH and added ammonium nitrate limestone on the nitrogen contents of perennial ryegrass and red clover grown in association.



The nitrogen content of the clover of series II behaved like that of series I. At pH-values of 6,4 and 6,8, it maintained a constant value of approximately 2,8 %, independent of the supply of fertilizer nitrogen. At pH 5,1 the N-content rose with increasing rate of supply of fertilizer nitrogen, while at pH 5,0 and 5,6 the nitrogen-content curve reached a minimum with 80 kg N per ha.

On 14 August, the cutting date of the third series of plots, the nitrogen-content curves of the grass showed characteristic differences between acid and neutral plots. At pH 4,8 the nitrogen content was proportional to the rate of fertilizer nitrogen, at pH 5,3 and 5,5 it reached a minimum with 80 or 120 kg N per ha, and on the neutral plots the nitrogen content was greatest at the lowest rate of nitrogen fertilization. The nitrogen content of the clover of series III was almost independent of the nitrogen fertilization; it was somewhat smaller at pH-values of 4,8 and 5,3 than at higher pH.

FIG. 6. Effect of soil-pH and added ammonium nitrate limestone on the yields of perennial ryegrass and red clover grown in association.

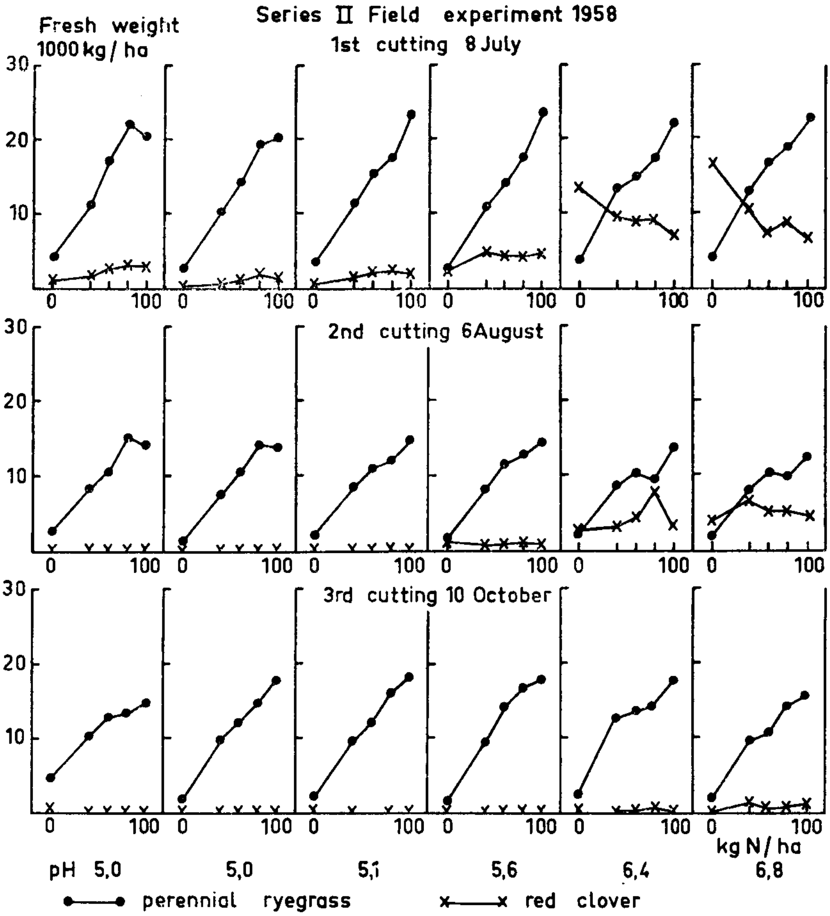


3.1.2. *Field experiment 1958*

The response of the r y e g r a s s of the first cuttings to fertilizer nitrogen was similar to that of the previous year (FIG. 6, 7 and 8). Maximum grass yields were lower, however, than in 1957, due to the lower amounts of fertilizer nitrogen applied. C l o v e r yields were also lower than in the preceding year. This may be seen from the results of Series I (FIGURES 6 and 9) which is comparable to Series I and II of 1957 (FIGURES 1, 2 and 4). As a result, the depressing effect of the clover plants on the grass yields at pH 6,6 and 7 (third cutting) was less pronounced than in the 1957 experiment.

A comparison in the a n n u a l p r o d u c t i o n of herbage between series I and III which were dressed twice with ammonium nitrate limestone and series II which was dressed three times, reveals that on the acid soils, the latter gave higher yields (FIG. 9).

FIG. 7. Effect of soil-pH and added ammonium nitrate limestone on the yields of perennial ryegrass and red clover grown in association.

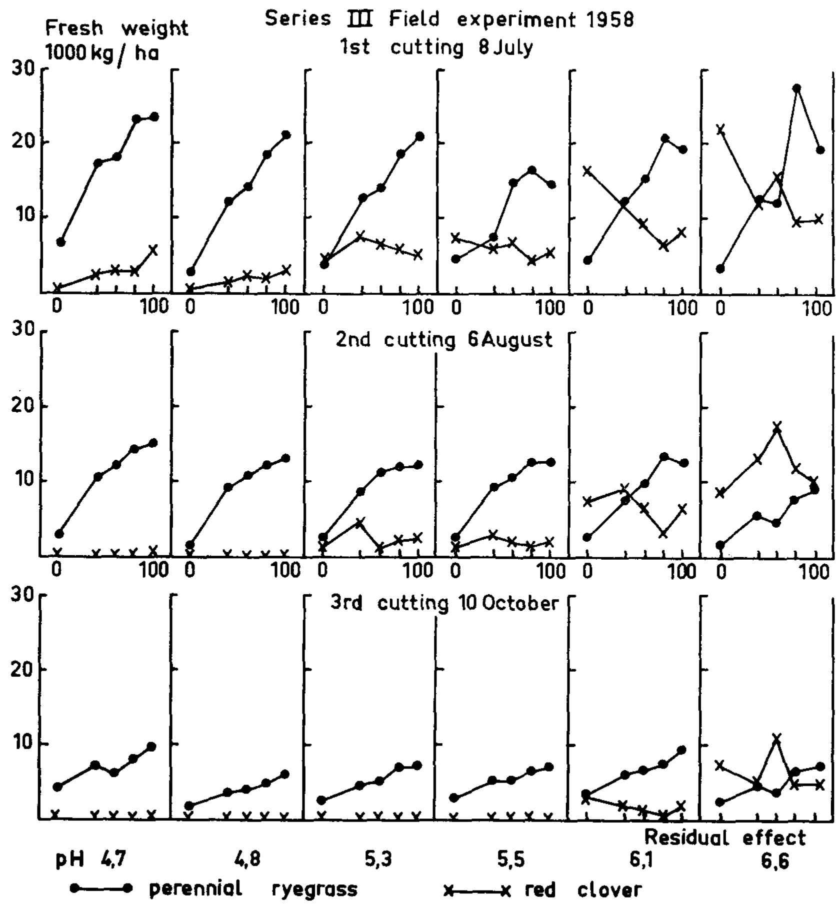


On the neutral soils, however, the differences in total herbage were insignificant because the increase in grass production with the increased nitrogen fertilization resulted in a decrease of yield of clover.

3.1.3. *Field experiment 1959*

In this experiment clover and grass plants were grown on separate plots. Details of the experiment are given under "Methods".
Weather conditions in the Netherlands in 1959 differed considerably from the more normal climatical conditions prevailing in 1957 and 1958. Normally, rainfall is relatively high and temperatures are moderate during the growing period of the crops but in 1959 the weather was extremely sunny, warm and dry so that the plots had to be watered frequently to maintain normal growth. Yields of herbage were lower than in 1957 and 1958, presumably due to the unfavourable growing conditions.

FIG. 8. Effect of soil-pH and added ammonium nitrate limestone on the yields of perennial ryegrass and red clover grown in association.



From the yield data (FIG. 10) it will be seen that on the acid plots clover and grass responded similarly to the applied fertilizer nitrogen. At pH 6,4 and 6,8, however, yields of clover considerably exceeded those of grass. The response of the red clover to added fertilizer nitrogen, even on neutral soil, may have been due to the abnormal weather conditions in 1959. In 1960, when lower temperatures and more humid weather conditions prevailed during the growing season, hardly any response of the red clover to added fertilizer nitrogen on neutral soil was observed.

FIG. 9. Effect of soil-pH and added ammonium nitrate limestone on the annual yields of perennial ryegrass and red clover grown in association.

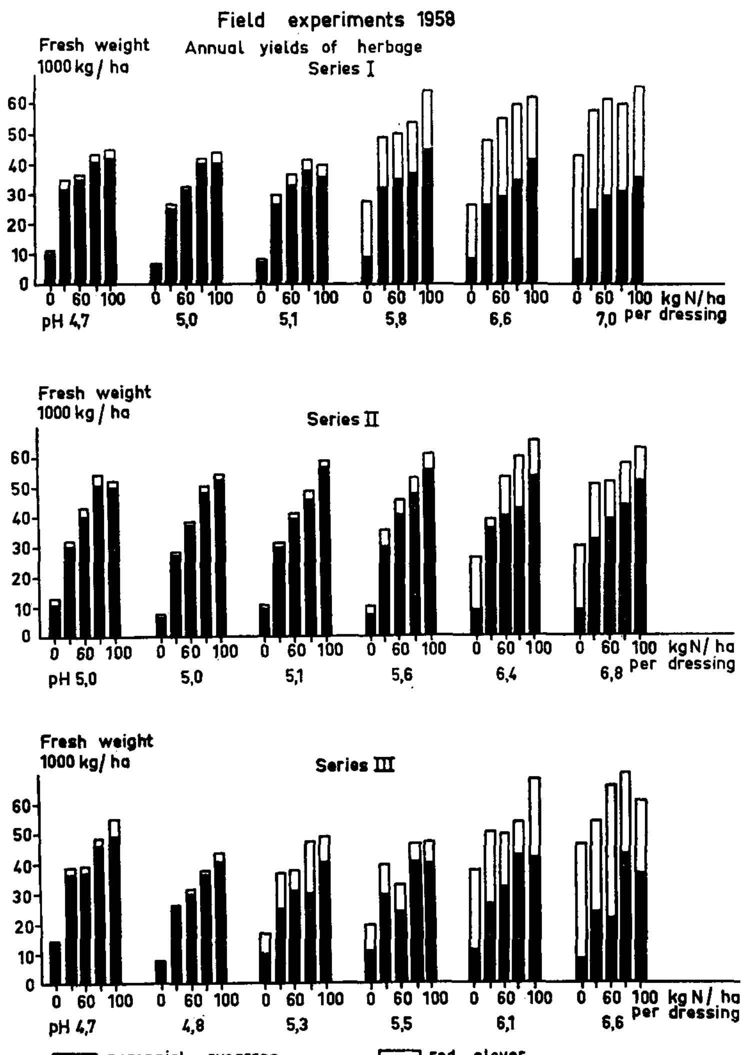
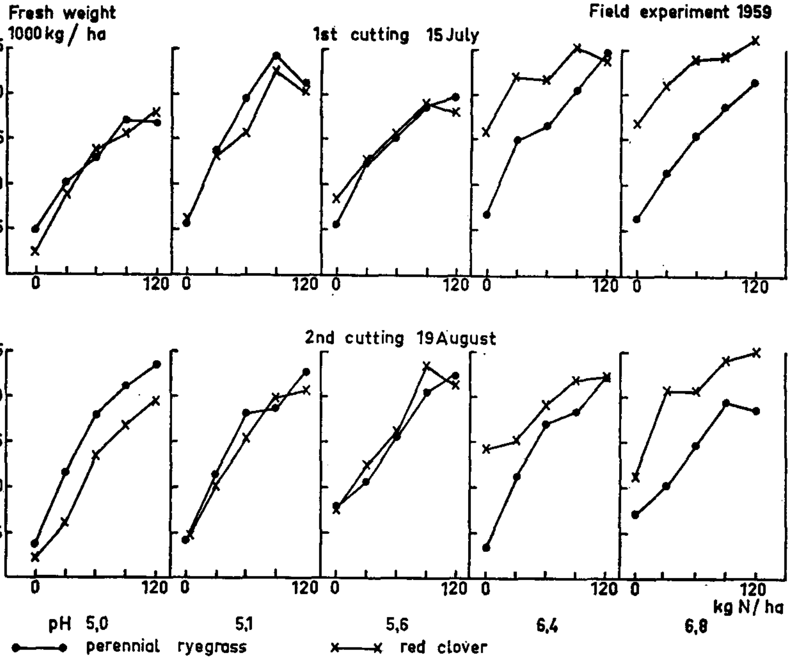


FIG. 10. Effect of soil-pH and added ammonium nitrate limestone on the yields of perennial ryegrass and red clover grown separately.



Examination of the root systems of the 1959 crop revealed that in July the plants on the neutral plots were amply nodulated whereas on the acid plots no nodules were found. In September, however, nodules were found on the roots of all plants, even on those from soil which was acid or dressed with fertilizer nitrogen.

3.2. Combined effects of mineral nitrogen, stable manure and inoculation with *Rhizobium trifolii* on the growth of red clover on acid and neutral soils

3.2.1. *Pot experiment 1957*

In this experiment the effect of fertilizer nitrogen, stable manure, stable-manure extract and molybdenum on the growth of red clover on acid soil was studied.

Without inoculation and without added nitrogen or stable manure, the growth of the red clover on the acid soil was very poor due to nitrogen deficiency. Inoculation with *Rhizobium trifolii* gave well nodulated plants which made adequate growth. In the presence of stable manure the growth of the inoculated plants was still considerably enhanced, the highest yields being obtained with the highest dose of stable manure (FIG. 11a, TABLE 2). Addition of stable-manure extract, in an amount equivalent to 130 g of fresh stable manure containing 15 mg N, also gave higher yields than the control pots (FIG. 11b). Molybdenum produced slightly higher yields than in controls.

Ammonium nitrate affected the growth of the clover plants on the inoculated acid soil in an entirely different way. In its presence the plants were dark green and their development was improved in proportion to the amount of added nitrogen. Initially no nodules were formed, which was in contrast to the control plants without added nitrogen. Gradually the plants turned yellow-green, because their ammonium nitrate had been used up and apparently no symbiotic nitrogen fixation was occurring. Some weeks later, however, the plants with the lowest amount of added nitrogen became green as a result of the formation of effective nodules. The higher the amount of nitrogen applied, the more retarded was the formation of nodules. The plants with the highest amount completely failed to form effective nodules when their nitrogen had been consumed so that they became severely nitrogen-deficient. Therefore the yield of the plants in this experiment was inversely proportional to the amount of nitrogen added at the beginning of the experiment (FIG. 11c, TABLE 2).

TABLE 2. Effects of NH_4NO_3, stable manure and molybdenum on yield (g dry weight per pot) of inoculated red clover, grown on acid soil

Treatment per pot	Yield of tops
Control (uninoculated)	0,9 [1]
„ (inoculated)	3,6 [1]
$Na_2MoO_4 \cdot 2H_2O$, 2,5 mg	4,4 [1]
21 mg N as NH_4NO_3	3,2 [2]
42 mg N „ „	1,6 [2]
84 mg N „ „	1,6 [2]
21 mg N as stable manure	4,7 [2]
42 mg N „ „ „	5,4 [2]
84 mg N „ „ „	6,3 [2]
15 mg N as stable-manure extract	6,0 [3]

Average values of the yields of four [1], two [2] and six [3] pots respectively.

3.2.2. *Pot experiment 1958*

In this experiment a comparison was made between the growth of red clover plants on neutral and acid soil, with increasing amounts of added KNO_3. Each series of nitrogen dressings was a. without added stable manure (a_1 uninoculated, a_2 inoculated with *Rhizobium trifolii*), b. with added stable manure (b_1 uninoculated, b_2 inoculated with *Rhizobium trifolii*). For comparison, one series of nitrogen dressings was added to pots containing soil from an acid plot which had been treated with stable manure a few years before starting the experiment (residual stable manure).

On the neutral soil (pH 7,0), the development of the clover was luxuriant and independent of the addition of nitrate or stable manure, or of inoculation. At the end of the experiment all root systems were well nodulated.

On the inoculated acid soil (pH 5,0) the development of the clover was satisfactory but considerably less than on the neutral soil. Added nitrate had practically no effect but stable manure considerably improved yields. The root systems of the plants of this series were well nodulated; the number of nodules was inversely related to the amount of added nitrogen.

On the uninoculated acid soil during the first two months the clover developed in the same way in each of the three series (no stable manure, fresh stable

manure, and residual stable manure), the plants responding clearly to nitrate. Without added nitrate, or with only small amounts, the leaves were yellow-green due to nitrogen deficiency. With larger amounts of nitrate, the leaves were darker green but not so healthy as on plants growing on inoculated acid soil or on neutral soil.
Approximately 2$^1/_2$ months after starting the experiment, however, the low-nitrate plants and those without added nitrogen growing in the soil with residual stable manure turned much darker green than their controls, undoubtedly due to symbiotic nitrogen fixation. The plants which had fresh stable manure showed a similar but less pronounced colour change. As a result of the improved nitrogen fixation, the plants of both these series made a much better growth than those of the control pots (see TABLE 3). At the end of the experiment the latter plants also improved in appearance; apparently they had succeeded in fixing some nitrogen.

TABLE 3. Effect of KNO_3 and stable manure on yield, fresh weight (g/pot) and nitrogen (mg/pot) in plants of inoculated and uninoculated red clover on neutral and acid soils (*single values*)

N added mg/pot	Neutral soil control[1]	Acid soil, inoculated		Acid soil, uninoculated		
		control	stable manure	control	stable manure	residual stable manure
0	670	385	370	4	53	365
150	740	330	375	6	270	415
300	—	390(2724)[2]	395(2778)	90(570)	275(1112)	380(2750)
450	735	355	435	35	240	385
600	—	370(2985)	445(2785)	195(1017)	175(915)	430(2727)
800	740	330	440	75	310	415
1000	—	380(2636)	455(3215)	75(569)	395(2348)	400(2433)
1500	735	355	465	160	260	395
2000	—	295(2098)	405(2440)	140(1082)	205(1350)	190(934)

1 Uninoculated; the value for uninoculated with stable manure and inoculated with and without stable manure are similar to those for uninoculated controls and are not recorded.
2 Total N in tops and roots (mg per pot).

Nodulation of these three series of plants was clearly related to their development and leaf colour. Plants grown on soil with residual stable manure bore many pink nodules, but fewer with more added nitrate. Plants treated with fresh stable manure had a small number of pink nodules but many small, white nodules. Plants from control pots with acid soil also had these small white nodules, which apparently had a very low nitrogen-fixing capacity. In some pots of this series a few pink nodules were formed which resulted in a better development of the plants.
The yields of the plants of this experiment are recorded in TABLE 3. In addition to fresh weights of tops, amount of nitrogen in the herbage is given for a number of pots. From these data it will be seen that by far the highest yields of tops were from the neutral soil and these were little affected by inoculation, stable manure or nitrogen fertilization, which were very important factors with acid soil.
Summarizing the results obtained, the various factors studied may be arranged in descending order of importance for the yield of red clover as follows: soil-pH, inoculation with an effective strain of *Rhizobium,* stable manure (residual effect more important than direct effect), and nitrogenous fertilizers.

The behaviour of red clover, and presumably other leguminous plants also, in response to the above-mentioned factors is quite different from that of perennial ryegrass which was included in some series of this experiment. The latter responded almost only to added nitrate (cf TABLE 4).

TABLE 4. Effect of KNO_3 on yield of perennial ryegrass, g fresh weight/pot, at different soil-pH with or without stable manure

N added mg/pot	Acid soil		Neutral soil	
	control	stable manure	control	stable manure
0	2[1]	10	5	10
150	15	20	20	20
300	35	40	40	40
600	75	90	90	100
1000	150	160	155	165
1500	215	220	210	240
2000	305	310	335	320

[1] Single values.

4. Discussion

The results obtained on the garden plots of different pH-values allow comparison of the effect of increasing amounts of fertilizer nitrogen on spring-sown grass-clover associations under conditions favourable or unfavourable for nitrogen fixation. At pH-values of approximately 6 and higher the effect of the fertilizer nitrogen on the annual yield of herbage was only slight, due to the luxuriant development of the clover without added nitrogen. With increased supply of fertilizer nitrogen the proportion of grass in the total herbage was considerably increased and that of clover reduced owing to the competitive effect of the grass.

On the acid soils, however, where the development of the clover, even without added nitrogen, was very poor, total yield of herbage was markedly increased by fertilizer nitrogen. It must be stressed that the poor response of the annual yield of herbage to added fertilizer nitrogen on neutral soil was obtained when the nitrogen was given to the first and third cuttings (series I and II 1957, series I 1958) but not to the second, when there was luxuriant development of the clover during midsummer which was greater on the plots which in the preceding growing period had grown with little or no fertilizer nitrogen.

In general the response of the annual yield of herbage to fertilizer nitrogen may be enhanced by cutting the sward earlier and by adding nitrogenous fertilizer more frequently. However, the ready alteration of the proportion of clover in the herbage on neutral soil from more than 90 to less than 20 % by applying large amounts of nitrogenous fertilizer (cf FIG. 3) indicates that a system of frequent cutting and fertilizer treatment would readily eliminate the red clover. This agrees with the experience of SCHEYGROND, VOS and SONNEVELD (1958) who found that red clover, being very susceptible to mowing and grazing management, disappeared very soon from a sward. This contrasts with the behaviour of a mixture of white clover and grass, where the persistence of the legume is favoured by close grazing and mowing systems owing to its prostrate growth habit. Frequent grazing tends to maintain white clover production even when relatively large amounts of fertilizer nitrogen are used (FRANKENA, 1937; WILLIAMS, 1952). In our experiments, however, using a red clover-perennial ryegrass mixture, a delay of the first cutting from 25 June to 11 July increased the annual yield of herbage on the neutral plots mainly due to a more vigorous regrowth of the clover (FIG. 4, Series III 1957).

Even under favourable conditions a clover-grass sward is distinctly later in spring growth than a pure grass stand dressed with nitrogen; therefore it may economically be better to use fertilizer nitrogen for the stimulation of grass growth for a purpose such as early spring grazing, even though subsequent clover production may be lowered (WALKER, 1956).

Presumably the best way of achieving high early yields of herbage of the desired composition on slightly acid or neutral soils with both red and white clover swards, would be to supply a moderate amount of fertilizer nitrogen in the spring, to cut relatively early, and to rely on the clover for fixing the required nitrogen for the production of herbage during summer and autumn. Such a system will change the proportion of clover in the herbage from a relatively low value in the first cutting to a high value in the second and subsequent cuttings (cf series I and II, 1957, where the first cutting was dressed with fertilizer nitrogen but no nitrogen was supplied to the herbage of the second cutting). On acid soils, however, where the fixation of nitrogen by the legumes is insignificant, nitrogenous fertilizer should be supplied throughout the growing season.

The pronounced response of the grass to fertilizer nitrogen on both the acid and neutral soils of our field experiments enabled the production of almost equal amounts of herbage on each type of soil. On the acid soils, however, maximal yields were obtained only at the expense of large amounts of fertilizer nitrogen, in contrast to the neutral soils where omission of nitrogen resulted in only slightly lower yields of herbage. This means that in 1957 the red clover on the neutral soils fixed more than 240 kg of nitrogen per ha. In 1958 less nitrogen was fixed than in 1957.

The depression of the yield of clover in the grass-clover association by large amounts of fertilizer nitrogen is clearly demonstrated in the 1957 and '58 field experiments. Since no such effect was observed in the 1959 field experiment and the 1958 pot experiment in which the clover was grown alone, it may be assumed that the inhibition of the clover in the association was due to suppression by the grass. Obviously this depended to a large extent on the competition for light between the grass and clover plants. This is supported by the more successful competition for mineral nitrogen by grass than by clover in an association of the two (WALKER *et al.*, 1956; ENNIK, 1960). In clover, however, the mineral nitrogen taken up does not promote growth since it substitutes for nitrogen fixed from the air (ALLOS and BARTHOLOMEW, 1955; MACAULIFFE *et al.*, 1958 and G. D. THORNTON, 1956). Thus grass responds more quickly to fertilizer nitrogen than does clover so that the latter becomes deprived of light. Clover plants are very sensitive to poor light conditions (BLACK, 1957).

Grass-clover associations may compete for growth factors such as potassium, phosphate and sulphate, as well as for light. The mere lack of space may also be a reason why the grass suppresses the clover, as shown for white clover by HOLMES and MACLUSKY (1955) and SCHEYGROND *et al.* (1958).

To decide if the grass component of the association is stimulated by nitrogen derived from the clover, a comparison should be made of the yield and the nitrogen content of the grass grown at a low level of fertilizer nitrogen with and without clover. For such a comparison the yield data of the acid and neutral plots might theoretically be used, since the growth of the clover on acid soil was very poor compared with the luxuriant development on neutral soil, whereas the response of the grass to the added nitrogen was apparently not affected by the pH of the soil. As may be seen from the yield data in FIG. 3, however, the competition of the clover on the grass at low nitrogen levels on neutral soil was so pronounced that considerably lower yields of grass were obtained at the higher soil-pH. Therefore the comparison of grass yields or total nitrogen in the grass from acid and neutral soil may be inadequate for demonstrating the supply of nitrogen from the clover. The typical differences in the nitrogen-content curves between grass from acid and neutral soils (cf FIG. 5) may be explained either by liberation of legume nitrogen at low levels of fertilizer nitrogen and at neutral soil reaction, or by a reduction of grass growth on neutral soil by competition from legumes, producing an increased percentage of N in the grass.

More decisive conclusions as to the effect of leguminous plants on the nitrogen supply of grass plants growing in association may be derived from a pot experiment with root systems separate and in contact (DILZ and MULDER, 1962). It was found that in summer during the period of intensive growth of the legumes, small but significant amounts of symbiotically-fixed nitrogen were supplied to the grass plants. Of the three legumes tested, lucerne released the largest amount (8 % of the total N in its tops) and red clover the lowest (1 % of the total N in its tops). In the autumn, after the legumes had been cut and regrown, these relative values for the released N were higher, particularly those of white clover. Much larger amounts of legume nitrogen became available to the grass plants after the legumes were killed.

The information on nitrogen fixation by red clover gained in the present investigation clearly shows that inoculation with an effective strain of *Rhizobium trifolii*, the presence of organic substances, and addition of nitrogenous compounds, may affect nodulation. On the uninoculated garden plots, where no clover plants had grown for at least 35 years, effective nodules formed freely when the soil had a pH about 6 or higher. At pH 5,2 or lower, practically no nodules occurred and between pH 5,2 and 5,8 nodulation was moderate.

When the soil was inoculated with an effective strain of *Rhizobium trifolii*, normal nodulation and nitrogen fixation occurred even on acid soil (pot experiment 1958, TABLE 3), demonstrating that the absence of nodules in the uninoculated plants was due to an insufficient number of Rhizobium cells and not to inhibition of nodule growth. In a recent investigation MULDER and VAN VEEN (1960) have shown that the absence of nodules in red clover on acid soil is due to the diminished multiplication of Rhizobium cells in the rhizosphere of plants growing on acid soil rather than to the entire absence of the bacteria from the soil.

Continued cultivation of red clover on uninoculated acid soils presumably leads to a gradual increase in the small number of Rhizobium cells which is apparently always present in these soils. This is supported by the gradual increase in nodulation observed during the growing period of the clover on the acid plots. This may have been due to the formation by the clover or the grass plants of unknown organic compounds promoting nodulation of red clover on uninoculated acid soil. The importance of such compounds was demonstrated in the field experiment, where the residual stable manure gave rise to luxuriant nodulation of the clover plants at a soil-pH of 5,0, and in the pot experiment of 1958 in which a beneficial residual effect as well as a direct effect of stable manure on nodulation were observed. Similar results have been obtained following the application of small amounts of Rhizobium and yeast extracts to acid soil (MULDER and VAN VEEN, 1960).

The inhibitory effect of nitrogen compounds on nodulation of red clover was shown in the field experiments (1957, TABLE 1) as well as in the pot experiments. Evidence was obtained that this effect was more pronounced when the clover was grown in small glass jars than when in the field. In the experiment in Neubauer glass jars with inoculated acid soil, the clover plants after consumption of the fertilizer nitrogen even became more nitrogen-deficient than those which had received no fertilizer nitrogen (FIG. 11c and TABLE 2). The inhibition of nodulation by nitrogenous compounds was apparently responsible for the absence of symbiotic nitrogen fixation during consumption of fertilizer nitrogen. The control plants given no nitrogen had formed effective nodules and showed no nitrogen deficiency.

In addition to its suppression of nodulation, the combined nitrogen may inhibit nitrogen fixation in nodulated plants. This was demonstrated by ALLOS and BARTHOLOMEW (1955), MCAULIFFE *et al.* (1958) and G. D. THORNTON (1956) in experiments using ^{15}N, and by our 1958 experiments in Mitscherlich pots (TABLE 3) where well-nodulated red clover on neutral and inoculated acid soil showed no response to increasing doses of fertilizer nitrogen up to 2000 mg N per pot. In these experiments, mineral nitrogen did not reduce clover yield, as it did in the above-mentioned experiment with Neubauer glass jars.

ACKNOWLEDGEMENT

The authors are indebted to Miss M. A. TH. STEEMERS and Mr. W. L. VAN VEEN for technical assistance, to Mrs J. A. BAKHUIS for valuable criticism, and to Dr. R. COOPER, Rothamsted Experimental Station, Harpenden, England, for reading and correcting the English text.

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