AGGREGATION OF TRIFOLIUM REPENS AT VARIOUS FERTILIZATIONS

J. G. P. DIRVEN, G. O. NIJLAND and K. WIND

Afdeling Landbouwplantenteelt en Graslandcultuur, Landbouwhogeschool, Wageningen

1. INTRODUCTION

The effect of fertilization on the productivity of *Trifolium repens* – in mono as well as in mixed culture – has been investigated in detail under Dutch conditions as well as under conditions elsewhere. In view of the agricultural aspects of such investigations, special attention was paid in the mixed cultures to the increase or decrease of *Trifolium repens* in the sward.

Hardly any of these studies were directed to the distribution of this species in the sod. As *Trifolium repens* spreads by stolons there is a tendency of growing in clumps. However, it may be interesting to see to what extent this aggregation is affected by other factors. In a grazing experiment with various fertilization treatments it was found that the distribution of *Trifolium repens* demonstrated obvious clumping. Visual observation suggested that this legume demonstrated more aggregation in the fertilized treatments than in the non-fertilized treatments. Preliminary frequency readings with units of varying size confirmed this suggestion. These observations gave rise to a more detailed study of the extent of aggregation in the various fertilization treatments with different methods. The results are presented in this paper.

2. MATERIAL AND METHODS

When interpreting the data obtained in studying the relationship between botanical composition and habitat factors, we are usually confronted by the link between the type of use and the fertilization status of the grassland. Generally, the fertilization status of pastures is better than that of hay fields. For a proper analysis of these two factors, in 1957 an experiment was set out on two grasslands with a rather poor fertilization status on basin clay soil on the experimental farm "De Ossekampen" at Wageningen. In this experiment the pasture treatments received a light fertilization and the hay treatments a heavy one. However, the observations described were limited to the pasture treatments.

2.1. Experimental fields

The botanical composition of both grasslands mainly was: *Festuca rubra, Agrostis tenuis* and *Anthoxanthum odoratum* with the important associates: *Holcus lanatus, Lolium perenne* and *Poa trivialis*. In plot 5 *Lolium perenne* and *Poa trivialis* are more important, and *Festuca rubra* and *Agrostis tenuis* occur

Fertilization in kg per ha on the various treatments

Plot	Treatment	N	P_2O_5	K_2O	CaO
5	0				
	Ca				1000
	NPK	60	40	60	
13	0				
	PK		40	60	
	NPK	60	40	60	

less frequently than in plot 13. The size of the pasture treatments is 0.18 ha. The fertilization program is outlined above. Fertilizer was applied as ammonium nitrate limestone, super phosphate, 40% potassium salts and lime marl. The nitrogen was applied in two equal applications; the first in spring, the second in summer. When the results of the soil analyses of 1958 and 1963 were compared it was found that the soil had not been enriched in the fertilized treatments. The pH-KCl in the layer 0–5 cm of the Ca-treatment increased from 4.9 to 6.4 in the period 1958–1963; in the other treatments the pH-KCl fluctuated between 4.5 and 4.9. Detailed data on soil analysis, fertilization, use, botanical composition and yield have been summarised by ELBERSE (1966) in a report on the period 1957–1965.

Grazing management consisted of 4 to 7 heifers at a time per treatment, about three times a year. To prevent mineral transport by excrement, the heifers were grazed on a non-fertilized strip for some days before putting them to pasture.

2.2. Measurements

The effect of the fertilizer applications on the yield was determined by cutting the grass three times a year under cages (area 5.04 sq.m). In the years 1957 and 1958 there were two cages per treatment; since 1959 however, there have been four cages per treatment.

The botanical composition was analysed by the 25 sq. cm-frequency and order method (DE VRIES 1949). The annual sampling was always carried out in May. For this 50 handfuls or borings of 25 sq. cm are taken of each treatment along the diagonal and two equidistant parallels.

The trend in the frequency percentages in the years 1957–1966 suggests that *Trifolium repens* increased somewhat in all the treatments. Between the various fertilization treatments, however, no significant differences in the frequency of occurrence of this legume could be demonstrated. This is mainly due to the rather small number of borings or handfuls taken from each treatment, because of which the frequency percentages will show a rather great standard error. Moreover these percentages are also affected by the more or less patchwise distribution of *Trifolium repens.*

To obtain a better understanding of the reaction of *Trifolium repens* to the various fertilizer treatments, in June 1967 detailed sampling was carried out along the diagonal and seven equidistant lines on each side of it. For this, first the presence or absence of *Trifolium repens* was determined in 1024 units of 400 sq. cm, 100 sq. cm, 25 sq. cm and 6.25 sq. cm. The sampling units were always situated concentrically to each other. Then 160 samples were taken along the same lines by the 20 point quadrat method. This gives 3200 points per 0.18 ha (0.45 acre). The 20 point quadrat apparatus was always placed at right angles to the sampling lines, after which the needles at a distance of 5 cm were lowered perpendicularly into the vegetation. The number of needles hitting *Trifolium repens* was recorded. The unit size in this method is equal to the area of a needle (0.07 sq. cm).

At the beginning of September 1969 the local frequency of *Trifolium repens* was determined per treatment. This was always done within sampling units of 25 × 25 cm, which were divided into 25 sub-quadrats.

The sampling units were distributed along the diagonal and the equidistant lines in such a way that the distance between the samples was equal to that between the parallels.

3. RESULTS

The relation between sample size and presence frequency of a certain plant species is dependent on the more or less patchwise distribution of the individuals in the area to be sampled. At the same percentage cover of a plant species the frequency percentage will increase to a larger extent by enlarging the sampling unit

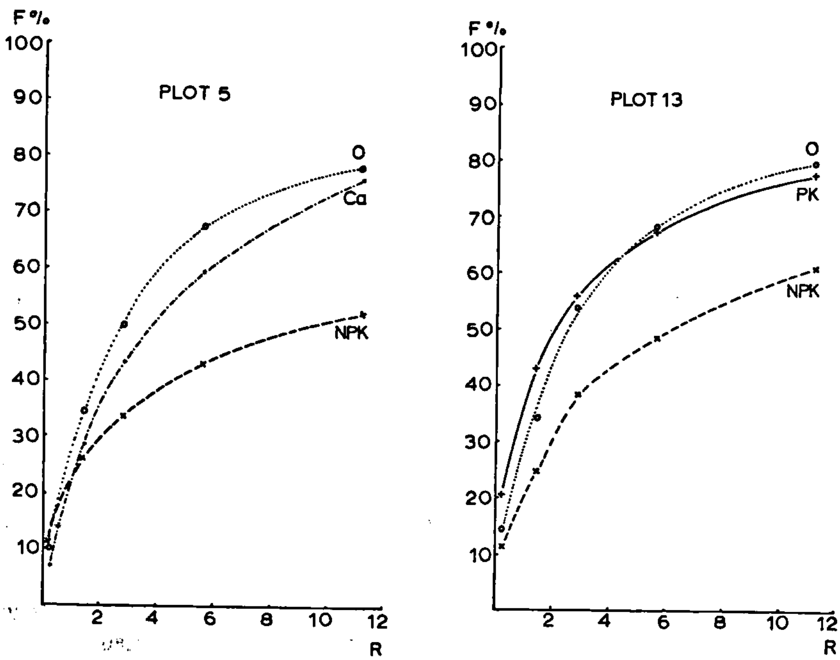

Fig. 1. Relation between presence frequency (F %) of *Trifolium repens* and the radius R (in cm) of the sampling units.

Table 1. Difference (d) between the frequency percentages of *Trifolium repens* in the various fertilization treatments and the two-tailed probability value (P) obtained by use of the Wilcoxon test

		point quadrat		6.25 cm²		25 cm²		100 cm²		400 cm²	
		d	p	d	p	d	p	d	p	d	p
plot 5	0–NPK	−0.9	0.53	+8.5	0.001	+16.5	***	+24.6	***	+26.3	***
	0–Ca	+3.0	0.040	+5.8	0.025	+ 6.6	0.008	+ 8.2	0.0013	+ 2.4	0.35
	Ca–NPK	−3.9	0.006	+2.7	0.29	+ 9.9	***	+16.4	***	+23.9	***
plot 13	0–NPK	+2.9	0.040	+10.1	***	+12.6	***	+19.8	***	+18.6	***
	0–PK	−6.2	***	− 8.3	0.0013	− 1.8	0.48	+ 0.7	0.78	+ 1.7	0.51
	PK–NPK	+9.1	***	+18.4	***	+17.4	***	+19.1	***	+16.9	***

*** $< 10^{-3}$

as the individuals of the plant species are less aggregated (ABERDEEN 1958). For this reason the presence frequency method was applied to quantify the degree of clumping of *Trifolium repens* in the trial plots. The results of this method are represented in *fig. 1.* The frequency percentages of the smallest unit size were obtained by the point quadrat apparatus. The differences between the frequency percentages of the various treatments were tested for significance with the Wilcoxon test. The data are listed in *table 1.* The distribution data of the point quadrat readings were compared with those of a binomial distribution with the same mean ($n = 20$). When there is no aggregation, e.g. in a random distribution, the distribution of the point quadrat readings can be approximated by a binomial distribution. Both distributions, that observed and the binomial one, are shown in *fig. 2.* The histogram of the binomial distribution, which is in fact discontinuous, for illustrative purposes is represented by a dotted line, as a continuous distribution.

The just mentioned data were compared in two ways with the binomial distribution. First, the standard error of the observations (S) was compared to that of the binomial distribution (σ)

$$\sigma = \sqrt{nq(1-q)}$$

where n is the number of terms of the binomium; and q the mean chance of positive observations.

The ratio S/σ is used as an index of aggregation. This value will be greater as aggregation increases.

It must be realized that the aggregations with a diameter corresponding to the width of the point quadrat apparatus (1 metre) are shown most critically. The quotients S/σ of the various treatments are mentioned in *table 2.*

Next, the results obtained by the point quadrat method were also compared with the chi-squared test to the relevant binomial distribution. In calculating the testing quantity χ^2, the various distribution classes are combined with the adjacent classes in such a way that the expected number of observations in each

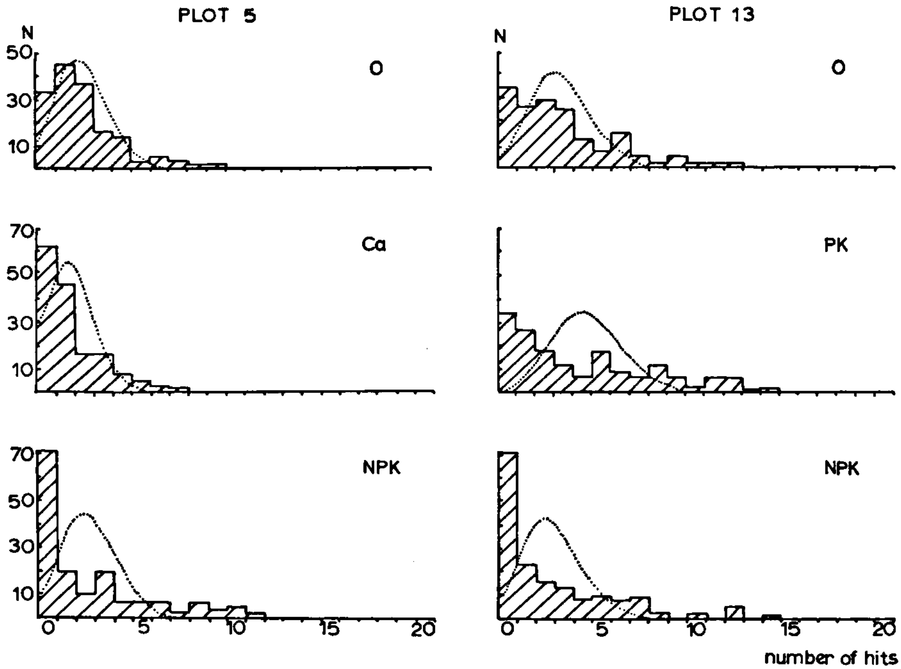

Fig. 2. Distribution of 160 readings (N) with the 20-point quadrat apparatus on the number of possible hits of *Trifolium repens*.

class is over 5 (FISHER & YATES 1948) and the number of classes for each treatment is the same. The results of this χ^2 test are shown in *table 2*.

The observations of the local frequency readings were tested in the same way against the relevant binomial distribution. The data obtained are represented in *fig. 3*. The testing quantities S/σ and χ^2 are mentioned in *table 2*.

The trend in the frequency percentages in *fig. 1* shows that the aggregation of *Trifolium repens* in field 5 is more considerable in the NPK treatment than in the

Table 2. Testing quantities S/σ and χ^2 of the point quadrat method and local frequency method with respect to the relevant binomial distribution.

		Point quadrat method 1967		Local frequency method 1969	
		S/σ	χ^2	S/σ	χ^2
plot 5	0	1.45	22	3.15	294
	Ca	1.58	43	3.58	349
	NPK	2.09	228	4.02	389
plot 13	0	1.78	48	3.37	291
	PK	2.24	119	4.01	455
	NPK	2.22	269	3.92	423

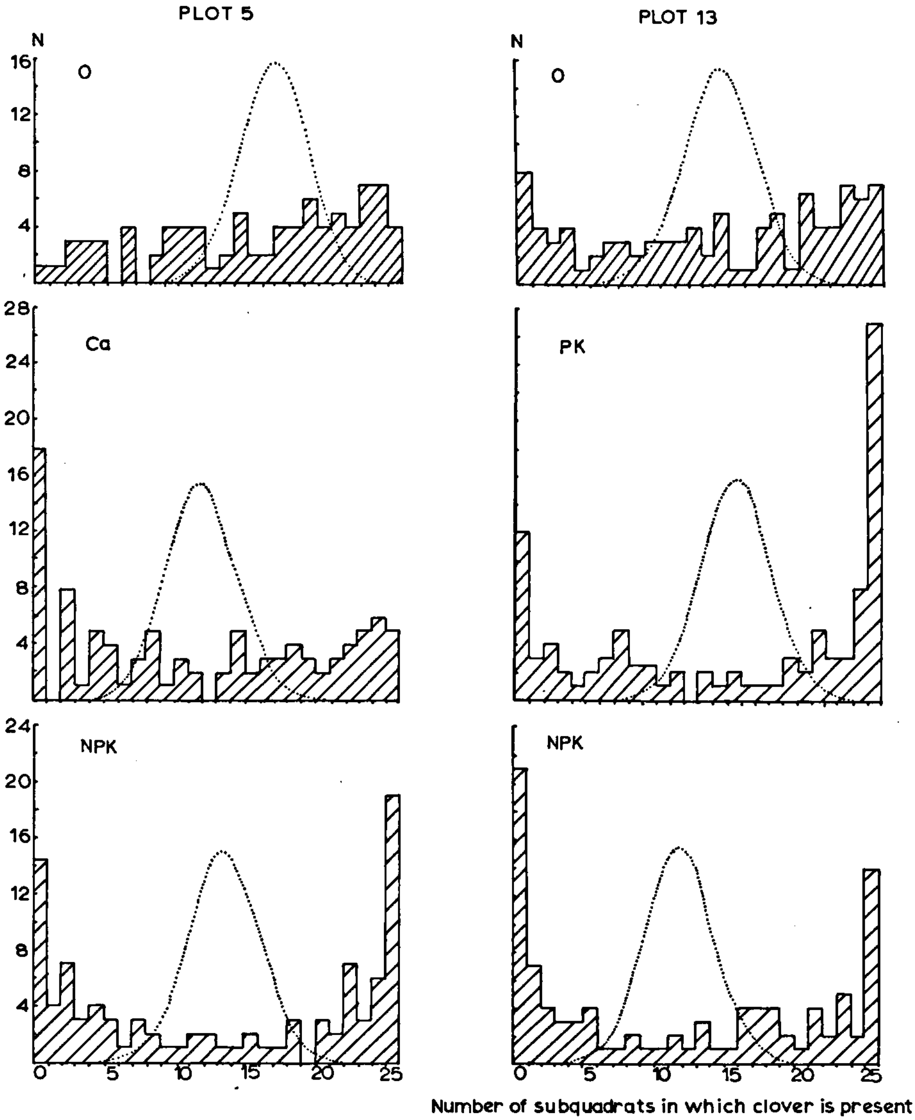

Fig. 3. Distribution (N) of the local frequency of *Trifolium repens* in the 25 sub-quadrats of 100 samples.

non-fertilized and Ca treatments. In the smallest unit the frequency percentage of *Trifolium repens* is lower in the latter treatments, but as the unit size increases the frequency percentages increase more than those in the NPK treatment. *Table 1* shows that from unit size 25 sq. cm the difference between the frequency percentages in the non-fertilized and the NPK treatments and between those of the Ca and NPK treatments are very reliable. No difference in aggregation could be demonstrated between the non-fertilized and the Ca treatments. The ratio S/σ and the quantity χ^2 of the point quadrat readings roughly agree with these conclusions.

Fig. 1 also demonstrates that the aggregation of *Trifolium repens* in field 13 is also greater in the NPK treatment than in the non-fertilized treatment. In the PK treatment the frequency percentage in the smallest unit size is higher than in the non-fertilized treatment. In the unit size 25 sq. cm the frequency percentages are almost the same in both treatments, the frequency percentage in the PK treatment being lower in the two larger unit sizes. Although the data in *table 1* do not prove these differences to be significant, they still indicate a somewhat greater aggregation of *Trifolium repens* in the PK treatment. A true difference in aggregation between the NPK and PK treatments was not demonstrated. The ratio S/σ and the quantity χ^2 of the point quadrat readings confirm these results, although the χ^2 of the PK treatment is rather lower than that of the NPK treatment.

Finally, the S/σ ratios and χ^2 values calculated from the local frequency readings also indicate that the aggregation of *Trifolium repens* in the NPK treatments is greater than in the non-fertilized treatments. The higher values of the S/σ ratio and the somewhat deviating trend in the χ^2 values with respect to the 20 point quadrat readings is due to the proportion of the area of the aggregations to the sampling units. The width of the point quadrat apparatus is 100 cm; the largest diameter of the local frequency readings 35 cm.

4. DISCUSSION

The greater aggregation of *Trifolium repens* in the fertilized treatments is a direct as well an indirect result of the fertilizers applied. For, if it is assumed that the non-fertilized habitat already shows patchwise variations and that plant species grow in the places most favourable to them, the vegetation patterns will come to correspond with the patchwise habitat variations in the course of time. The effect of fertilization on a certain plant species may be most evident in those places where growing conditions are most favourable, so that the patchwise differences in the occurrence of the mentioned plant species may increase. Since the fertilization level in this experiment is low, the mentioned differences may be less pronounced.

A greater effect on aggregation of *Trifolium repens* is probably due to the yield increasing effect of fertilization and the allied increase in the number of pasture days. For the defecation and urine discharges of the cattle leads to a concentration of minerals at certain places in the sward and as a result there will be patchwise great differences in soil fertility (VAN DER KLEY & VAN DER PLOEG 1955). The treatments were trimmed once a year, but the dung pats were not spread. Thus rank grass growth may temporarily occur in the dung and urine places, which are not grazed by cattle. Because of the better growth of the

Table 3. Average dry matter yields and average number of pasture days on the various fertilization treatments, both converted to ha^{-1} $year^{-1}$

Treatment		1957–1961		1962–1966
		kg dm	pasture days	kg dm
plot 5	0	8600	394	6600
	Ca	9000	528	9200
	NPK	9800	578	9800
plot 13	0	7300	417	6900
	PK	8900	533	8100
	NPK	9500	600	10000

grasses *Trifolium repens* will be suppressed, whereas in the closely cropped parts it will show fair development (ENNIK 1957). Since the number of dung and urine places will increase with a greater number of grazing days, the question arises whether this might also stimulate the aggregation of *Trifolium repens.*

To test this supposition in *table 3* an outline is shown of the dry matter yields and as far as known, the number of pasture days on the various treatments.

Table 3 shows that averaged over the period 1957–1961 there is a close correlation between the dry matter yields and the number of grazing days in the various treatments. In the next period 1962–1966 the yield differences between the non-fertilized and NPK treatments increased. The number of pasture days on the separate treatments, unfortunately, was not recorded for this five year period, but the number of heifers put to graze on the NPK treatments in most cases was 50%, and on the Ca and PK treatments 25% higher than on the non-fertilized treatments. The grazing period was almost the same for all treatments. The greater aggregation in the NPK treatments with respect to the non-fertilized ones can be explained for the greater part as a result of differences in pasture days. Because of the occurrence of temporarily rank patches the occurrence of *Trifolium repens* may be locally less frequent. However, the lower aggregation in the Ca treatment does not quite fit in, although the results of the local frequency readings indicate that the aggregation of *Trifolium repens* in this treatment is greater than in the non-fertilized treatment.

It is striking that the greater aggregation occurred in the treatments fertilized with P and K.

The preceding clearly shows that the cause of the more or less patchwise growth of *Trifolium repens* is much more complex than assumed initially. In this it was assumed that the grass would show rank growth in the dung and urine places and that this would suppress *Trifolium repens.* Of course this holds only for the fertile grasslands, in which *Lolium perenne* is an important species in the sward. But in our grazing experiment the fertilization status (of the soil) was less favourable and the sod was mainly formed by less productive species, like *Agrostis tenuis*, *Anthoxanthum odoratum* and *Festuca rubra.* As a result grass growth in the dung and urine places will not be so rank and because of the fertilizers applied *Trifolium repens* will be able to survive better even in these places than in the other parts of the field. Especially the high potassium content of the urine may have a favourable effect on the growth of *Trifolium repens*, because on soils with a low potassium content grass will show a higher uptake of potassium than white clover, and under these conditions *Trifolium repens* will not be able to survive so well.

Finally, the results of the present investigation clearly show that by applying routine methods in botanical grassland research important happenings in the grass sward are not recorded. It was also demonstrated that the effect of fertilizers on the distribution of plant species in many cases is not a direct but an indirect effect.

ACKNOWLEDGMENT

The authors wish to thank Miss A. H. van Rossem for translating the manuscript.

REFERENCES

ABERDEEN, J. C. E. (1958): The effect of quadrat size, plant size and plant distribution on frequency estimates in plant ecology. *Aust. J. Bot.* **6**: 47–58.

ELBERSE, W. TH. (1966): Invloed van gebruik en bemesting op botanische samenstelling en produktie van verwaarloosd grasland. *Verslagen IBS* no. **40**.

ENNIK, G. C. (1957): Een vergelijking van de invloed van standweiden en omweiden op de botanische samenstelling van grasland. *IBS Jaarboek* **1957**: 27–37.

FISHER, R. A. & F. YATES (1948): *Statistical tables for biological, agricultural and medical research.* Oliver and Boyd, London.

KLEY, F. K. VAN DER & H. VAN DER PLOEG (1955): Graasgewoonten en voedselopname van Nederlandse rundertweelingen II. Voedselgebruik en beweidingssysteem. *Landbouwkundig Tijdschrift* **67**: 620–627.

VRIES, D. M. DE (1949): Survey of methods of botanical analysis of grassland. *Rep. 5th Intern. Grassld Congr. Neth.*: 143–153.