

Some Genetic Aspects of the Symbiotic Relationship Between White Clover (*Trifolium repens*) and *Rhizobium Trifolii*

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Summary. The results of experiments with white clover (*Trifolium repens*) in which time of nodulation and seedling plant weight or vigour were measured are reported. Experiments 1 and 2 were conducted in artificial growth medium in test tubes with controlled inoculation and experiment 3 in soil without controlled inoculation. Experiment 1 which was preliminary in nature showed the extent of the variation for time of nodulation after inoculation with *Rhizobium trifolii*. It was evident also that plant vigour and the number of days to nodulation were negatively correlated. Experiment 2 forms the major part of the results and is concerned with the analyses and interpretation of the diallel cross progeny of twelve plants selected from experiment 1. The results indicated a rather complex genetic picture for the two characters measured, namely days to nodulation and seedling plant weight at 80 days. Reciprocal (both general and specific) as well as additive (g.c.a.) and non-additive (s.c.a.) effects were present. Experiment 3, in which seed from 22 families of the diallel cross was sown in soil without controlled inoculation, indicated that the results obtained under the laboratory conditions of experiment 2 and those obtained in soil conditions were not correlated. The implications of these results in relation to selection of improved varieties of the host species are discussed.

Introduction

The symbiotic relationship between legume and nodule bacteria is one of considerable biological importance. The genetic aspect of this relationship is the main item of interest in this contribution.

NUTMAN (1946a, b; 1954a, b; 1959) working with red clover (*Trifolium pratense*) has demonstrated by genetic analysis and selection experiments that nodule size and number, earliness and lateness of nodulation and effectiveness of response all show genetic variation. Similarly JONES (1960; 1962a, b; 1963; JONES and BURROWS (1968) working with white clover (*Trifolium repens*) and GIBSON'S (1962) studies on lucerne (*Medicago sativa*) have shown that genetic variation was present for the characters studied.

The main objectives of the experiments reported here were: (a) to investigate in some detail the types of genetic variation controlling two characters, number of days to primary nodulation and seedling plant weight at 60–80 days; (b) to determine to what extent the observations on seedling plants under laboratory conditions may be of use in a plant breeding programme.

Materials and Methods

The legume species used throughout was white clover (*Trifolium repens*), variety S. 100. In the first two experiments (numbered 1 and 2) the clover seedlings were grown on nitrogen-free agar medium similar to that described by PURCHASE and VINCENT (1949). Before sowing, the seeds were surface sterilised in mercuric chloride and washed in distilled water. The seeds were germinated on agar plates at 25 °C. The germinated seeds were transferred to 6 in. test-tubes containing nitrogen-free agar medium and after five days each seedling was inoculated with *Rhizobium trifolii* strain J. 12. Previous

work had shown that this strain gave an effective response with white clover.

All experiments were conducted in a glasshouse during the summer and autumn months of 1962, 1964, 1965.

Experiment 1

This was a preliminary experiment carried out in the summer of 1962, the object of which was to determine the range of effectiveness of response with strain J. 12 and to provide plants for further work. Five hundred seeds of S. 100 white clover were grown as described above, the number of days to primary nodulation was recorded and the effectiveness of the symbiosis was visually assessed after 60 days. This assessment was based on height, number of leaves and general vigour of each plant in a manner similar to that described by NUTMAN (1954a).

Experiment 2

Experiment 2 was based on the diallel cross progeny of 12 parents selected from the plants of experiment 1. It was observed (experiment 1) that while there was a correlation between time of nodulation and plant vigour, many plants which were slow to nodulate were among the most vigorous group and vice versa. Plants showing various combinations of the two characters were selected as parents for the diallel cross. The parents were not therefore a random sample from the initial population of plants.

Selfed progeny could not be obtained in the diallel set of crosses since the incompatibility mechanism in white clover effectively prevents self-fertilisation; reciprocal crosses were kept separate. The crosses were made by hand without emasculation and all parents were tested for self incompatibility by self pollinating approximately 500 florets on each parent plant. All parents were highly self sterile. For each cross about 250 florets were pollinated.

A sample of seed from each family was surface sterilised and germinated in petri-dishes. Fifteen seedlings from each family were transferred to nitrogen-free agar medium in numbered test tubes and inoculated with *Rhizobium*

trifolii strain J. 12. The experimental design consisted of a single block in which each plant was located at random. Randomisation was performed before inoculation.

The root system of each plant was examined each day until the first nodular swelling was observed. This was taken as the time of primary nodulation. The number of days from inoculation to primary nodulation and plant fresh weight at 80 days were recorded for each plant.

Experiment 3

This experiment was conducted to determine what correlation, if any, was present between the results obtained in test-tubes and those observed under soil conditions. A small sample of seed from each family of the maternal arrays of parents 4 and 11 were germinated. Ten germinated seeds of each family were sown in soil in 5 1/2 in. pots. The soil used in this experiment was a light textured sandy soil of low nitrogen status (Screen series; GARDINER and RYAN 1964). Suitable amounts of potassium, phosphorus and trace elements were added and the pH was raised with lime to about 6.9. The seed was not inoculated in this experiment, nodulation was effected by the rhizobium in the soil. The experimental design was similar to that for experiment 2, i.e. single plant randomisation of all the 220 plants in the experiment. The plants were grown in the glasshouse in the summer and autumn of 1965. Three harvests were taken from each plant, fresh weight and dry weight for each harvest was determined.

Statistical Analysis

The Model: The analysis and interpretation apply only to the group of parents which were selected in a non-random manner as described in the previous section. The appropriate analysis was therefore that of a model I or fixed effects model as outlined by EISENHART (1947). The statistical model is the same as Model A of EISEN et al. (1966) and the definition of the parameters follows that of EISEN et al. (1967).

The Model:

$$y_{ij} = \mu + g_i + g_j + s_{ij} + m_j + r_{ij} + e_{ij}$$

where

μ = overall mean

$g_i (g_j)$ = general combining ability effect (g.c.a.) of i^{th} (j^{th}) parent

s_{ij} = specific combining ability effect (s.c.a.) of the $i j^{\text{th}}$ cross

m_j = general reciprocal effect (g.r.e.) of the j^{th} parent

r_{ij} = specific reciprocal effect (s.r.e.) of the $(i j)^{\text{th}}$ versus the $(j i)^{\text{th}}$ cross

e_{ij} = Random error

The effects were subject to the following restrictions:

$$\begin{aligned} \sum_i g_i &= 0 \\ s_{ij} &= s_{ji}; \quad \sum_{i \neq j} s_{ij} = 0 = \sum_{j \neq i} s_{ji} \\ \sum_j m_j &= 0 \\ r_{ij} &= -r_{ji}; \quad \sum_{i \neq j} r_{ij} = 0 = \sum_{j \neq i} r_{ji} \end{aligned}$$

e_{ij} were assumed to be $N(0, \sigma^2)$

The difference between this model and that of GRIF-FING (1956; Method 3, fixed effects) is that the reciprocal sum of squares are partitioned into general and specific reciprocal differences. EISEN et al. (1966) has discussed the relationship between the estimates of the parameters derived from both models. The latter does not give the expectations of mean squares of the present model and these are therefore presented in Table 1.

Table 1. Sums of squares and expectation of mean squares for diallel analysis

Source of variation	D.F.	S.S.*	Expectation of mean squares
General combining ability (g.c.a.)	$p - 1$	S.S.g.c.a.	$\sigma^2 + 2(p - 2) \left(\frac{1}{p - 1} \right) \sum_i \left(g_i + \frac{1}{2} m_j \right)^2$
Specific combining ability (s.c.a.)	$\frac{1}{2} p(p - 3)$	S.S.s.c.a.	$\sigma^2 + 2 \left(\frac{2}{p(p - 3)} \right) \sum_{i < j} s_{ij}^2$
General reciprocal effects (g.r.e.)	$p - 1$	S.S.g.r.e.	$\sigma^2 + \frac{p}{2} \left(\frac{1}{p - 1} \right) \sum_j m_j^2$
Specific reciprocal effects (s.r.e.)	$\frac{1}{2} (p - 1)(p - 2)$	S.S.s.r.e.	$\sigma^2 + 2 \left(\frac{2}{(p - 1)(p - 2)} \right) \sum_{i < j} r_{ij}^2$
Error (within family)	$N - p^2 - p - 1$	S.S.w.	σ^2

* S.S.g.c.a. = $\frac{1}{2(p - 2)} \sum_i (X_i + X_{.i})^2 - \frac{2}{p(p - 2)} X^2$

S.S.s.c.a. = $\frac{1}{2} \sum_{i < j} (X_{ij} + X_{ji})^2 - \frac{1}{2(p - 2)} \sum_i (X_i + X_{.i})^2 + \frac{1}{(p - 1)(p - 2)} X^2$

S.S.g.r.e. = $\frac{1}{2p} \sum (X_i - X_{.i})^2$

S.S.s.r.e. = $\frac{1}{2} \sum_{i < j} (X_{ij} - X_{ji})^2 - \frac{1}{2p} \sum (X_i - X_{.i})^2$

Estimates of parameters: Equations for the estimation of the parameters are given by EISEN (1966). The variances of these estimates and of the difference between them are as follows:

$$\text{Var } g_i = \frac{(p-1)^2}{p^2(p-2)} \sigma^2; \quad \text{Var } (g_i - g_k) = \frac{2(p-1)}{p(p-2)} \sigma^2;$$

$$\text{Var } m_i = \frac{2(p-1)}{p^2} \sigma^2; \quad \text{Var } (m_i - m_k) = \frac{4}{p^2} \sigma^2;$$

$$\text{Var } s_i = \frac{(p-3)}{2(p-1)} \sigma^2; \quad \text{Var } (s_{ij} - s_{ik}) = \frac{(p-3)}{p-2} \sigma^2;$$

$$\text{Var } (s_{ij} - s_{kl}) = \frac{(p-4)}{p-2} \sigma^2;$$

$$\text{Var } r_{ij} = \frac{(p-2)}{2p} \sigma^2; \quad \text{Var } (r_{ij} - r_{ik}) = \frac{(p-1)}{p} \sigma^2;$$

$$\text{Var } (r_{ij} - r_{kl}) = \frac{(p+2)}{p} \sigma^2.$$

Significance tests: As can be seen from Table 1 there is no ratio of mean squares that gives a test of the g.c.a. effects. A test of these effects was derived as follows:

Since the estimates of the g_i effects (\hat{g}_i ; Table 7) are $N \left[0, \frac{(p-1)^2 \sigma^2}{p^2(p-2)} \right]$ under the null hypothesis and

the correlation between the g_i 's is $-\frac{1}{p-1}$ then

$$\frac{\sum \hat{g}_i^2}{(p-1)^2 \sigma^2 / p^2 (p-2)} = \chi^2 \quad \text{for } (p-1) \text{ d.f.}$$

it follows that $\frac{p^2(p-2) \sum \hat{g}_i^2}{(p-1)^3 \hat{\sigma}^2}$ is an F variable (since it is the ratio of two chi-squares) with $(p-1)$ and $(N - p^2 - p - 1)$ degrees of freedom, where p = number of parents, N = total number of observations in the diallel analysis and a tilde superscript (\sim) indicates an estimate of the parameter concerned. Tests of significance for the other effects were obtained in the usual manner.

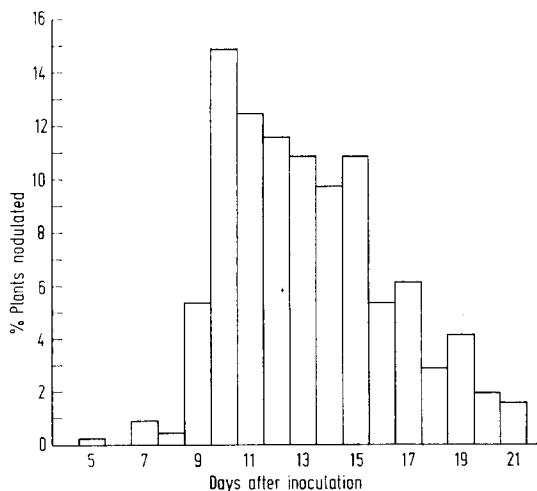


Fig. 1. Frequency distribution of plants of experiment 1 based on the number of days to primary nodulation after inoculation

Results

Experiment 1

The nodulation pattern is shown in fig. 1. The distribution was slightly positively skewed, a result which was also found by JONES (1962a). After 60 days the plants were divided into six groups on the basis of a visual assessment of the effectiveness of response (this assessment was based on the general vigour, height and number of leaves of each plant). Analysis of variance of days to nodulation for these groups showed that the variance between groups was greater ($P < 0.001$) than that within groups. These results indicated that effectiveness of response and the time of primary nodulation were correlated to some degree.

Experiment 2

The crosses between two plants, parents 1 and 12 gave very few seeds. It is possible that these plants had the same pair of incompatibility alleles. In addition some families in the maternal array of parent 12 had poor germination. For the purposes of the diallel analysis both the maternal and paternal arrays of this parent were excluded.

Not all families had 15 plants as intended at the start of the experiment. This was due mainly to poor germination. In addition six plants were lost during the period from nodulation to harvesting. This accounts for the variation in the number of degrees of freedom for the within-family component. Plant numbers per family are summarised in table 2.

Table 2. Number of plants per family

	Days to nodulation	Plant fresh weight
No. of families with 15 plants	93	87
No. of families with 14 plants	14	20
No. of families with 13 plants	2	2
No. of families with 4 plants	1	1
Total no. of plants	1,621	1,615

Table 3. Preliminary analysis of between and within family variation

Days to primary nodulation			Plant fresh weight (mg)			
Item	D.F.	M.S.	F.	D.F.	M.S.	F.
Between families	109	62.43	6.78***	109	33604.10	10.39***
Within families	1511	9.21		1505	3235.83	
	1620			1614		

* F - ratio significant at $P = 0.05-0.01$

** F - ratio significant at $P = 0.01-0.001$

*** F - ratio significant at $P < 0.001$

These symbols have the same meaning in all subsequent tables

Preliminary Analyses: A preliminary analysis of between- and within-family variation for days to nodulation and plant fresh weight is given in table 3. The between-family component was highly significant in both analyses, further analysis of this component was therefore desirable.

Diallel Analyses: The family means for both characters are given in table 4 and 5 and the diallel analyses are summarised in table 6. Since family

means were used in the diallel analyses, the error M.S. or within-family variance (from table 3) was adjusted accordingly by dividing by the harmonic mean of the number of plants per family (SNEDECOR 1956 p. 385).

For both characters the test for g.c.a. effects as outlined in a previous section showed that these were highly significant (P 0.001). The remaining three items in the diallel analyses were also highly significant when tested against the error M.S.

Table 4. Character: Days to nodulate: Family means
Female parent No.

	1	2	3	4	5	6	7	8	9	10	11	\bar{X}_i
Male parent No.												
1		8.73	9.07	8.27	7.69	8.40	8.73	8.13	8.93	8.53	8.67	85.15
2	7.86		8.27	8.60	10.80	8.73	8.33	8.87	9.40	10.13	11.93	92.92
3	8.13	10.13		8.00	8.64	10.80	8.40	8.07	10.00	9.57	7.93	89.67
4	7.29	8.40	8.07		8.71	10.73	8.71	9.60	10.40	7.27	8.93	88.11
5	8.20	10.21	7.13	7.87		8.67	6.60	7.33	8.53	10.69	6.87	82.10
6	6.40	7.27	7.80	8.20	8.60		10.20	11.00	11.53	12.80	14.47	98.27
7	9.07	9.47	6.93	7.73	7.87	8.40		10.00	6.87	8.13	11.20	85.67
8	7.00	9.40	9.33	7.00	9.13	9.00	9.00		10.40	8.73	7.87	86.86
9	8.33	11.73	9.67	8.13	10.60	9.47	9.00	7.80		10.87	11.67	97.27
10	6.93	9.93	6.20	8.57	7.47	9.53	11.00	9.67	9.14		9.27	87.71
11	7.87	11.57	14.47	8.40	10.07	18.20	17.47	12.67	12.20	15.13		128.05
\bar{X}_i	77.08	96.84	86.94	80.77	89.58	101.93	97.44	93.14	97.40	101.85	98.81	
$X_{.i} - \bar{X}_i$	-8.07	3.92	-2.73	-7.34	7.48	3.66	11.77	6.28	0.13	14.14	-29.24	
$X_{.i} + \bar{X}_i$	162.23	189.76	176.61	168.88	171.68	200.20	183.11	180.00	194.67	189.56	226.86	

Table 5. Character: Plant fresh weight in mg.: Family means
Female parent No.

	1	2	3	4	5	6	7	8	9	10	11	\bar{X}_i
Male parent No.												
1		244.9	214.1	216.7	227.0	227.5	249.2	242.0	249.1	190.1	201.3	2261.9
2	205.0		254.7	193.8	167.8	171.2	211.9	210.4	240.0	191.9	228.0	2074.7
3	242.9	235.1		273.7	204.7	222.4	201.8	244.1	196.9	239.8	213.2	2274.6
4	231.0	272.1	283.5		267.1	240.5	222.4	216.7	220.8	129.3	54.8	2138.2
5	250.8	179.9	240.2	226.1		249.5	239.4	266.7	248.5	179.1	232.9	2313.1
6	275.0	276.6	314.0	261.0	240.7		227.6	215.9	212.1	203.4	170.9	2397.2
7	261.3	209.3	253.5	253.5	225.3	272.9		189.5	258.4	198.3	122.2	2244.2
8	265.0	226.9	246.9	258.0	230.2	206.1	215.8		222.4	221.7	240.7	2333.7
9	268.2	228.4	228.6	285.1	269.6	245.5	221.6	217.9		209.4	235.9	2410.2
10	300.0	190.9	297.2	253.4	193.3	234.2	108.1	218.9	234.7		190.6	2221.3
11	268.8	231.2	160.6	220.7	226.5	29.7	44.0	139.6	197.7	118.1		1636.9
\bar{X}_i	2568.0	2295.3	2493.3	2442.0	2252.2	2099.5	1941.9	2161.7	2280.6	1881.1	1890.5	
$X_{.i} - \bar{X}_i$	306.1	220.6	218.7	303.8	-60.9	-297.7	-302.4	-172.6	-129.6	-340.2	253.6	
$X_{.i} + \bar{X}_i$	4829.9	4370.0	4767.9	4580.2	4565.3	4496.7	4186.0	4495.4	4690.8	4102.4	3527.4	

Table 6. Diallel analysis of variance

Item	Days to primary nodulation			Plant fresh weight mg.		
	D.F.	M.S.	F.	D.F.	M.S.	F.
General combining ability (g.c.a.)	10	17.602 ¹		10	7642.133 ¹	
Specific combining ability (s.c.a.)	44	2.922	4.59***	44	1901.275	8.49***
General reciprocal effect (g.r.e.)	10	6.564	10.30***	10	3148.612	14.02***
Specific reciprocal effect (s.r.e.)	45	1.923	3.02***	45	1222.452	5.44***
Error ² (within family)	1511	0.637		1505	224.520	

¹ see text for test of significance

² Error appropriate to family means

Additive effects: Estimates of the additive effects (g_i and m_j) are given in table 7. The general combining ability and general reciprocal effects were of the same order of magnitude (disregarding sign) for days to nodulate, while the latter effect was in most cases of greater importance in determining plant weight.

Table 7. Estimates of parameters g_i and m_j for both characters

Days of nodulate				Plant weight mg.			
g_1	-0.94	m_1	-0.73	g_1	8.90	m_1	27.83
g_2	0.04	m_2	0.36	g_2	-12.76	m_2	20.05
g_3	-0.39	m_3	-0.25	g_3	9.43	m_3	19.88
g_4	-0.53	m_4	-0.67	g_4	-4.87	m_4	27.62
g_5	-1.12	m_5	0.68	g_5	10.88	m_5	-5.54
g_6	0.63	m_6	0.33	g_6	17.83	m_6	-27.06
g_7	-0.68	m_7	1.07	g_7	0.79	m_7	-27.50
g_8	-0.61	m_8	0.57	g_8	12.05	m_8	-15.64
g_9	0.48	m_9	0.02	g_9	20.98	m_9	-11.78
g_{10}	-0.43	m_{10}	1.28	g_{10}	-2.14	m_{10}	-30.93
g_{11}	3.61	m_{11}	-2.66	g_{11}	-61.08	m_{11}	23.05
S.E.	$g_i = \pm 0.24$			S.E.	$g_i = \pm 4.54$		
S.E.	$m_j = \pm 0.32$			S.E.	$m_j = \pm 6.10$		
S.E.	$(g_i - g_j) = \pm 0.36$			S.E.	$(g_i - g_j) = \pm 6.73$		
S.E.	$(m_i - m_j) = \pm 0.48$			S.E.	$(m_i - m_j) = \pm 9.04$		

A feature of these results was that for most parents where both the g.c.a. and g.r.e. effects were large (i.e. > twice the S.E.) they were of opposite sign. Parents 1 and 4 for days to nodulate and parents 1 and 3 for plant weight were exceptions in this respect. In so far as the additive effects determine response to selection it would be necessary to select parents of good general combining ability and to determine whether these parents should be used as male or as female parents.

Non-additive effects: Estimates of the non-additive effects (s_{ij} and r_{ij}) are given in tables 8 and 9. For both characters many of these estimates are relatively large. The crosses in the array of parent 11 and to a lesser extent parent 10 had a high frequency of large and highly significant non-additive effects. A higher frequency of significant (i.e. > twice the S.E.) non-additive effects was observed for plant weight than for days to nodulation.

General reciprocal effects: If these effects were directional, i.e. of either maternal or paternal origin, then the variances of the male and female arrays should differ. Assuming that these general reciprocal

Table 8. Days to nodulate estimates of parameters s_{ij} (top right) and r_{ij} (bottom left)

		Parent No.										
		1	2	3	4	5	6	7	8	9	10	11
Parent No.	1		0.09	1.13	0.74	0.74	-1.38	1.06	-0.10	0.15	-0.46	-2.00
	2	-0.11		0.20	-0.07	1.77	-2.31	-0.46	-0.06	0.55	0.31	-0.04
	3	0.23	-0.63		0.19	-0.12	-0.28	-0.97	0.24	0.55	-1.11	0.14
	4	0.47	0.61	0.17		0.72	0.31	0.02	0.27	0.41	-0.64	-1.97
	5	-0.96	0.13	0.29	-0.25		-0.68	-1.13	0.04	0.56	0.36	-2.32
	6	0.47	0.74	1.21	0.76	0.21		-0.64	0.23	-0.09	0.86	3.96
	7	-1.07	-0.93	0.08	-0.38	-0.83	0.53		0.68	-1.71	0.21	2.90
	8	-0.09	-0.37	-1.04	0.68	-0.84	0.88	0.75		-0.36	0.02	-0.98
	9	-0.07	-0.99	0.04	0.80	-0.70	1.19	-0.54	1.58		0.01	-0.14
	10	-0.21	-0.45	0.92	-1.63	1.31	1.16	-1.54	-0.83	0.23		0.42
	11	1.36	1.69	-2.06	1.26	0.07	-0.37	-1.27	-0.78	1.07	-0.96	
S.E.'s	$s_{ij} = \pm 0.50$; $(s_{ij} - s_{ik}) = \pm 0.75$; $(s_{ij} - s_{kl}) = \pm 0.70$											
	$r_{ij} = \pm 0.51$; $(r_{ij} - r_{ik}) = \pm 0.76$; $(r_{ij} - r_{kl}) = \pm 0.87$											

Table 9. Plant fresh weight. Estimates of parameters s_{ij} (top right) and r_{ij} (bottom left)

		Parent No.										
		1	2	3	4	5	6	7	8	9	10	11
Parent No.	1		-16.09	-34.64	-28.86	-12.99	3.18	24.44	5.50	-0.21	18.88	40.83
	2	23.84		7.30	5.79	-52.49	1.38	5.34	-3.80	0.89	-9.22	60.93
	3	-10.43	9.89		29.33	-25.99	23.57	0.28	0.94	-42.66	45.78	-3.88
	4	-7.04	-42.93	-8.76		8.59	16.55	21.01	3.22	7.96	-20.95	-42.60
	5	4.78	6.74	-5.04	37.08		11.73	16.24	15.15	14.89	-25.27	50.18
	6	3.70	-29.14	-22.32	17.09	15.16		37.95	-18.49	-11.55	11.14	-75.41
	7	21.61	25.07	-2.16	12.00	18.03	-22.44		-9.58	16.91	-37.20	-75.35
	8	10.23	9.59	16.36	0.98	23.30	-0.81	-19.08		-20.12	12.71	14.51
	9	10.25	21.72	-0.02	-12.45	-7.43	-24.34	10.54	0.32		3.61	30.30
	10	-25.57	25.99	-3.29	-32.78	5.60	-13.47	46.82	9.04	-3.08		0.54
	11	-31.36	-3.10	24.71	-80.67	-11.10	45.54	13.83	31.20	1.68	9.26	
S.E.	$s_{ij} = \pm 9.48$; $(s_{ij} - s_{ik}) = \pm 14.13$; $(s_{ij} - s_{kl}) = \pm 13.22$											
	$r_{ij} = \pm 9.59$; $(r_{ij} - r_{ik}) = \pm 14.29$; $(r_{ij} - r_{kl}) = \pm 16.29$											

effects were entirely of maternal origin then the expectation of the variance of the female arrays is $\sigma^2 + (p - 2) \sum g_i^2 + (p - 1) \sum m_i^2$ and that of the male arrays is $\sigma^2 + (P - 2) \sum g_i^2$. These expectations are reversed if the effects were of paternal origin (TOPHAM 1966). The variance ratio of the larger/smaller array mean square was not significant for either character. There was no evidence of any overall trend in the direction of these general reciprocal effects.

Covariance analysis: The correlation between days to nodulation and plant fresh weight was highly significant, $r_{(1613)} = 0.48 \pm 0.0218$. In table 10 the analysis of between- and within-family variation in plant fresh weight after correction for covariation with days to nodulation is given. It was evident that there was a considerable amount of variation in this character which cannot be explained by variation in days to nodulation. This was not surprising since other factors such as nodule size, number and distribution will influence the efficiency of the symbiotic relationship between host and *Rhizobium* and thereby affect the growth rate of the individual plant. The proportion of the total sum of squares for plant weight that is attributable to variation in days to nodulate is measured by r^2 which in this case is 0.23 (or 23 per cent).

Table 10. Analysis of variance of plant fresh weight after correction for covariation with days to nodulation

Item	D.F.	M.S.	F.
Between-Families	109	21 705.2	7.80***
Within-Families	1504	2 783.4	
	1613		

Experiment 3

The analysis of dry matter yields summed over all 3 harvests is summarised in table 11. No difference between array 4 and 11 was found but the variation between families within each array was significant. Maternal arrays 4 and 11 were chosen for this experiment because they differed widely in terms of days to nodulation and plant weight in experiment 2. There was, however, no evidence that the array

Table 11. Analysis of dry matter yield (g) per plant accumulated over three harvests

Source of variation	D.F.	M.S.	F.
Array 4 V. array 11	1	3.59	1.09
Between-families within array 4	10	11.02	3.35***
Between-families within array 11	10	9.79	2.98**
Within families	197 ¹	3.29	

¹ one plot was missing

means differed in experiment 3. Analysis of the individual harvests gave results similar to those for the total plant weight (table 11). A correlation analysis for the fresh weights of the 22 family means of experiments 2 and 3 gave a value of r which did not differ significantly from zero ($r_{(20)} = -0.196 \pm 0.219$).

Discussion

The main conclusions which can be drawn from these experiments are: (i) There was genetic variation present for both characters studied. (ii) This variation was complex, reciprocal differences as well as additive and non-additive effects were present. (iii) Plant fresh weight at 80 days and time of nodulation were correlated. (iv) Performance under soil conditions was not correlated with the results obtained in artificial growth medium.

While the non-additive effects were relatively large, particularly for plant weight, the estimates of general combining ability indicate that selection should be effective. The analyses suggest that the general reciprocal effects are of considerable importance, especially so for plant weight. These results do not agree with those reported by JONES (1966) who found no evidence for reciprocal differences. In order to utilise this variation in general reciprocal effects it would be necessary to control the direction in which crosses are made. This would limit to some extent the choice of breeding system which could be used. There is, however, insufficient information regarding the inheritance of these effects, which in statistical terms are additive. It is, therefore, not possible to predict what effect selection would have on this variation.

Parents 10 and 11 were taken from the two most ineffective groups of experiment 1. The progeny of these two plants were also the least effective in experiment 2. There was no segregation pattern to suggest that the ineffective response was simply inherited as for example was found for the „i“ and „i e“ factors in red clover (NUTMAN 1954, 1957). The progeny of these plants did however show large reciprocal differences (both general and specific) for both characters, plant 11 was much better as female than as male parent while the reverse was shown by parent 10. Further breeding tests would be necessary to determine the nature of these reciprocal differences.

The data of experiment 3 indicated that there was no correlation between the results obtained when the same families were grown in artificial medium in test-tubes under controlled inoculation and those where the families were grown in soil without controlled inoculation. The lack of correlation could result from an interaction between plant and growth medium, between host and *Rhizobium*, or a complex interaction of all three variables. If the interaction between plant and growth medium is the major fac-

tor then selection under the artificial conditions of experiments 1 and 2 would not be expected to result in improved performance under field conditions. If on the other hand the interaction was that between host plant and inoculum then selection for effective response using a single strain of *Rhizobium* may result in a highly specific host/*Rhizobium* combination. Specific host/*Rhizobium* strain interaction was a notable feature of the results obtained by NUTMAN (1954b) in red clover and by GIBSON (1962, 1964) in lucerne and in subterranean clover. The value of such a specific combination would depend on the success of inoculation with the desired *Rhizobium* before sowing. The results of field inoculation experiments of JONES et al. (1964) and JONES and DAVIES (1966) conducted on upland acid and peat soils showed a response to inoculation and the introduced effective *Rhizobium* appeared to persist and compete well with the indigenous strains. These soils are known to have a low frequency of effective *Rhizobium* strains compared to fertile soils with a satisfactory base status (JONES 1962b, 1963; JONES et al. 1964; HOLDING and KING 1963). On fertile soils white clover is normally sown without inoculation. Selection of a clover variety for effective symbiotic response with a single *Rhizobium* strain might not therefore be of much value for general agricultural purposes if this response is highly specific to that particular *Rhizobium* strain.

Although it is not possible from the experiments reported here to distinguish between the possible causes of the lack of correlation between the results obtained in experiments 2 and 3, they do suggest that selection on the basis of symbiotic effectiveness in artificial media using one strain of *Rhizobium* may not show increased response under field conditions.

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Zusammenfassung

In drei Versuchen wurde beim Weißklee (*Trifolium repens*) die Zeit bis zur Knöllchenbildung sowie das Gewicht der Pflanze bzw. deren Entwicklungsgeschwindigkeit ermittelt. Bei Versuch 1 und 2 erfolgte die Kultur in Reagenzgläsern auf künstlichem Nährboden nach kontrollierter Impfung und bei Versuch 3 im Boden ohne Impfung. Zunächst wurde im Versuch 1 die Variabilität der Zeitdauer bis zur Knöllchenbildung nach Impfung mit *Rhizobium trifolii* untersucht, und es zeigte sich eine auffällige Beziehung zwischen der Entwicklungsgeschwindigkeit der Knollen und der Pflanze. Den wesentlichsten Teil

der Ergebnisse lieferte Versuch 2, in dem die Nachkommenschaften dialleler Kreuzungen von 12 in Versuch 1 selektierten Pflanzen hinsichtlich der Kriterien Anzahl Tage bis zur Knöllchenbildung und Pflanzengewicht nach 80 Tagen geprüft wurden. Die Resultate ergaben ein recht kompliziertes Bild von der genetischen Veranlagung der beiden untersuchten Merkmale. Es wurden reziproke Unterschiede (sowohl allgemein wie spezifisch) sowie additive und nicht-additive Wirkungen festgestellt. Schließlich wurden im Versuch 3 die Nachkommenschaften aus 22 diallelen Kreuzungen in Erde ohne Impfung geprüft und die Ergebnisse mit denen aus Versuch 2 verglichen. Es wurde festgestellt, daß sie nicht übereinstimmen.

Zum Schluß wird die Bedeutung dieser Ergebnisse im Hinblick auf die Selektion von verbesserten Formen von Wirtspflanzen diskutiert.

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