# PHYSIOLOGICAL STUDIES ON THE GERMINATION AND EARLY SEEDLING GROWTH OF CLOVERS

By R. M. Greenwood

Chemist, Plant Chemistry Laboratory, Department of Scientific and Industrial Research, Palmerston North.

## INTRODUCTION

In the field numerous factors operate to influence the germination and establishment of clovers, and Mr Sears, in the final paper in this series, shows how complex the problem is. It is impossible by field observation alone to disentangle the effects of all these factors and to decide in any particular case just which of them have been of importance in giving the observed result. It is thus essential for a better understanding of the behaviour of clover seedlings that certain variables, for instance nutrient supply, should be held constant while studying the effect of controlled variation in another, for example light intensity, and this is only possible under controlled conditions in a laboratory. This does not mean that the results of such findings will be of direct and immediate application in practice, but rather that without. a knowledge of the fundamental reactions of seedlings to such factors as light, temperature, and nutrient supply, progress in the field will be hampered.

## PHASES OF SEEDLING GROWTH

It is desirable to begin by considering briefly the phases of seedling growth of clovers up to establishment. These can be divided into (1) Imbibition, during which water is taken in and the seed swells, (2) Germination, when obvious growth starts and the young root breaks through the seed coat (3) Growth in the dark up to the time when the seedling normally emerges from the soil; and finally (4) Growth in the light up to the time that the seedling can be said to be established, which can roughly be taken as the time when pronounced branching has occurred.

In this paper I cannot deal fully with all these phases, but shall confine my remarks to the following aspects :-

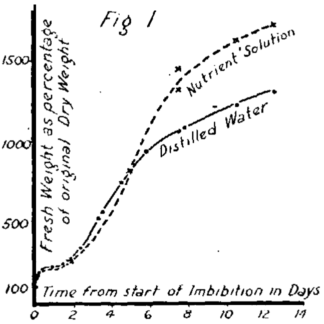
1.. The effect of nutrients on seedling growth in the dark.
2. Growth under optimum conditions in the field, together with a consideration of the effect of seed size on seedling size, and
3. Growth of seedlings under controlled artificial light in a cabinet.

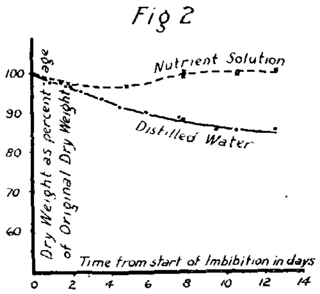
So far subterranean clover only has been studied, as its seeds are larger and easier to handle individually. Later, when the responses with this species have been obtained, it will be possible to run through the other species more quickly.

## THE EFFECT OF NUTRIENTS ON SEEDLING GROWTH IN THE DARK

In the study of the growth of clover seedlings in the dark there are several methods of measuring growth. Fresh and dry weights of uniform groups of seedlings may be obtained, thus giving a measure of the water content and the total bulk of tissue. If the seedlings are divided into their different organs before weighing, a better picture of development is obtained, the distribution of weight between the different portions of the seedling giving a measure of the rate of mobilisation of seed reserves.

Results by all these methods show that the presence of nutrients has a marked effect both on the total development of the seedling and on the relative development of its parts, even at this early stage. Thus when the fresh weights of seedlings grown in the dark at a uniform temperature (in this case 68 degrees F.) are plotted against time, it is seen (Fig. 1)



that in the presence of nutrients the fresh weight, after a slight initial lag, increases more rapidly and to a greater extent than when. the seedlings develop in distilled water. The dry weights are plotted on a different scale (Fig. 2), and it can be seen that the dry weights of seedlings grown in distilled water fall steadily with time, as would be expected, due to losses in dry weight from respiration. In the presence of nutrients, however, no consistent loss of weight occurs. This is not because of a decreased rate of respiration, but because the' rapid intake of nutrients balances the loss of weight due to respiration. Thus measurements of the ash as a percentage of the original dry weight of the seed (which gives a measure of the overall mineral content of the seedlings) show 14.6 per cent. for seedlings grown in nutrient solution for 7½ days and 16.2 per cent. for those grown for 12½ days, compared with 3.6 per cent, for the dry seed or for the seedlings grown in distilled water.

When seedlings which have been grown for 7½ days in nutrient solution or in water are divided into seed coats, seed leaves, stem tissue, and roots, and the dry weights and ash contents compared (Table 1), it is seen that in the presence of nutrients there has been a drop in the dry weight of the seed leaves—which indicates a more rapid mobilisation of seed reserves, as the seed leaves make up most of the weight on the seeds-and a marked increase in the dry weight of the stems and roots. The ash content shows a marked uptake of mineral nutrients, particularly in the stems and roots.

**Table 1. Distribution of Dry Matter and Ash in Various Tissues of Seedling Clovers grown for 7) days in the dark at 68deg. F. expressed as percentage of original dry weight of the seed.**

| | Grown in Distilled Water | | Grown in Nutrient Solution | |
|---|---|---|---|---|
| | DM % | Ash % | DM % | Ash % |
| Seed Coats | 20.0 | .34 | 19.7 | .51 |
| Seed Leaves | 34.8 | 1.02 | 29.7 | 3.89 |
| Stem Tissue | 29.5 | 1.89 | 42.0 | 8.04 |
| Roots | 6.5 | .21 | 8.8 | 2.13 |
| TOTAL | 90.8 | 3.46 | 100.2 | 14.57 |

The fact that the presence of nutrients has a marked effect on seedling development can be better understood when the analysis of dry seed is compared with that of typical clover herbage (Table 2).

**Table 2. Comparison in Mineral Content between Subterranean Clover seed and Clover Merbage, given as percentage of dry weight.**

| | Ash | Ca | K | P | N |
|---|---|---|---|---|---|
| Seed | 3.7 | .12 | 1.05 | .54 | 5.76 |
| Herbage | 9.6 | 1.33 | 2.69 | .43 | 4.78 |

It is seen that the percentage of calcium in particular is remarkably low in seeds, and potash is also low. Thus it can be said that for the optimum development of seedlings, nutrients are required right from the time of germination.

## GROWTH UNDER OPTIMUM CONDITIONS' IN THE FIELD

In any study of the growth of plants under controlled conditions it is of value to know the rate and type of growth to be expected under the best conditions outside. To this end subterranean clover seeds were sown at March 31, 1950, in 8in. pots containing rich potting soil mixture and the pots sunk to ground level outside. They were watered as required and plants thinned so that. no appreciable 'competition occurred between them. In order at the same time to study the effect of seed size, the one sample of seed was screened into a number of different size classes and three of these selected having mean seed weights of 3.5, 6.4 and 10.4 mg., the seeds sown being individually weighed and tagged.

Four harvests were made 4, 5½, 8, and 17½ weeks after sowing. At each harvest whole pots were taken and emptied out, the seedlings separated, and the roots washed free of soil, and fresh and dry weights of the individual seedlings obtained. If the mean dry weights of the seedlings at the different harvests are corrected to a seed weight of 11 mg. and plotted against time (Fig. 3), 'a typical growth curve is obtained showing that the larger the seedling the greater the increase in weight during any one time interval.

Fig 3

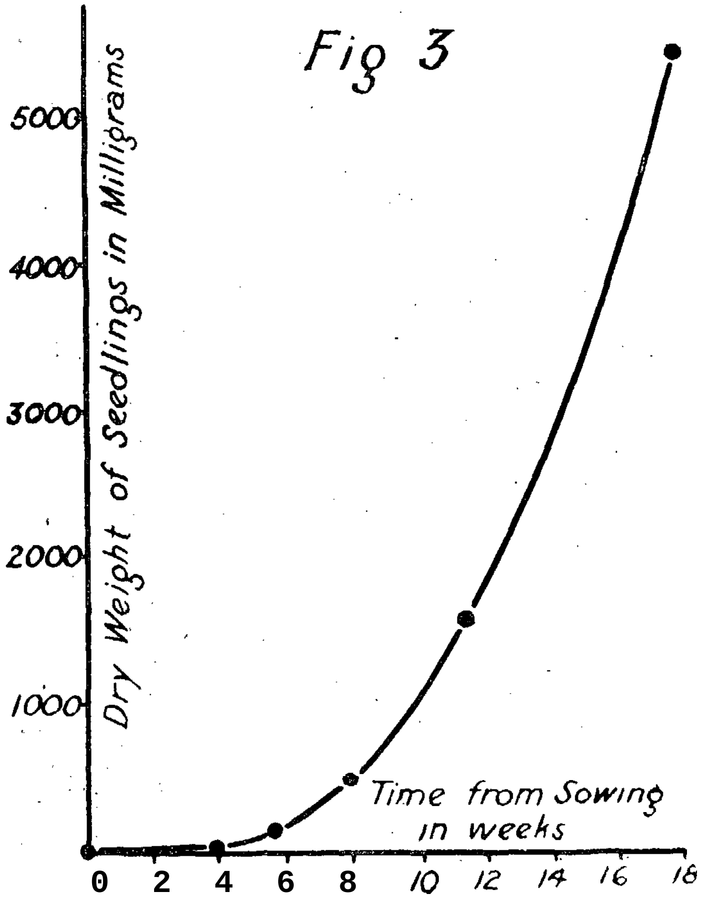
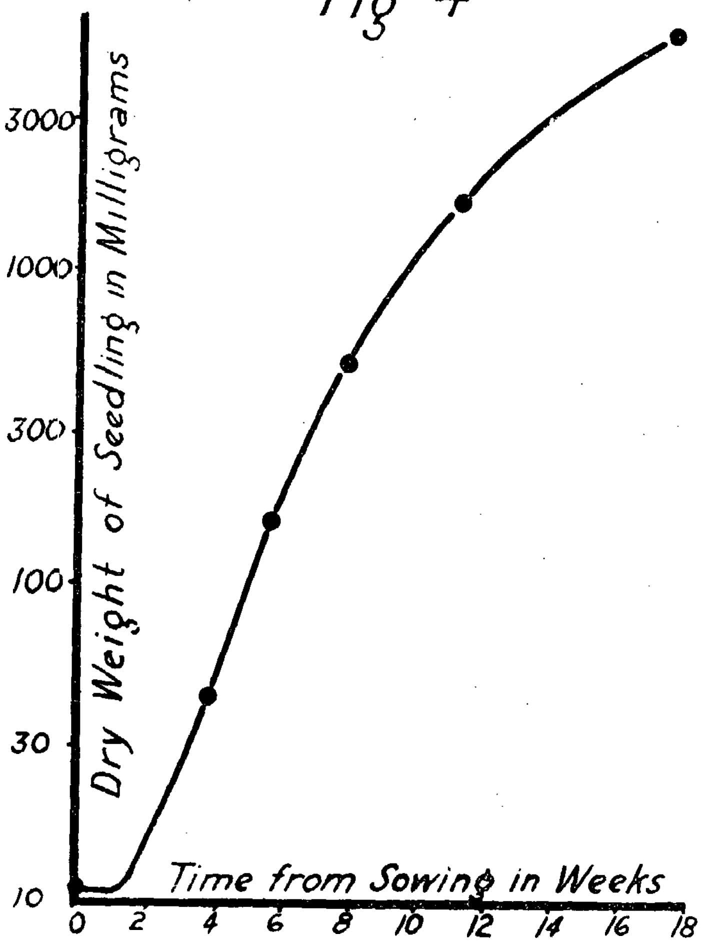

5000
4000
3000
2000
1000
Dry Weight of Seedlings in Milligrams
Time from Sowing in weeks
0
2
4
6
8
10
12
14
16
18

Fig 4


Dry Weight of Seedling in Milligrams
3000
1000
300
100
30
10
Time from Sowing in Weeks
0
2
4
6
8
10
12
14
16
18

If, however, these same figures are plotted on logarithmic paper against time (Fig. 4), on which scale a straight line indicates that over that period the rate of growth has followed a uniform rate of compound interest, it can be seen that after allowing for an initial lag period of about ten days, the rate of increase is almost constant till the third harvest. After this the rate drops. This drop, however, may well be explained by the fact that this was the winter period, with lower temperatures and shorter days, so that had conditions remained the same throughout, it is probable that the rate of growth would have remained the same for a longer period, possibly until flowering began.

## SEED SIZE

As regards the effect of seed size on plant size, it was found that for the first two harvests the weights of the seedlings were almost in direct proportion to the weights of the seeds. Not only were the individual leaves larger on the plants from the larger seeds, but also these plants were more forward in that the number of leaves and also the amount of root branching were greater. The effect of seed size was still marked at the third harvest, though by this time other variables had caused a greater spread in the seedling weights. At the fourth harvest the effect of other variable factors masked to a large extent the effect of seed weight. The fact that seed size can influence seedling size for such a long period, at least eight weeks, is of interest, though I would not like at this stage to comment on whether large or small seed would be of more value in practice, sown on the basis of equal weight of seed. The other clover species do not have such a range of seed sizes as does subterranean clover, and the effect of seed size would thus not be such moment with them.

## GROWTH OF SEEDLINGS UNDER CONTROLLED ARTIFICIAL LIGHT IN A CABINET

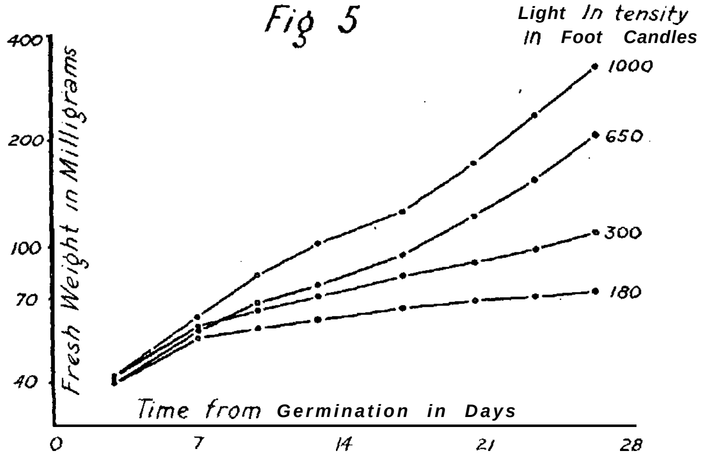
When one is interested in the growth of seedlings under conditions of controlled light and temperature, it is not possible to use. sunlight, as there are such very great variations from day to day in intensity due to clouds, etc., and also seasonal changes both in maximum intensity and in length of daylight. Also the large amount of heat associated with sunlight makes it almost impossible to control temperature accurately. Thus for studies of this kind artificial light must be used. I do not mean to imply that it is possible with artificial light to reproduce exactly the same type of growth as in sunlight, but with modern fluorescent lamps, although the maximum intensity obtainable is low (only about a tenth of full midday sunlight), rapid, healthy, and uniform growth of seedlings can be obtained, and thus the effects of any factors on the rate of growth can be readily determined. It must be realised, too, that such light intensities are by no means uncommon in the field. For instance, it was found that the light intensity at ground level under a medium dense 6in. pasture was reduced to about one fiftieth of that of full sunlight, and such a light intensity would definitely limit the growth of seedlings.

For the purpose of these growth studies a bank of fluorescent lamps has been built up at the Dominion Physical Laboratory which fits into a temperature controlled, water-jacketed cabinet. As the number of seedlings that can be grown in the cabinet at one time is limited, a method of growing them in such a way that each seedling may be weighed at intervals a number of times is a great advantage, as in this way much more definite information can be obtained from the same number of seedlings. The seedlings are thus grown with their roots in nutrient solution and are held in slots cut in sheet rubber in such a way that they can be removed periodically, the roots blotted, weighed rapidly, and returned to the solution.

To illustrate the use of the cabinet, which has been running for only a short time, an experiment on the effect of light intensity on seedling growth at constant temperature (68deg. F.) can be described. In this experiment the day length was set at 14 hours, and 4 jars of seedlings were grown at light intensities of approximately 1000, 650, 300 and 180 foot candles.

In the graph (Fig. 5), the mean fresh weights of four seedlings at each light intensity are plotted on a logarithmic scale against time. It can be seen that after an initial period of adjustment the light intensity has a profound effect on the rate of growth, and that the rates of growth remain constant within limits for the duration of the experiment. At the highest rate of growth seedlings double their weight in about 8 days, while at the lowest rate of growth they would take about 50 days to double their weight.

Fig 5




This experiment was in the nature of a trial run, and it should be possible in future to get more even rates of growth than these, the kink in the top curve for instance being due in part to excessive crowding of the plants at that stage.

A photograph of the seedlings after 27 days' growth shows the marked effect of light intensity, higher light giving a larger, more robust plant with more leaves and a better developed root system.

Although conditions were very different in this experiment from conditions outside during the experiment described before, notably in that the temperature was higher and more uniform, the day length longer, and the light intensity less, nevertheless it is of interest to note that the rate of growth of the seedlings at the highest light was slightly greater than that of the seedlings grown outside. The type of growth was very different, however, in that more and smaller leaves were formed and the plants were more upright, though temperature probably has a considerable effect here.

## FACTORS AFFECTING EARLY SEEDLING GROWTH

In conclusion the factors which may affect early seedling growth can be listed as follows:—

1. First there are the factors of microclimate and soil under which the seedling develops; the microclimate of the seedling is very different from the conditions a few feet above the sward that we nor-

mally experience. These factors include light, regarding which both intensity and duration per day are important, temperature-both mean temperature and fluctuations in temperature-and moisture relations above and below ground. Also there is the nutrient status of the seedlings, with regard to all essential plant nutrients, and for an understanding of this a knowledge of the nutrient requirements of the seedlings and the reserves contained in the seed is required.

2. Then there are the biotic factors, those concerned with the effects of other forms of life on the clover seedlings ; and these include the effects of diseases, pests such as slugs, aphides, and caterpillars, birds, and the defoliations and trampling by stock. Plant competition is obviously of great importance for seedlings growing up in a sward, but for the most part this effect may be analysed out into its effects on the microclimate. Thus under a grass cover wind velocities will be less and thus effective humidities higher, temperatures more uniform, and above all light intensities will be considerably reduced.

3. Thirdly there are the factors due to the seed itself. There is the species of seed; obviously different species of clover might be expected to give different responses under the same conditions, but so may different strains within the one species. Then there is the effect of seed size, as has been described. Finally there is the effect of the vigour or vitality of the seed, an aspect of which Mr Hyde has already spoken.

The effects of many of these factors may be studied in the laboratory under controlled conditions and the results then applied to the field and used in the interpretation of results obtained there.