THE PASSAGE OF WHITE CLOVER SEEDS THROUGH THE BODY OF SHEEP AND THE EFFECT ON GERMINATION CAPACITY

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INTRODUCTION

To maintain a high rate of production from pastures one essential is an adequate supply of nitrogen, and this supply can be assured most economically by introducing strong-growing clovers into the sward. Sufficient clover plants can be readily established on arable. land by employing a suitable seed mixture when the pasture is renewed. On unploughable land, however, there are no opportunities for the complete renewal of pasture, and other methods of introducing clovers have to be found.

In New Zealand there are large areas of unploughable land where pastures are deficient in good clovers and the improvement of these pastures is an urgent problem. This question is being approached in several wavs, one of which is the study of natural means of introducing clovers. The present article deals with part of this work and concerns the role of sheep in the dissemination of clovers.

Several workers have made observations on the effects of animal digestion on seeds and have found that seeds of many leguminous plants may pass uninjured through the digestive tract of sheep and cattle. In no case, however, has sufficient attention been devoted to the significance of the hard seed condition in relation to survival. The possibility of using animals for disseminating seed of pasture plants has been discussed by overseas workers from time to time. In 1932 a German worker drew attention to a practice of farmers in Germany who, having observed that some ripe white clover seed passes uninjured through the

alimentary canal of animals, turned young animals, sheep for preference, into fields where white clover plants were in full seed. The sheep were subsequently transferred to a field where it was desired to establish white clover.

In view of the overseas work on the subject we wanted to look further into the matter to see what the position was under New Zealand conditions, and I will now discuss briefly the experimental work we have carried out.

This work was carried out using 4-year-old wethers at the Grasslands Division area in Palmerston North. We observed what clover seed the animals ate when grazing on clover pasture, and fed them with samples of white clover seed, both commercial scarified samples and hand-harvested unscarified seed. The main point of interest was, of course, the quantity and condition of the seed which the animals passed. This entailed the collection of all the faeces of the experi-

Fig. l.-Experimental animal in 10ft. x 10ft. cage on pasture containing a liberal quantity of white clover seed-heads. Note harness and faeces collection bag.

mental animals, and meant that we had to extract all the clover seed contained in the faeces.

The faeces were collected in bags attached to the animals by harness (Fig. I.)

METHODS OF EXTRACTION

The total quantity of dung from each sheep was collected daily, weighed, and mixed. A. 1000-gram sample for seed analysis was taken by subdividing the total quantity of dung into small heaps and then taking up these at random until the required weight was obtained. The sample of dung was then placed in a cloth bag and thoroughly macerated under water. After the pellets were broken down the bag was emptied into a bucket and placed under a tap where by flotation and decantation the light fibrous material and mucilaginous matter was washed away, leaving the coarser fibres and seed remaining in the bucket. During the flotation process a number of empty seed coats as well as some clover embryos from partly masticated seed floated to the surface, showing that in the process of grazing or cud chewing damage occurred to the seed being consumed. It is possible that in the process of maceration some swollen seeds were damaged. The small quantity of fibre and seed remaining was readily air dried and the seed separated out by sieving.

The seeds recovered were found to vary greatly in colour and were separated into two classes.

1. Seeds of normal colour, yellow to reddish brown.
2. Seeds of abnormal colour, olive green to black.

It was found that class 1 consisted of hard seeds, that is, seeds which did not absorb moisture readily because of the impermeability of the seed coat, and class 2 were permeable seeds and had absorbed stomach juices. The seeds of the two classes were air dried and weighed separately.

The first question that posed itself was whether sheep really did eat mature white clover seed heads readily. Close observation of sheep grazing in small enclosures soon showed that they consumed seed heads freely, sometimes even showing a preference for them over more lush feed.

The next question was whether the seed consumed passed through the animal. Examination of the faeces by the method already outlined showed that seeds were present in large numbers. The mean daily output of

seed per sheep during the period when grazing on the clovery sward was 1891 milligrams of normal coloured seed and 676 milligrams of discoloured seed.

The viability of the seed was, of course, a matter of special interest. Some of the seed was extracted as previously described from a 1000-gram sample, classified according to colour, then weighed and tested for germination. In the germination tests the seeds were classified as follows:

a. Seeds germinating in 16 days.
b. Abnormal growths.
c. Hard seeds.
d. Dead seeds.

The results of the germination tests revealed that the seeds of normal colour were almost all hard seeds, only 5 per cent. germinating within 16 days (the remainder being in the hard condition). Of the discoloured seeds only 11 per cent. were viable and none was hard. The non-viable seeds included 9 per cent. which produced useless abnormal growths. To ascertain whether they were viable the hard seeds from several samples of dung were scarified between sheets of sandpaper and returned to the incubator. These hard seeds had failed' to germinate after 28 days on moist filter paper at 15 degrees C. After scarification 92 per cent. of the seeds germinated in 7 days, 7 per cent. produced abnormal growths, and 1 per cent. remained hard., The abnormal growths appeared to be due to injury suffered during scarification.

In view of these results it was considered reasonable to assume that all hard seeds recovered from dung were viable.

Generally speaking it was the hard seeds only that passed through the animal without injury, whereas the permeable seeds were either non-viable or produced abnormal growths. At this stage of the investigations it was clear that the animals did freely graze the mature clover heads, seed did appear in the faeces, and this seed was viable, although germination was delayed because of the impermeability of the seed coat.

FEEDING OF KNOWN QUANTITIES OF SEED IN CAPSULES

The previous experiment has indicated that only the hard seed passed through the digestive tract of sheep without considerable loss of viability. To in-

vestigate this point further experiments were carried out in which two different lots of seed were fed to sheep.

1. Hand-harvested white clover, which was not subjected to any scarification in cleaning or harvesting, and tested 2 per cent. germination in X6 days, the remaining 98 per cent. being hard seed.
2. Scarified seed (this was a sample of commercial white clover which in the process of machine harvesting and dressing had become scarified to the extent that the sample germinated 93 per cent. in 16 days, with 2 per cent. hard seed, 2 per cent. abnormal growths and 3 per cent dead seeds).

Four sheep were grazed for 7 days on a pure grass sward. At the end of this period, the faeces were examined and found to be free from clover seed. Faeces collection bags were attached to the sheep and 10 gelatine capsules containing approximately 1 gram of seed per capsule were injected by phenothiazine gun into each sheep's throat, Nos. 1 and 2 sheep receiving the scarified seed and sheep Nos. 3 and 4 unscarified hand-harvested seed.

Immediately following dosing the sheep were returned to a clover-free pasture. Faeces were collected daily for 7 days, when the dung of three of the four sheep contained no further seed. The white clover seed was recovered from the dung and classified according to colour. Germination tests were carried out on the seeds of normal colour recovered from each sheep.

Table I-CAPSULE FEEDING TRIAL

Sound seed recovered in the faeces of sheep fed clover seed on 19/2/50 and thereafter grazed on clover-free pasture.

	Scarified Seed		Unscarified Seed	
Days from dosing	Sheep No. 1 fed 10906mg.	Sheep No. 2 fed 10953mg.	Sheep No. 3 fed 10361mg.	Sheep No. 4 fed 10225mg.
1	26	35	299	898
2	247	49	1827	926
3	27	5	73	849
4	14	49	11	275
5	4	0	21	378
6	0	0	0	52
	318mg.	138mg.	2231mg.	3378mg.

Table 2-Germination of White Clover Seed before and after passing through sheep.

Seed Sample	Eefore Feeding			Collected from Dung		
	% Germination	% Hard Seeds	% Abnormal Growth	% Germination	% Hard Seeds	% Abnormal Growth
Unscarified Seed	2	98	0	4	96	0
Scarified Seed	93	2	3	19	80	1

Of the scarified sample 3.7 per cent. was recovered and of the unscarified sample 29 per cent. was recovered. It is especially noteworthy that 456 milligrams of hard seeds were recovered from the two sheep fed with 21859 milligrams of the scarified sample. This commercial seed contained approximately 2 per cent. of hard seeds and it appears that all this seed passed uninjured through the sheep. Of the hand-harvested seed of which 98 per cent. was hard seed, 29 per cent. was recovered.

All of this seed was viable, although most of it was in the hard condition. The results indicate that a large proportion of the seed ingested may be destroyed, and that the survival rate of hard seeds in the digestive tract of the sheep is much greater than that of permeable-coated seeds, even when these seeds are mature.

To determine whether any seed remained in the sheep's stomach and intestines after 6 days, when three of the four sheep showed no trace of seed in their dung, it was decided to kill No. 4 sheep which had been fed hand-harvested white clover seed. All stomachs and intestines were opened up, the contents washed, and seed recovered. The seed was distributed in the digestive tract as follows:-

Place found	Milligrams of seed
Rumen	0
Reticulum	12
Omasum	1307
Abomasum	99
Small intestine	76
Large intestine	65
	1559mg.

Most of the seed found in the animal's digestive tract was lodged between the "leaves" and folds of the omasum. In view of this, it is possible that small

quantities of seed would pass out for a considerable time after ingestion. All the seed recovered from the' digestive tract of the slaughtered animal was in the hard condition.

Continuing the investigations still further, the question arose, what proportion of the seed present in ,a clover pasture was eaten by the animals and what proportion passed as viable seed in the dung?

The conditions in the last experiment were different in several respects from those where seed is consumed in natural grazing. The seed was not intimately mixed with other feed consumed and -it did not receive any initial mastication. To follow up this point two experimental sheep were used and, as in the previous experiment, they 'were pre-grazed on a pure ryegrass sward for 7 days to ensure that their faeces contained no clover seed. The sheep were then placed in two 100 sq. ft. cages on clover pasture. Before shifting the sheep into the cages, estimations were made of the quantity of seed available in each cage. A square foot. quadrat was placed at random on the pasture to be grazed and all white clover seed heads, whether mature or immature, were plucked from the sample area. Four such. samples were taken from each 100 sq. ft. enclosure. The cages were shifted daily and each day sampling of the herbage for clover seed content was carried out in the above manner before grazing.

An estimation was made of the total quantity of clover seed in the herbage available to the sheep and also the proportion of hard seed. The clover seed heads in each sample were separated into ripe and unripe heads. Seed from the ripe heads was rubbed out and placed in water. for 24 hours. The hard seeds which had not absorbed water were then easily separated by sieving. These hard seeds were weighed separately. The permeable seeds from the ripe heads together with all the seeds from unripe heads were brought to the air-dry condition and weighed.

Simultaneously, with the estimation for total quantities of seed available, faeces samples were collected and the clover seed washed from the dung. The two experimental sheep were given fresh clover areas to graze on each. of the 4 consecutive days, when they were once more returned to a pure. grass sward. Faeees sampling continued for a further 7 days, by which time the 'dung showed that no further seed was passing through.

The quantity of seed recovered in the dung represented only a small proportion of the seed available in the feed. From the first animal 4.3 per cent. of the available seed was recovered. Of this 3.3 per cent. was hard- seed and 1 per cent. swollen, discoloured seeds. Of the hard seeds available 7 per cent. was recovered as hard seed. From the second animal only 0.76 per cent. of the seed available in the feed was recovered, 0.65 per cent. being hard seed and 0.11 per cent. swollen, discoloured seed. Of the hard seed available to the animal 1.86 per cent. was recovered as hard seed in the dung.

There was a large difference in the proportions of seed recovered from the two sheep. It was observed that the second animal was restless and there was a greater wastage of feed through trampling down the herbage.

In order to determine whether sheep under normal grazing conditions would select clover seed heads when grazing a clover pasture 4 sheep were harnessed with faeces collection bags and then grazed on an area containing a relatively poor white clover seed head content.

The faeces collected revealed that even under poor seed crop conditions the sheep passed an average of 3 grams of clover seed per day per animal.

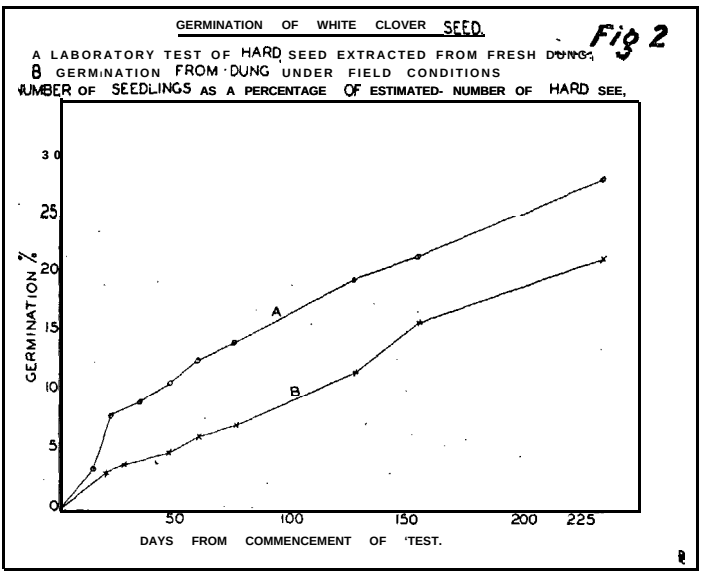
It was now clear that the dung of sheep grazing on clover pastures contained significant amounts of viable clover seed. However, another question arose. Would these seeds germinate in the dung in the field and what delay could be expected before seedlings appeared ?

A 1500-gram sample of dung containing clover seed was tested for seed content and found to contain 5464 hard seeds and 1514 soft, swollen seeds. Twelve 50-gram samples of this dung were then placed on sterilised soil in pots which had been sunk into the ground to endeavour to simulate natural soil conditions. The estimated clover. seed content of each 50 grams of dung was 546 hard seeds and 151 immature seeds.

Counts were made periodically of the clover seedlings that emerged, and as the plants became established they were carefully removed to allow for counting the future seedlings.

Concurrently seed extracted from a sample of the same lot of dung was submitted to a laboratory germination test. The results of the laboratory germina-

tion tests and field counts are shown in the accompanying graph (Fig. II.)



In recording the germination of seed in the dung it was assumed that only those seeds which passed through the animal in the hard condition were capable of remaining viable through the period of fermentation of the dung.

In the laboratory the rate of germination of hard seeds extracted from fresh dung was comparatively rapid in the first three weeks and thereafter remained nearly constant at a lower level. The rate of germination of the seeds from dung in the pots of soil was approximately the same as that in the laboratory after the first 3 weeks. It is possible that the difference in the germination curves is due to the non-survival of those seeds which became permeable in the dung during the first 3 weeks when the dung was rotting.

It was apparent that many of the seeds in dung remained in the soil still in a viable but dormant condition long after the dung had rotted away. These seeds and others which under natural pasture conditions ripen and fall to the ground must accumulate in the surface soil.

To obtain some reasonably sound idea of the quan-

tity of dormant clover seeds in pasture,. soil samples are being collected from various localities and from pastures of different age, composition, and management. This work is being undertaken in collaboration with Mr E. 0. C. Hyde of Plant Chemistry Laboratory. The results collected to date are shown in the accompanying table (3) and are calculated from 100 random soil samples taken to a depth of 2in. from each paddock.

Table S-Buried Clover Seed Populations in Soil from Various Localities.

Area	Species	Seeds per sq. ft.	lb. per acre
Manawatu dairy pasture	White clover	300	15
	Suckling clover	22	1
		322	16
Manawatu hill-country pasture	White clover	82	4
	Suckling clover	278	12
		360	16
Rangitikei sheep and cattle pasture, light grazed	White clover	2150	108
	Suckling clover	135	6
		2285	114
Rotorua 1½yr. old pasture			
(a) Clover-deficient parts	White clover	20	1
(b) Clover-dominant parts	White clover	5020	251
Auckland gumland scrub	White clover	100	5
	Suckling clover	130	5
	Lotus hisvnidus	920	13½
	Lotus major	50	1½
	Clustered clover	15	½
		1215	25½

Area	Species	Seeds per sq. ft.	lb. per acre.
Coastal sand, 20-yr. old pasture	White clover	10	½
	Suckling clover	350	11
	Subterranean clover	680	350
	Clustered clover	650	20
		1690	381½
Low-lying doastal sand, 12-yr. old pasture	White clover	75	3¾
	Suckling clover	780	31
	Subterranean clover	670	345
	Clustered clover	115	3¾
	Strawberry clover	145	21
	English trefoil	30	3½
	Haresfoot trefoil	25	3
		1840	411
Havelock North self-sown paddock	White clover	20	
	Suckling clover	140	t
	Subterranean clover	960.	560
	Striated clover	90	11
	Clustered- clover	670	22
		1880	600

The large quantities of seed lying dormant in some soils would imply that the practice of sowing the normal quantity of 2 or 31b. of white clover seed per acre was unnecessary. In fact, some farmers in the past have relied on volunteer clover and have neglectd to sow clover seed. This practice is unsound for several reasons.

1. The strain represented by the dormant clover seeds may be a poor one.
2. The! depth at which the seeds lie after cultivation may not allow for a good strike, even where seed is present in large quantities.
3. Buried seeds are in the hard condition and only a small proportion may germinate over the period of pasture establishment.

In view of the results presented in the table the dormant clover seed content of soils should be con-

sidered in land used for high-grade seed production if contamination by inferior strains is to be avoided.

DISCUSSION

The present work indicates the importance of the hard seed condition in relation to the natural dissemination of white clover by animals. Sheep graze freely on the seed heads of white clover, consuming ripe and unripe heads apparently without discrimination. Most of the permeable seeds from unripe heads are destroyed in the process of mastication and digestion, and the few that are viable at defecation do not appear to survive the period of fermentation of the dung. The impermeable seeds from ripe heads have a higher survival rate, although it is clear that a large proportion of these may likewise be destroyed.

Where sheep were fed hard seeds of white clover in capsules, 32 per cent. of the seed was recovered in the dung. Sheep which were similarly fed with seed containing only 2 per cent. of hard seed destroyed almost all the permeable seed, while practically the whole of the hard seed passed through the animals undamaged.

The feeding of seed in capsules introduces several factors which make it impossible to compare the results directly with those obtained under grazing conditions. First, there is no initial mastication, and secondly, the seed, being free from the seed heads, may not be regurgitated and chewed with the cud in the normal manner. The important result of these experiments is that the non-survival of the permeable seeds was not due to any weakness due to immaturity, because mature scarified seed was used.

The rate of germination of white clover in dung on soil in the field is very similar to the rate of germination of hard seed in the laboratory. Only in the first four weeks of the testing period was the rate in the laboratory higher than that in the field. This may have been due to the non-survival of some seedlings in the fermenting dung or to desiccation of some germinating seeds. Apparently it is only the hard seeds remaining after the fermentation of the dung which are of ecological and agronomic significance. These seeds remain viable and are effective in the dissemination of white clover. The germination of these seeds is delayed by the impermeability of the seed ‘coat. Germination is spread evenly over a long

period- and in this climate there appears to be little seasonal fluctuation in the rate of germination. The fact that some of these seeds germinate at all seasons of the year may help to ensure the survival of a proportion of the seedlings in the field.

In all experiments it was found that clover seed began to appear in the faeces one day after feeding with seed commenced? and continued for at least five days after feeding with seed was discontinued. The fact that seed was still present in the alimentary canal of a sheep slaughtered 6 days after feeding with seed indicates that the passing of seed may continue over a still longer period in diminishing quantities.

In the present studies measurements were made of the quantity of ripe seed present in a pasture and the quantity of viable seeds passed by the animals grazing the pasture. It was estimated that hard seed available in the feed amounted to 98lb. per acre and that under a rate of stocking where the feed was grazed down in one day the amount of viable seed distributed through the faeces of the sheep was approximately 4.5lb., comprising about 4,000,000 seeds.

There is a great difference between animals in regard to the quantity of seed in the faeces.

The present work indicates that under New Zealand conditions it is practicable to graze areas of white clover which are in the ripe head stage with large flocks of sheep and the stock could then be turned out on to clover-deficient hill country. It is possible that two or more grazings could be made during a flowering season. The method outlined could be adopted where ploughable country is farmed in conjunction with hill country and where climate, topography, and lack of machinery make the harvesting of white clover seed impracticable.

Though the proportion of clover seed disseminated in- this way 'is not great, the seed which is destroyed cannot be regarded as entirely wasted because of the high food value of the seed which is digested.

The ability of stock to disseminate seed on hill country could also be exploited by feeding clover hay with 'a high seed content. This practice is more promising than the feeding of commercial seed in chaff, bran, or other such media. It has been shown that it is mainly the hard clover seeds which survive the passage through the animal. Most of the mature seed in clover hay is in this condition but on the other hand

in all commercial samples most of the seed is rendered permeable to moisture by scarification in the process of harvesting- and cleaning and hence readily digested by the animal.

The natural impermeable condition of clover seed is of significance not only in regard to survival in the passage through the animal, but also because of its intermittent germination spread over a long period. In these experiments where dung containing hard seeds was sown, clover seedlings continued to appear throughout the autumn, winter, and spring. Where seed is sown on an established pasture, conditions are liable to be much less favourable for the survival of seedlings than on a seed-bed prepared by cultivation. 'Under these conditions it may be advantageous to have the seed germinate over a long period rather than all together on the first occasion when temperature and moisture conditions are favourable.

Striking examples of the effectiveness of delayed germination in ensuring survival of clover have been noted. on hill country in old stands of subterranean clover. Successive lots of seedlings emerge during summer and autumn only to be destroyed by subsequent periods of drought. Seedlings emerging later, when there is a continuity of moisture supply, persist and ensure a satisfactory stand.

Further work is being carried out to determine the possibility of harvesting clover with a high proportion of hard seed and using this class of seed for oversowing hill pasture.