STUDIES OF DORMANCY IN THE SEEDS OF SUBTERRANEAN CLOVER (*TRIFOLIUM SUBTERRANEUM* L.)

I. BREAKING OF DORMANCY BY CARBON DIOXIDE AND BY ACTIVATED CARBON

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Summary

Two new methods for breaking the dormancy of subterranean clover (*Trifolium subterraneum* L.) seeds are described, viz. treatment of imbibed seeds with a low concentration of carbon dioxide, and with activated carbons.

Responses to these treatments are found widely throughout the species.

Between approximately 0·5 and 5 per cent. carbon dioxide no differences in response occur.

The activated carbons do not appear to adsorb inhibitors, as is their usual action, and the evidence is consistent with the supposition that they produce carbon dioxide which then initiates germination.

The relevance of these findings to some aspects of seed testing and field practice is indicated.

I. INTRODUCTION

It is well known that, in common with those of many other leguminous species, the seeds of subterranean clover (*Trifolium subterraneum* L.) may either be hard, or possess a post-harvest dormancy of shorter or longer duration, or both. The delay in germination occasioned by either of these features has considerable agronomic significance, and information on the nature and origin of these conditions, as well as on mechanisms responsible for release from them should be of value.

Aitken (1938) established the morphological basis of hardness of seed, as well as the influence of conditions during seed maturation on the development of this character, and Loftus Hills (1942, 1944*a*, 1944*b*, 1944*c*, 1944*d*) has contributed most to knowledge of dormancy in this species. He showed that dormancy was a varietal character, and listed the relative dormancy for most of the strains then current, as well as the times necessary for passage out of dormancy. Delayed harvest was found to reduce the proportion of dormant seed, and an earlier finding of Woodforde (1935), that exposure of imbibed seed to low temperature accelerates release from dormancy, was confirmed. Loftus Hills also noted that removal of the testa had a similar effect.

The present work is concerned solely with dormancy and in this paper two further, and highly effective, methods of breaking dormancy will be described.

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II. Methods

Seed was obtained from spaced plants field-grown at Canberra. At commercial ripeness, tops and burrs were harvested, dried at a temperature not exceeding 30°C, and threshed on a light, machine-operated rubber thresher. Seed was stored in the laboratory in closed (but not sealed) containers which were frequently opened during the course of the investigation.

In order to avoid the complication of any residual hard-seededness, all seeds were subjected either to percussion or to light scarification at the time of test. Such seeds were immersed in a shallow layer of boiled mains water for 1–2 hr, and during the subsequent 1–4 hr only swollen or swelling seeds were taken into germination trials.

Standard or control conditions were provided by setting out replicates of 50 or 25 (rarely) seeds in 10-cm diameter petri dishes on two 9-cm circles of filter paper moistened with 4 ml of boiled mains water.

Gas treatments were applied in two ways. In the first, replicates of 30–50 seeds were placed on moistened filter paper in tubes of approximately 45 ml capacity, through which gases of the required composition were passed at the rate of 10–20 ml per minute. The gas mixtures were obtained from an apparatus of the type described by Bailey (1954). When carbon dioxide content was varied, oxygen was maintained constant at 21 per cent.,* the deficit being supplied by nitrogen. When only oxygen content was varied, nitrogen was appropriately adjusted. In the second method, replicates of 50 or 25 (usually) seeds were placed on moistened filter paper in flat-sided bottles of approximately 160 ml capacity. Each bottle was perfused, sufficiently to ensure 5–10 exchanges, with a gas mixture of the required composition, and then sealed by a washer and screw cap. Alternatively, an amount of gas necessary to give the required concentration was displaced into a bottle which was then sealed. When carbon dioxide was so added concomitant decreases in oxygen and nitrogen concentrations ensued. It is appreciated that respiratory and other processes would cause changes in the concentrations thus established; but it will later be seen that these are unimportant in relation to the magnitude of the effects produced by the initial concentrations of the gases.

For tests involving the use of adsorbents, replicates of 25 seeds were placed in 10-cm diameter petri dishes in the usual way, and each seed was covered with a small, standard scoopful of the particular adsorbent. The weight of adsorbent applied per seed varied with the nature of the adsorbent—for the activated carbons (the most important class investigated) it was of the order of 0·02 g.

Unless otherwise stated all containers were held in the dark at 22 ± 1°C and examined in either diffuse daylight or weak fluorescent light at approximately 24-hr intervals, for at least so long as germinations were frequent. Seeds whose radicles showed positive geotropism were counted as germinated and removed, except from sealed containers. For the latter, daily germination counts accurate to ± 1 were made through the walls, and precise counts were made at the termination of the experiment.

*The composition of gas mixtures is stated throughout as per cent. by volume.

For many of the results presented the differences between treatments are readily apparent, and only estimates of variability between replicates are given. Where it was desirable to carry out analyses of variance it was necessary to transform the percentage values. The angular transformation was employed.

III. Results

(*a*) *The Time Course of Germination in Dormant Samples*

Most of the results to be presented have been obtained with the highly dormant strain Burnerang, and with Mt. Barker which shows moderate dormancy. As a background to these results it is desirable to present information on the germination patterns found in such dormant varieties.

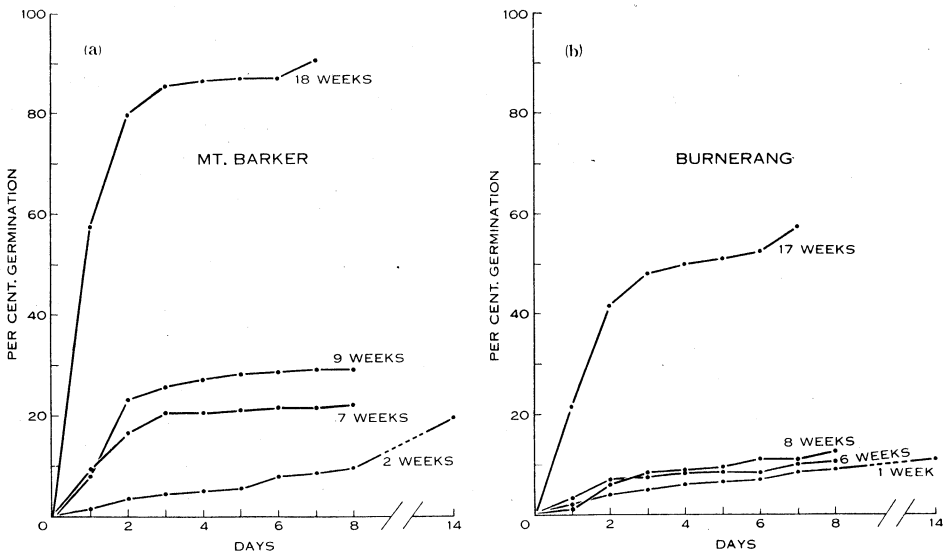

Fig. 1.—The time course of germination. (*a*) Mt. Barker strain, 2, 7, 9, and 18 weeks after harvest; (*b*) Burnerang strain, 1, 6, 8, and 17 weeks after harvest.

Both the daily germination during any one test, and the general level of germination at different points in storage life show interesting changes. From Figure 1 it may be seen that germination in both strains is low initially and increases with increasing rapidity. The initial rate of increase is greater in Mt. Barker than Burnerang. For the later tests it also seems that, under the conditions employed here, asymptotic germination values are reached in 7–8 days or less; but longer periods are required in the earliest test.

It seemed probable that, in any seed sample, rate of germination and absolute germinability should be positively correlated, and this supposition has been further examined.

Estimates of rate have been obtained by calculating the rate index according to Bartlett (1937). This is a somewhat arbitrary measure which takes into account the numbers of seeds which have germinated at successive observations, in relation

to the final number germinated. In effect, it measures the rate of germination of those seeds which actually germinate. Another estimate of rate has been obtained by finding the time required for a sample to reach 50 per cent. of its asymptotic germination. These values were derived, by interpolation, from the time-course curves of individual replicates. Where a true asymptote was not reached the final value recorded was used for this purpose. Both methods of estimating rate are relatively insensitive, especially when germination is low, and the consequent variability in small samples is high.

These data are collected in Table 1, and trends in the expected direction are present on both rate bases. For Mt. Barker, the difference between the rate index initially and the rate index for the three later storage points taken together is significant ($P<0{\cdot}05$); but the smaller difference is not significant for Burnerang.

TABLE 1

AMOUNT AND RATE OF GERMINATION AT INTERVALS FROM HARVEST

Germination and rate index at day 7 of test

Strain	Time from Harvest (weeks)	Germination (%)	Rate Index	Time to 50% of Final or Asymptotic Value (days)
Mt. Barker	2	8·5±1·0	0·629±0·105	8·1±1·39
	7	21·5±2·4	0·876±0·038	1·3±0·27
	9	29·0±4·7	0·829±0·004	1·4±0·06
	18	90·5±1·7	0·905±0·025	0·8±0
Burnerang	1	8·5±2·6	0·706±0·072	3·0±0·79
	6	10·0±2·4	0·777±0·041	2·0±0·52
	8	11·5±3·0	0·622±0·118	3·0±0·86
	17	57·5±2·6	0·802±0·037	1·3±0·15

It is clear that, in this instance, any relation between rate index and per cent. germination would be markedly curvilinear, with the curve concave to the germination axis.

Another set of data is presented in Figure 2. Here, at one storage point, germination was raised to different levels by different dormancy-breaking treatments, and the correlation between treatment per cent. germination and treatment rate index is 0·959 ($P<0{\cdot}05$). In this case the relationship is linear over the range of values obtained.

It should be recorded that, in the several sets of data available for analysis, significance was not invariably achieved in the apparent trend, and the causes of such variation are not known. A relationship appears to be better established when comparisons are made within one strain for one trial, rather than between strains and trials. Moreover, the precise form of such a relationship is variable, and it is likely that this is a reflection of differences in post-harvest maturation in relation to the exact actions of the dormancy-relieving agents.

Further work would be necessary to clarify these features, and for the present it is warranted to conclude only that the occurrence of a higher rate index may be taken as supporting evidence of a reduction in dormancy status.

(*b*) *Carbon Dioxide and Germination*

(i) *The Effect.*—Results of preliminary experiments suggested that an increased partial pressure of carbon dioxide facilitated the germination of dormant seeds, and this was substantiated in direct tests.

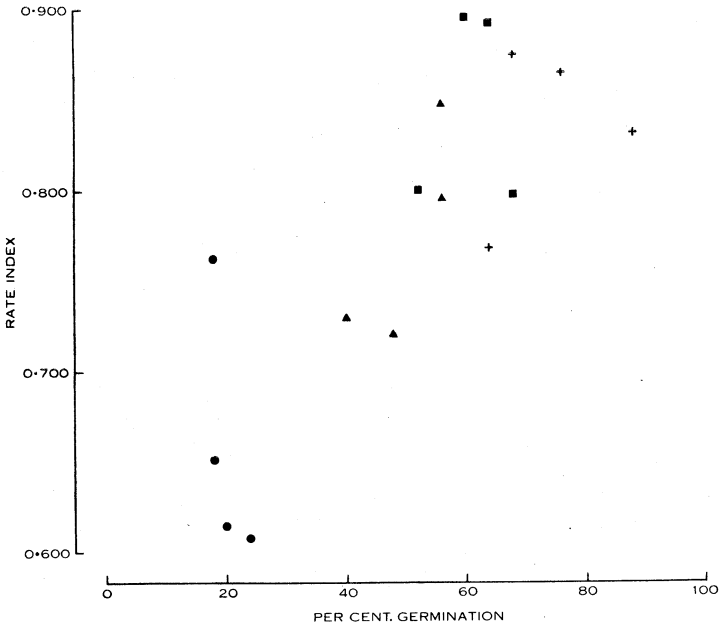

Fig. 2.—Relation between per cent. germination and rate index, Mt. Barker strain, following control treatment (●) and treatment with three different activated carbons (▲, ■, +).

In these experiments, in order to avoid any ambiguity which may have been introduced if volatile metabolites (in addition to respiratory carbon dioxide) were produced by the seeds, tests were conducted in gas streams of the required composition.

The results of one typical experiment are presented in Table 2. In this experiment, seeds were first exposed to standard germination conditions, and those which germinated were removed daily for 8 days, at which time germination had virtually ceased. The remaining, i.e. most dormant, seeds were randomized between control and carbon dioxide treatments. The removal of the readily germinable seeds accentuated treatment effects. Practically no further germination occurred when the seeds were exposed to air, either still (dishes) or moving (tubes); but nearly all seeds exposed to carbon dioxide promptly germinated.

Essentially similar results were obtained in four other experiments, in which the seeds were exposed, with or without the pre-incubation period, to air streams enriched to contain from 0·3–4·5 per cent. carbon dioxide. In one experiment the carbon dioxide was derived from A.R. reagents.

This simple type of experiment provides unequivocal evidence that carbon dioxide is an agent which initiates germination.

The marked response to carbon dioxide in so low a concentration as 0·3 per cent. suggested the possibility that the respiratory carbon dioxide evolved by dormant seeds, if allowed to accumulate in sealed vessels, could itself initiate germination. This proved to be so (e.g. per cent. germinations for Mt. Barker on day 2 of test: open vessels, 46 $\pm$8; sealed vessels, 96$\pm$2). This effect is usually enhanced

TABLE 2

EFFECT OF CARBON DIOXIDE ON THE GERMINATION OF DORMANT SEEDS

Values are cumulative per cent. germinations. Mt. Barker strain. Preliminary incubation period 8 days, during which 21 per cent. germinated. Carbon dioxide concentration 0·3–0·4 per cent.

Day	Tube 1		Tube 2		Tube 3		Tube 4		Control Dishes	
	Gas	Germination (%)	Gas	Germination (%)	Gas	Germination (%)	Gas	Germination (%)	1	2
1	Air	0	Air	0	CO_2	92	CO_2	86	0	0
2	Air	0	Air	0	CO_2	92	CO_2	91	0	0
3	Air	0	Air	0	CO_2	96	CO_2	100	0	0
4	Air	0	Air	0	CO_2	96	CO_2	100	4	0
5	Air	4	CO_2	71					4	0
6	Air	4	CO_2	81					4	0
7	Air	4	CO_2	86					4	0

by the presence in the germination sample of one or more non-dormant seeds. Such seeds germinate promptly, and the carbon dioxide output of the seedling rises sharply, with the consequent rapid initiation of germination of the remainder. However, even when the non-dormant seeds are excluded by pre-incubation, as described above, a similar result is obtained after a somewhat longer time lag.

(ii) *Quantitative Aspects.*—This autocatalytic action of respiratory carbon dioxide indicates that investigation of quantitative aspects should be made in gas streams. However, as appropriate facilities were not available, approximate data were obtained by exposing seeds in sealed bottles to atmospheres of known initial carbon dioxide content.

Some estimate of the drift from these initial concentrations may be made. If the respiratory output of carbon dioxide is taken to be 1 μl/hr/seed (a value in fair agreement with that calculable from the data of Black (1955) on dry weight losses during germination of subterranean clover, and also with that reported by Stiles

and Leach (1932) for the respiration of *Lathyrus odoratus* L. early in germination), the initial per cent. compositions in the present experiments would rise daily by 0·4 units.

TABLE 3

RELATION BETWEEN GERMINATION AND INITIAL CONCENTRATION OF CARBON DIOXIDE

Initial CO_2 Concn. (%)	Cumulative Germination (%)		
	Day 1	Day 3	Day 6
Atmospheric*	0±0	4±4	5±5
2·5	8±4	94±2	98±2
5	4±4	86±10	94±2
10	2±2	74±2	84±4

*Not sealed.

From Table 3 it is seen that the main treatment effects are apparent before gross changes in carbon dioxide concentration would have occurred. Only when the initial concentration exceeds 5 per cent. do inhibitory effects occur, and between this value and 0·3 per cent. (the lowest concentration investigated by the gas stream method) a broad optimum exists. The standard carbon dioxide concentration adopted for later experiments was exposure to an atmosphere initially containing 2·5 per cent. carbon dioxide.

TABLE 4

EFFECT ON VIABILITY AND DORMANCY OF EXPOSURE TO 100 PER CENT. CARBON DIOXIDE

Duration of Exposure (days)	Germination during Exposure (%)	After Transfer to Air		
		Immediately Germinable (%)	Dormant (%)	Not Viable (%)
1	0	67	26	7
4	0	73	3	24
8	0	31*	0	69

*Many abnormals.

As would be expected, very high concentrations of carbon dioxide inhibit germination. However, Table 4 shows that a surprisingly high percentage of seeds retain viability for as long as 4 days' exposure to 100 per cent. carbon dioxide. There is evidence that shorter exposures induce some secondary dormancy.

A further finding is that reduction of oxygen tension does not interfere with the stimulation of germination induced by carbon dioxide in the optimal range,

until the partial pressure of oxygen falls to approximately half that of the normal atmosphere.

(c) *Activated Carbon and Germination*

(i) *The Effect*

Treatment of dormant seed with activated carbons also brings about rapid and high germination.

Of approximately 20 carbon samples tested, all have given some response (with the possible exception of No. 2 of Table 5). The numbered carbons of Table 5 were prepared from a uniform, chemically pure sample by heat activation at different

TABLE 5

EFFECT OF ACTIVATED CARBONS ON AMOUNT AND RATE OF GERMINATION

Germination and rate index at day 7 of test. Mt. Barker strain

Activated Carbon No.	Germination (%)	Means of Transformed Per Cent. Values	Rate Index
Nil	46·7	43·1	0·480
"Norit FNX"	93·3	77·8	0·855
1	84·0	67·6	0·643
2	42·7	40·4	0·678
3	73·3	59·6	0·704
4	89·3	71·0	0·729
5	86·7	69·3	0·704
6	93·3	75·2	0·808
7	88·0	69·9	0·755
8	93·3	75·2	0·701
9	86·7	68·9	0·667
10	82·7	67·1	0·772
Least significant difference ($P<0·05$)		14·0	0·075
,, ,, ,, ($P<0·01$)		19·0	0·094

temperatures under nitrogen, to give a series with characteristics ranging from acidic (No. 1) to alkaline (No. 10). Taken as a whole, the carbons markedly increased both the amount of final germination (although at the time of test the control germination was substantial), and also the rate of germination. In this case differences between the various carbons scarcely achieved significance. However, significant differences have been observed between other samples of carbons, tested on other occasions, e.g. those of Figure 2. It is not known whether these two types of response represent real differences between the carbons themselves, or are caused by differences in the dormancy status of the seeds.

An indication of the rapidity with which the carbons initiate germination is the shortness of time they require to be in contact with seeds. The data of Table 6 were obtained in an experiment in which seeds were first covered with carbon in the usual manner, and at intervals replicates were washed free of carbon, and thereafter

treated as the controls. A significant increase in germination results from as little as 4 hours' contact.

(ii) *Mechanism*

While the wide applicability and high efficiency of this treatment in overcoming dormancy is securely founded, the mechanism by which the result is achieved is less surely known. The most probable actions of carbons are firstly, removal of inhibitor(s) either by adsorption or destruction, and secondly, production of carbon dioxide. The evidence for such actions may now be examined.

TABLE 6

EFFECT OF DURATION OF CONTACT OF ACTIVATED CARBON WITH SEEDS ON DORMANCY BREAKING

Values are mean cumulative per cent. germinations. Activated carbon, "Norit FNX". Mt. Barker strain

Contact Time (hr)	Day 3		Day 6	
	Germination (%)	Means of Transformed Per Cent. Values	Germination (%)	Means of Transformed Per Cent. Values
0	12	19·1	20	26·1
1	16	23·4	24	29·3
2	16	23·4	16	23·4
4	40	39·3	50	45·0
6	52	46·2	58	49·8
Permanent	90	71·7	98	84·3
Least significant difference ($P<0·05$)		16·4		15·0
,, ,, ,, ($P<0·01$)		24·9		22·7

(1) *Inhibitor Adsorption.*—This is usually taken to be the action of activated carbons when they are effective in promoting seed germination. If this were so in the present case, it might reasonably be expected that other adsorbents could be found with at least qualitatively, even if not quantitatively, similar actions. However, no such adsorbent has been found among those tested.

The results of such trials are not presented in detail. Some adsorbents corresponding to Figure 3, curve 1, had a deleterious effect on germination (e.g. magnesium trisilicate, Fuller's earth "Fulmont", Fuller's earth "Tonsil AC"); others, Figure 3, curve 2, were relatively indifferent (e.g. bentonite, kieselguhr, aluminium hydroxide). Another group, Figure 3, curve 3, improved germination, some to a marked extent (e.g calcium carbonate, "Cellite", cellulose powder, activated alumina). However, it will be noted that this action arose after a marked lag phase, in sharp distinction to the action of activated carbons and carbon dioxide. It is suggested that the adsorbents of this group are merely more effective in retarding the diffusion of respiratory carbon dioxide away from the seeds, the germinations of which are thus initiated in a manner akin to sealing in a germination vessel.

(2) *Destruction of Inhibitors.*—Garten and Weiss (1955) have pointed out that some activated carbons produce peroxides, and it may be that these are then able destructively to oxidize inhibitors. Table 7 shows that there is, in fact, a promotive effect by hydrogen peroxide. However, this effect is seen only at very high concentrations which could scarcely correspond to the equivalent concentrations found in carbons. At such concentrations there is no effect. The high concentrations were observed to loosen the testas, and it seems that the hydrogen peroxide has acted chemically only to produce the result of mechanical removal or loosening observed by Loftus Hills (1944*a*).

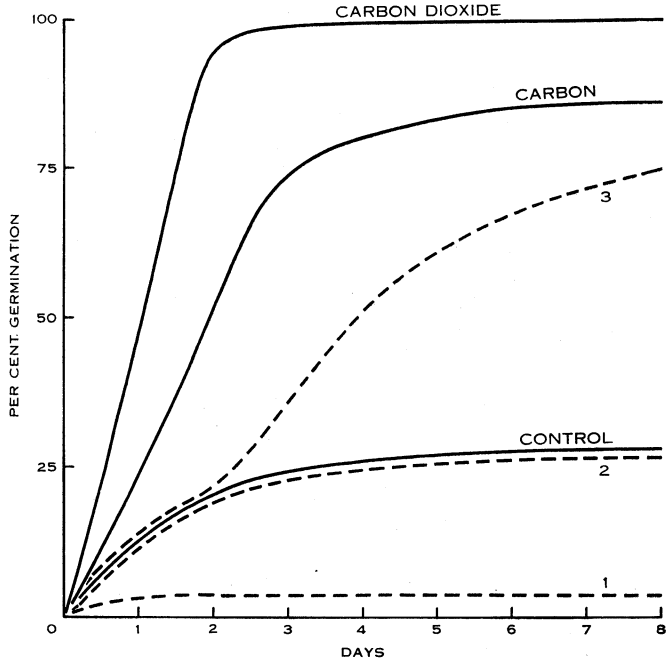

Fig. 3.—Schematic representation of the time course of germination of dormant seeds treated with carbon dioxide, activated carbon, and other classes of adsorbents (curves 1, 2, and 3).

(3) *Production of Carbon Dioxide.*—Only inferential evidence is available to support this hypothesis—that only carbons produce the effect is indeed suggestive.

However, activated carbon, previously wetted and allowed to equilibrate, is no less effective in promoting germination than carbon applied dry, and hence becoming wetted around the seeds (e.g. per cent. germinations for Mt. Barker on day 3 of test: control, 44 ± 8; dry carbon, 76 ± 4; wetted carbon, 72 ± 12). This means that any carbon dioxide which could be evolved by the activated carbon does not arise by displacement of previously adsorbed carbon dioxide, and presumably must arise by direct oxidation. Such a reaction can occur, though at the relatively low temperatures of physiological experiments the rate would be low (see Jones and Townend (1946) for a discussion on the formation of a carbon–water–

oxygen complex, and the conditions determining its breakdown to carbon monoxide and carbon dioxide in differing proportions).

TABLE 7

EFFECT OF HYDROGEN PEROXIDE ON GERMINATION

Values are cumulative per cent. germinations. Mt. Barker strain

Concentration (M)	Day 2	Day 6
0	6±6	16±4
3	0±0	0±0*
6×10^{-1}	62±6	92±0
$1{\cdot}2 \times 10^{-1}$	18±2	52±16
$2{\cdot}4 \times 10^{-2}$	2±2	20±4
$4{\cdot}8 \times 10^{-3}$	0±0	10±2
$9{\cdot}6 \times 10^{-4}$	12±8	28±8

*Seeds killed.

It was thought that information might be obtained by incubating seeds in the presence of alkali, which might be expected to absorb carbon dioxide, respiratory and

TABLE 8

GERMINATION RESPONSES OF STRAINS TO CARBON DIOXIDE, ACTIVATED CARBON, OR LOW TEMPERATURE

Values are mean per cent. germinations. Initial carbon dioxide concentration, 2·5 per cent.; activated carbon, "Norit A"; low temperature treatment, 1 day at 10°C

Strain	Day 3				Day 6		
	Control	CO_2	Carbon	Cold	Control	Carbon	Cold
Portugal, C.P.I. 19465*	12	93	63	29	14	75	30
Algiers, C.P.I. 19455	4	98	69	40	26	73	66
Cyprus, C.P.I. 19448	20	100	54	53	32	76	67
Turkey, C.P.I. 15077A	30	96	71	75	38	75	84
Burnerang	35	100	90	77	38	96	79
Canary Is.	34	94	77	75	46	81	80
Northam First Early	42	99	55	48	58	65	78
Morocco, C.P.I. 19458	53	100	90	98	65	94	98
Greece, C.P.I. 19479B	65	100	51	84	70	69	89
Daliak	58	100	65	90	72	74	93
Portugal, C.P.I. 19472	78	100	89	95	80	94	96

*Commonwealth Plant Introduction number.

otherwise, and so nullify or reduce the effects of sealing and application of carbon. Experiments to investigate this were conducted in petri dishes fitted, after the

fashion of Conway vessels, with central wells, which contained either alkali or water. Seeds were arranged in the annular space and could be covered with carbon. The dishes were either merely covered (open), or provided with sealed cover glasses. In some experiments all possible treatment combinations were employed.

The data are too voluminous for presentation; but the following more important results can be stated: (1) Sealing always accelerated germination. (2) Application of carbon always accelerated germination; but the pattern of response varied in different treatment combinations. (3) Alkali always decreased the rate of germination in sealed systems in the absence of carbon. (4) Alkali decreased the rate of germination in sealed systems in the presence of carbon in one out of four trials.

(*d*) *Varietal Response*

The extent to which the above findings apply throughout the species is indicated by the data of Table 8 for a number of standard strains, together with introductions from the Mediterranean region. The trial was conducted approximately 4 months after harvest, by which time a natural spread of dormancy already existed. The strains are listed in Table 8 in decreasing order of dormancy as judged by the final control germination.

In all cases treatment with carbon dioxide brought about prompt and practically complete germination. Treatment with activated carbon or low temperature also increased both the speed and amount of germination; but not so markedly as did carbon dioxide. Of these latter two treatments there appeared a tendency for carbon to be the more effective on the more dormant samples, and cold on the less dormant.

IV. Discussion

It seems warranted to conclude that the germination responses described above, to both carbon dioxide and activated carbon, occur throughout the species.

The suggestion that activated carbons produce their effects by production of carbon dioxide can scarcely be regarded as proven, and it is clear that direct proof would be difficult to obtain. However, the case for this hypothesis is relatively strengthened by the following considerations which militate against the possibility of inhibitor adsorption: (1) There is little direct evidence for the existence of an inhibitor. Such an inhibitor would almost certainly be water soluble; but in this work leaching with water for periods up to 6 days did not produce clear-cut effects on germination which could be referred unambiguously to the leaching itself. It is most probable that any possible inhibitor is not located in the testa (where such inhibitors are usually, though not invariably, to be found), for it has been observed that, on occasions when the usually efficacious dormancy-breaking treatment of testa removal failed, the embryos could be promptly germinated by covering with activated carbon. (2) The rapidity of action of the carbons renders inhibitor adsorption less likely, since it must now be assumed that any inhibitor would have to diffuse from the embryo through the testa. (3) Considerable variation in the response to carbon was observed in the experiments involving sealing of vessels and placement of alkali. Whilst these inconsistencies cannot be fully interpreted, they

probably reflect such complexities in the system as the formation and breakdown of carbon complexes, self sorption by the carbon of carbon dioxide, and diffusion-limited absorption of carbon dioxide by the alkali. Had only the adsorption of an inhibitor been involved, a uniform response to carbon would be expected, and this was not found.

This appears to be the first occasion for which such an explanation has been suggested for the action of activated carbons, an interpretation which focuses attention on the action of carbon dioxide as the primary phenomenon. Carbon dioxide is usually held to induce secondary dormancy (Thornton 1953), and stimulation of germination by it has only rarely been recorded (Anderson 1933; Thornton 1935, 1936). However, in these cases, much higher concentrations of carbon dioxide

TABLE 9

EFFECT OF AGGREGATION OF SEED ON GERMINATION

Values are per cent. germinations at day 4 of test. Mt. Barker strain

No. Seeds per Pile	Seeds Only	Seeds + "Primer"
1	1·4	14·4
5	50·0	74·7
15	96·0*	96·2*

*No significant difference. All other possible comparisons significant at $P < 0{\cdot}001$.

were involved—for both *Xanthium* and lettuce the lowest effective concentration reported by Thornton was 5 per cent., and the effectiveness increased under certain conditions as the concentration rose to 80 per cent. This contrasts sharply with the very low concentration effective on subterranean clover, and it seems that quite distinct phenomena are involved. Discussion on possible mechanisms involved in the action of carbon dioxide will be deferred to a later paper where data on temperature relationships will be presented.

This extreme sensitivity of subterranean clover seeds to traces of carbon dioxide suggests that it may be made the basis of a dormancy-breaking treatment valuable in routine seed testing. In any event, it is clear that considerable variation in germination must result from hitherto unsuspected variables, such as the use of open germination trays compared with petri dishes, the number of seeds in relation to the capacity of germination container, the frequency of inspection, the intervals at which germinated seeds are removed, and the precise composition of the atmosphere in the incubators. The data of Table 9 are presented as an extreme case. Individual seeds or aggregations of five or 15 seeds were placed in small petri dishes of approximate capacity 7 ml, one seed or aggregate per dish. In a similar set the germination was "primed" by placing a seed previously germinated to have a radicle approximately 2 mm long beside the single seed, or at the centre of the aggregation.

The treatments were extensively replicated. The quite dramatic variation in germination recorded fully supports the above conclusion.

Lime-pelleted subterranean clover seeds germinate more rapidly and produce larger seedlings than non-treated seeds (Myers, unpublished data), and this appears to be another example of differences in germination behaviour interpretable on the basis of data presented in Section III.

One further implication merits mention. Some mechanism to ensure delayed and irregular germination appears to be common in the seeds of wild annual plants. This character has largely been lost in species of agronomic value in the course of their domestication, as a result of either unconscious or deliberate selection. Delayed germination in subterranean clover may be desirable or undesirable according to the location in which it is grown, and the management adopted, and attention may be directed to this character in breeding programmes.

The data collected by Russell (1950) show clearly that the soil atmosphere, especially under pasture, contains carbon dioxide to at least the minimal concentration required to initiate germination in imbibed, dormant subterranean clover seeds, and usually beyond this into the optimal range. It seems, therefore, that, under field conditions, once subterranean clover seeds soften, they will imbibe water at the next opportunity, and then germinate promptly. (Under certain conditions the most highly dormant varieties only may not show this pattern, nor need it be seen if soil temperatures are too high—provisos to be amplified in a later paper.) Preliminary tests have shown that imbibed seeds, planted in potting soil, germinate and establish fully at times when laboratory germination trials indicate considerable residual dormancy. If this conclusion is correct, it would appear more profitable to direct attention to the character of hard-seededness, rather than dormancy, in any attempts to modify varietal characteristics of delayed germination.

V. Acknowledgments

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VI. References

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