Japan in 1684 probably from China, where until to-day no true *M. japonica* has been collected, but an allied plant occurs, *M. Bealei*, which has been taken for the other one in this country. The beautiful garden Chrysanthemum, which is the national flower of Japan, was certainly introduced from China, yet the wild form, the original of the garden variety, grows in the south-western parts of Japan. Nowadays a great number of European and American plants are introduced into Japanese gardens, while quantities of Japanese plants are exported to foreign countries and especially to England, where it always gives me the intensest joy to see the plants of my native land.

# THE ECOLOGY OF *CALLUNA VULGARIS.*

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[Plate III and Two Text-Figures].

THE Ling (*Calluna vulgaris*) is usually recorded as forming definite heath communities, in competition with other plants, only on poor and often acid soils, such as those which occur on Bagshot sands, or less typically on the washed-out soils sometimes found overlying limestone and chalk.

A somewhat anomalous distribution of *Calluna* on parts of the chalk downs of Berkshire and Wiltshire has been described in a previous communication.[1]

In that paper it was recorded that sporadic communities of this plant occur on the higher parts of the downs, forming patches of typical heath vegetation quite different in character from the so-called "chalk heath" of the South Downs and elsewhere.

The distribution of these communities is roughly determined by the overlying deposits of clay-with-flints, which give rise in this neighbourhood to fertile loamy soils, with a low percentage of calcium carbonate. The soil in question has the appearance of a

[1] Rayner, M. C., and Jones, W. N. "Preliminary Observations on the Ecology of *Calluna vulgaris* on the Wiltshire and Berkshire Downs." New Phytologist, Vol. X, 1911, p. 227.

fertile loam, and the conditions generally give the impression that the heath flora has become established on the more fertile soils of the area, in competition with the surrounding vegetation.

Mechanical and chemical analyses support this view and indicate that the soil monopolized by *Calluna* is in every respect a good fertile loam with a high percentage of available mineral matter (*loc. cit.*, pp. 236, 237).

A low percentage of calcium carbonate excepted, there is no evident factor to account for the successful competition of the heather; the soil reaction is neutral, and the only feature of note chemically is the rather high ratio of magnesia to lime.

With regard to the significance of this ratio as it affects plant growth, there is at present a conflict of opinion and the evidence adduced by different observers is somewhat contradictory.

Two distinct problems were suggested as a result of these observations :—

(i.) The nature of the soil conditions which allows *Calluna* to compete successfully with other plants on small areas of fertile soil.

(ii.) The significance of calcium carbonate as a factor determining the spread of *Calluna* from those sharply defined areas to the surrounding "down" soil.

These problems do not appear to be capable of explanation in terms of current hypotheses, nor do these hypotheses throw clear light on the causes which determine the calcicole and calcifuge habit exhibited in varying degrees by so many plants.

The present investigation was undertaken primarily in order to study the biological significance of the sharp delimitation of *Calluna* under the conditions described, and secondarily, in order to ascertain whether the facts obtained in the course of the investigation might throw light on the larger question of the ecology of calcicole and calcifuge plants in general.

(*a*). Experimental work was begun with two objectives in view. Sand and water cultures were grown in the hope of obtaining evidence as to the effect of alteration in the proportions of lime and magnesia in the culture solutions.

Water culture experiments carried on continuously for twelve months pointed to the conclusion that further work in this direction was premature at this stage of the enquiry and unlikely to lead to definite results. They were therefore discontinued: the results obtained are briefly summarized at the end of this paper.

(*b*). Pot cultures were started using seed and fresh soils from the field, with a view to detailed observations on the behaviour of seeds and seedings under experimental soil conditions. Full analyses of the soils are available and have been recorded in a previous paper (*loc. cit.*). In these analyses the soils are referred to as "heather area" and "soil over chalk" respectively. For the sake of brevity they will be known as "heather" soil and "chalk" soil in the present paper. The former is apparently a good fertile loam although the percentage of calcium carbonate is low: the latter is a typical chalk-down soil containing 41·8% calcium carbonate.

It is convenient to deal with the results of these cultures under two heads:—(A) Germination, (B) Growth.

A. *Germination.* Seed was collected in the capsules during October, dried in the laboratory at air temperature and stored in paper bags. When required it was rubbed down, sieved, and the seed picked out by hand.

The seed is very small, with a rough testa, abundant oily endosperm and a minute straight embryo. Sowings were made in pots of fresh soil, under glass, using approximately the same number of seeds per pot, and records kept of the rate of germination, the number of seedlings, and the growth and condition of the roots and shoots at successive stages.

In the autumn, germination begins 25—30 days after sowing, and is usually rather slow and irregular. In the spring it takes place more uniformly, 16—19 days after sowing.

In "heather" soil, seeds germinate at the same rate and exhibit about the same germination capacity as on blotting paper in a germinator. As compared with this, the rate of germination is greatly retarded and the germination capacity markedly lowered in the "chalk" soil. A majority of the seeds do not germinate. Germination of the remainder is delayed—seedlings first appearing after two or three months and then in very small numbers. Seeds continue to germinate irregularly for six months or longer, but the total germination capacity is extremely low as compared with the controls.

In order to compare the behaviour of *Calluna* seed in this respect with that of other plants, sowings were made, in the two soils, of Foxglove (*Digitalis purpurea*) as a representative calcifuge, and of Kidney Vetch (*Anthyllis vulneraria*) as a calcicole. The latter plant is abundant on the down soil in the field.

In each case germination is simultaneous and the germination capacity similar in the two soils.

The phenomenon of retarded germination in a soil unfavourable to the growth of the plant does not appear, therefore, to be one which occurs generally in the case of calcicole and calcifuge plants.

Heating *Calluna* seed for some hours at a temperature of 67°C. to 70°C. hastens germination, the acceleration so produced being more marked in the autumn.

Seed so treated germinates more evenly and about seven days sooner than unheated seed.

Heating for a longer period, *e.g.*, twenty hours at 70°C. affects the germination capacity adversely, but not the vitality or rate of germination of those seeds which germinate.

No difference can be detected in the behaviour of seeds germinated at once after heating and those kept for several weeks before sowing.

Partial sterilization of the soil, by treatment with vapour of carbon bisulphide and by heat, gives somewhat irregular germination results, which are summarized in Table I. The soil for these experiments was treated as follows:—

Small quantities of soil were spread out in carbon bisulphide vapour for three or four days, then exposed freely to the air for several days, placed in small sterile pots and watered with distilled water, Other samples of soil were subjected to intermittent heating at 92°C. and 98°C. on five successive days, moistening with sterile water between each heating. The seed used for these experiments was in all cases untreated, and control seeds of oat placed in the pots germinated normally.

Reference to the tabulated records shows that the behaviour of the seedlings when the *unfavourable* soil is subjected to partial sterilization is noteworthy and in striking contrast with their reaction to the same soil untreated. The germination capacity remains low as in untreated soil, but the rate of germination is accelerated. The resulting seedlings develop a larger and more normal root system (Plate III, Fig. 1d).

B. *Growth.* Seedlings of *Calluna* are small and slow-growing. The primary root elongates slowly, but soon after germination several adventitious roots grow out in rapid succession from a thickening at the base of the hypocotyl.

The roots are almost transparent and provide beautiful material for observing the details of root structure. Root hairs are absent; the cortex consists of a few cell rows only, and the whole root is extremely slender and delicate.

TABLE I.—GERMINATION OF *CALLUNA* SEED.

| | | | Seedlings First Appear. | Number (Approximate). | Remarks. |
|---|---|---|---|---|---|
| Moist Air. | *Autumn.* | 1 Control (seed untreated) | 28 days | 42% | Germination of untreated seed is very irregular in autumn. |
| | | 2 Ditto... | 26 ,, | | |
| | | 3 (Seed heated 4 hours at 67°C.) | 17 ,, | 50% | Germination more uniform. A majority of the seeds germinated simultaneously. |
| | *Spring.* | 4 Control (seed untreated) | 16 ,, | 2 Seedlings | In the spring acceleration is less marked, but germination takes place more uniformly in the case of heated seed. |
| | | 5 (Seed heated 4 hours at 70°C.) | 16 ,, | 40% | |
| Untreated Soil. | *Late Autumn.* | *Heather Soil* (1) | 23 days | 100 seedlings | Approximately the same number of seeds sown in each case (250-300). In pots 1 and 2 seedlings appeared uniformly in pot, and majority ultimately germinated. In pots 3 and 4, 15-20 seedlings had appeared at the end of 6 months and represented the total germination capacity. |
| | | ,, ,, (2) | 21 ,, | 50 ,, | |
| | | *Chalk Soil* (3) | 68 ,, | 5 ,, | |
| | | ,, ,, (4) | 112 ,, | 3 ,, | |
| Partially Sterilized Soil. | *Heather Soil.* | Control | 53 days | Numerous seedlings | Germination extremely slow, possibly owing to spell of very cold damp weather. |
| | | $CS_2$ Vapour | 94 ,, | 4 seedlings | Result not confirmed. |
| | | Heated | 50 ,, | Numerous | More numerous and rather more vigorous than control. |
| | *Chalk Soil.* | Control | 112 days | 2 seedlings | Total germination capacity not materially affected by either treatment; remains very low. |
| | | $CS_2$ Vapour | 50 ,, | 2 ,, | |
| | | Heated | 55 ,, | 1 ,, | |

From the earliest stages, whether growing in moist air or in soil, the roots are infected with an endotrophic mycorhiza. Branched hyphæ project from the surface and many of the cortical cells contain the characteristic "knots" of mycelium.

The plumule is undeveloped in the resting embryo and growth of the shoot is slow, especially in the early stages.

In the "heather" soil seedlings grow normally; the leaves are bright green and a vigorous much-branched root-system is rapidly formed (Plate III, Fig. 1a).

Partial sterilization of this soil by carbon bisulphide, as described above (p. 62), gives an impetus to vegetative growth and the seedlings, for a time, compare favourably with the controls. Examined microscopically the roots of such seedlings are cleaner and fungal infection is rather less conspicuous (Plate III, Fig. 1b).

In the "chalk" soil, growth is practically inhibited.

The few seedlings which germinate remain in the seed-leaf stage for many months. Sometimes no other leaves are formed: those which do appear are small and chlorotic, and the whole shoot usually becomes bright red in colour. Root growth is almost completely checked; many lateral roots begin to develop, but their tips are strongly recurved and often show brownish discoloration (Plate III, Fig. 1c).

Seedlings may remain in this condition for months, making repeated but ineffectual efforts to form an adequate root system: if transplanted to the other soil, they at once recover and grow normally. Examined microscopically, these abnormal roots are remarkable, not only for the retarded growth and peculiar curvatures which they exhibit, but for the association with them of dense colonies of bacteria. The latter are especially conspicuous about the tips, which are usually invested with dense bacterial sheaths.

Bacteria are also abundant in and about many of the cortical cells and around the apices of young emerging lateral roots (Plate III, Fig. 2b, Fig. 3).

In "chalk" soil sterilized by either of the methods described on page 62 root development is more normal for a time. Bacterial growth appears to be retarded; a more normal root system is developed and reddening of the shoot takes place rather more slowly. This improvement, however, is temporary, and eventually the same symptoms appear as in roots in untreated soil (Plate III, Fig. 1d). In all these abnormal roots, mycelium, though present, is scanty in amount, few hyphæ project from the root and they are often crowded with bacteria.

The effect of watering seedlings growing in "heather" soil with a watery extract of "chalk" soil has been tried, with the following results :—

After four months of regular treatment seedlings in small pots do not differ markedly from the controls. After six to seven months treatment, seven out of eight seedlings so treated begin to show signs of injury. Growth was stunted, the foliage yellowish and discoloured, and the leaves dead on some of the older shoots (Plate III, Fig 4).

Examined microscopically, the roots of these seedlings show in a rather less marked degree the same peculiarities as have been already described for roots growing in "chalk" soil. Root growth is evidently checked, the tips show marked curvatures and are often invested with a dense mantle of bacteria. Mycorhizal growth is poorly developed and the general appearance of the roots quite characteristic.

Seedlings growing in larger pots responded to similar treatment much more slowly. After treatment for the same length of time, the growth and general condition of the shoot was practically unaffected.

Many of the root-tips, however, when examined microscopically, showed characteristic association of the mycelium with bacteria, the relations of the hyphæ with the latter being much more evident in such early stages than they are later when the tip has become invested in a dense sheath of bacterial growth.

Other seedlings growing in "chalk" soil were watered with an extract of "heather" soil prepared similarly to that used in the last experiment. This experiment was begun shortly after germination, before the ill effects of the unfavourable soil conditions had become marked, and was continued for six months. At the end of that period the controls—watered with distilled water—were dead; the seedlings in the three pots under treatment were alive ; the characteristic purpling of the leaves had appeared, but most of the plants had made four to six leaves on the main axis.

The change in the root system was more remarkable and is shown in Plate III, Fig. 5.

As is evident from a comparison of this photograph with that of the seedlings shown in Plate III, Fig. 1c, the root system is many times larger than that of a seedling from untreated soil, During part of the time occupied by the experiment, growth of the roots had apparently been almost normal and resulted in the formation of a more fully developed root system.

Several points of interest are suggested by these observations:

(i). Since the unfavourable and favourable soil conditions respectively can be supplied in a filtered, unboiled soil extract, they are either of a chemical or biological nature.

(ii). If the conditions be biological, and the injurious effect can be produced by supplying an organism present in the unfavourable soil simultaneously with the conditions which favour its growth, all the plants so treated might fairly be expected to behave in the same way.

Unfavourable symptoms appear, however, much more slowly in plants growing in large pots, thereby suggesting that the unfavourable factor is chemical in nature and that as soon as a certain concentration in the soil is reached, an unheathy condition of the roots is induced owing to disturbance of their normal relations with the micro-organisms present,—a view confirmed by the results of the treatment when an extract of "heather" soil is used for watering seedlings growing in "chalk" soil.

Whether the unfavourable effect is a *direct* result of the bacterial colonies which beset the roots, or whether it is *indirect* and due to the effect of these colonies on the micorhizal fungus, remains an open question, as does also the important point as to whether the pathogenic effect of the bacteria is dependent on a change in the chemical condition of the soil, the organism being present in both cases, or whether the latter is peculiar to the soil on chalk.

This experimental work, combined with examination of the roots of large numbers of seedlings grown under different soil conditions, suggests that the relation between the roots of *Calluna* and the micro-flora of the soil in which it is growing is an intimate one; that the balance between these biological factors may be very delicate, and only maintained in equilibrium, *i.e.*, in such relations that the result is either beneficial or indifferent to the plant, under definite soil conditions.

It is only by attacking this larger problem that a clue can be found to the soil conditions which determine growth in any given locality such as the one under consideration, where the soil differs in respect to certain constituents over a small area.

In the case of such plants as *Calluna* and its Ericaceous allies, the relation of the roots to the soil is evidently rendered more complex by the presence of a mycorhizal fungus, of which the full significance to the plant is still in need of demonstration. The details of fungal infection, and the ability of the plant to germinate and grow without its fungal partner have also yet to be described in the case of *Calluna*.

An obligate association of definite races of bacteria with the roots of plants has already been suggested[1], and it would seem not unreasonable to expect that such associations would become much more complex in the case of a plant, the roots of which constantly contain mycorhiza. It is claimed that several species of nitrogen-fixing fungi have been isolated and cultivated from the mycorhiza-bearing roots of various Ericaceous plants, including *Calluna vulgaris*, but absolute demonstration that this was the case could not be made, since the seedlings were never obtained in a sterile condition.[2] Similar relations are suggested far *Vaccinium corymbosum*,[3] and have indeed already been inferred for most plants which form root associations with either fungi or bacteria.

Before the main problem of the relations of the mycorhizal fungus to the plant and of the former to the soil and its bacterial flora can be attacked, it is necessary, therefore, to learn whether the association of the plant with its mycorhizal fungus is an obligate one, and for that purpose to determine the following points:—

(i). At what stage in the life-history of the plant, and from what source, does infection of the roots take place?

(ii). Is it possible (*a*) to germinate, (*b*) to grow *Calluna vulgaris* without infection of the roots under sterile conditions?

Having a knowledge of these facts, it should be possible to infer the existence, or not, of fundamental relations between the plant and special micro-organisms of the soil; to determine if any of the latter are indispensable for its growth, and whether the soil preferences and peculiarities of *Calluna* are directly associated

[1] Gottheil, O. "Botanische Beschreibung einiger Bodenbakterien." Centralbl. f. Bakteriologie, Bd. 7, p. 430.

[2] Ternetz, C. "Ueber die Assimilation des atmosphärischen Stickstoffes durch Pilze." Jahrb. f. wiss. Bot., Bd. 44, 1907, p. 353.

[3] Coville, F. V. "Experiments in Blueberry Culture." U.S. Dept. Agric. Bureau of Plant Industry, Bull. No. 193, 1911.

with them, or are specific to the plant, apart from its symbiotic relations with any other organism.

It has already been mentioned (page 64), that the roots of seedlings when germinated are always infected with mycorhizal fungus.

Microscopic examination of young seedlings, protected against infection from without during germination, shows that emergence of the radicle is accompanied by growth of fungal hyphæ from the testa. Some of these hyphæ grow across to the root and infect the seedling root, most commonly, *via* the cells of the root cap. This mode of infection appears to be very regular and characteristic. Bacteria are also associated with the seed-coat, but these are not conspicuous in seeds germinated under healthy conditions.

Infection of the seed-coat by fungi and bacteria occurs commonly in many seeds, such infection taking place after the seeds have been exposed to the air.[1] In the case of *Calluna* seeds, in addition to this superficial infection, a fungus is present, which is identical morphologically with the mycorhizal fungus present in healthy roots.

So far as could be ascertained from examination of fresh material, endosperm and embryo are free from infection in the resting seed. Microtome sectons of the seed pointed to infection of the testa only, and therefore to the possibility of sterilizing the seeds. The delayed infection observable in heated seeds (page 64) also supported this view, which has now been confirmed as correct, by germinating seedlings in a sterile condition, after adequate sterilization of the seed-coat.

It remains to enquire the source of infection and at what stage of seed development it takes place.

Examination of the ovary of the flower after fertilization reveals the fact that, in common with other parts of the flower the ovary wall is infected with fungal hyphæ.

Infection of the young seed-coat takes place from this source while the seeds are still enclosed in the ovary.

A full account of the details of this infection of the seed-coat, and of the mode of infection of the flower—whether from without at the period of flowering or from within by a more widespread distribution of the mycorhizal fungus in the plant than has been commonly assumed—is reserved for a subsequent paper.

Fungal infection of the testa is difficult to observe in the

[1] Arcichovskij, V. "Ueber die Methoden zur Gewinnung mikroorganismenfreier Samen." Centralb. f. Bakt., Bd. 36, 1912, Nos. 15—18.

resting seed, but in favourable cases the presence of hyphæ and more rarely of isolated spores can be demonstrated. The evidence at present available, points to infection of the coat by spores as relatively rare. This may, to some extent, account for the differential results obtained when attempting to sterilize seed. Effective sterilization is doubtless more difficult in the case of seeds, to which spores adhere so closely that they are not dislodged by washing or centrifuging. Attempts to sterilize the seed by immersion in hot water at 70°C., 60°C., and 52°C. respectively for fifteen minutes led to all the seeds being killed by the treatment. Seeds soaked in ·01% corrosive sublimate for five minutes always gave a large proportion of infected seeds when sown.

The following method was then tried and although sterile seedlings were not obtained, it yielded interesting results in another direction.

Soil extracts of the two soils were prepared, using 25 grams of fresh soil to 300c.c. distilled water; agar, in the proportion of 1·5% was added and the media sterilized in the usual way.

Seed was sterilized by soaking for thirty minutes in ·01% corrosive sublimate, washed repeatedly with sterile water and sown with a sterile pipette in Petri dishes of the above media, which were kept for some days at a temperature of 37°C. At the end of five days the two sets of plates were strikingly different in appearance, this observation applying both to the sterilized cultures and to the controls for which unsterilized seed was used.

The results are summarized in Table II.

TABLE II.—SEED CULTURES IN AGAR SOIL EXTRACTS.
TEMP. 37°C. 5 DAYS.

| | SEED TREATED CORR. SUB. ·01% | CONTROL (SEED UNTREATED). |
|---|---|---|
| (A) "Heather" Soil Extract. | Many seeds apparently sterile. Some with a zone of mycelium. Plates sterile except in the immediate neighbourhood of seeds. | Majority of the seeds surrounded by a zone of mycelium. A few seeds apparently unaffected. |
| (B) "Chalk" Soil Extract. | Almost every seed or group of seeds with a zone of bacterial growth. Mycelium almost or entirely absent. Plates otherwise sterile. | Majority of seeds surrounded by bacterial colonies. Mycelium, if present, always accompanied by vigorous bacterial growth. |

Since a majority of the seeds in each plate were infected, the Petri-dishes were opened and the growths examined microscopically. In the "chalk" soil cultures, well-defined bacterial colonies were present round all seeds but three, in a total of about one hundred seeds.

Hyphæ were absent from the outer limits of many of these colonies. In others mycelium was present but generally showed attenuated growth and the hyphæ were invested with a sheath of bacteria (Plate III, Fig. 6).

Masses of bacteria occurred in close association with the mycelium, which was rarely present in sufficient amount to alter the macroscopic appearance of the colony.

In the "heather" soil cultures a vigorous zone of mycelium was present around every infected seed. Mycelial growth round "sterilized" seeds was apparently pure but had not reached the sporing stage.[1]

Bacterial colonies, closely resembling those in the "chalk" soil cultures were present in many of the growths. These were not sufficiently prominent in any single case to affect the macroscopic appearance, which was always of a mycelial character.

The close association of bacteria with the hyphæ, which was a striking feature in the other plates, was not observable.

At this stage of development the difference in the two sets of plates was very striking:—

The cultures for which an extract of "heather" soil had been used were practically free from bacterial growth; in those for which an extract of the "chalk" soil was used they were the most prominent feature.

It was the striking contrast presented by this set of plates, in conjunction with the appearances noted in the roots of young seedlings growing in soil cultures, that first suggested the possibility of a biological relation between the mycorhizal fungus and soil bacteria.

It is significant that the mycelium grows vigorously in a medium made up with an extract of the one soil and is practically eliminated by bacterial growth in that made with an extract of the other soil, the seeds in both cases having had precisely similar treatment. The appearance of the hyphæ in the latter case strongly suggests that given certain conditions of nutrition, bacteria, constantly

[1] A fungus invariably present in these colonies has since then been identified with the species occurring as mycorhiza in the roots.

associated with the seed, can multiply and become pathogenic to the fungus which is also present on the seed coat. The same inference is suggested by the behaviour of the mycorhizal hyphæ on the roots in pot cultures of the "chalk" soil.

It is recognised, of course, that these pathogenic relations may be secondary in both cases, and determined by inability of the mycelium to thrive under the conditions supplied. In either case they seem to be pertinent to the inquiry. A large proportion of the seeds in these cultures germinated, and no difference was apparent either in rate of germination or in germination capacity. This difference of behaviour as compared with soil cultures may be due to the position of the seeds on the surface of the media, but requires further investigation. The seedlings grew for a month or more, but soon showed symptoms of starvation in all the cultures, due no doubt to the paucity of food material in the soil extracts.

The next attempt to sterilize seed was successful.

Similar extracts were prepared, made up with agar, sterilized, and placed in sterile Petri dishes. Seeds were soaked for thirty minutes in water, precautions being taken that the seed coats should be thoroughly wetted. They were then immersed for short periods (two minutes or less) in a 1% solution of corrosive sublimate, washed as before in many changes of sterile water and sown, fewer seeds being placed in any one dish. Care was taken to make the conditions as aseptic as possible and the cultures were placed under a bell-glass in a small greenhouse, all the surroundings having previously been washed down with a strong solution of Jeyes' fluid in water.

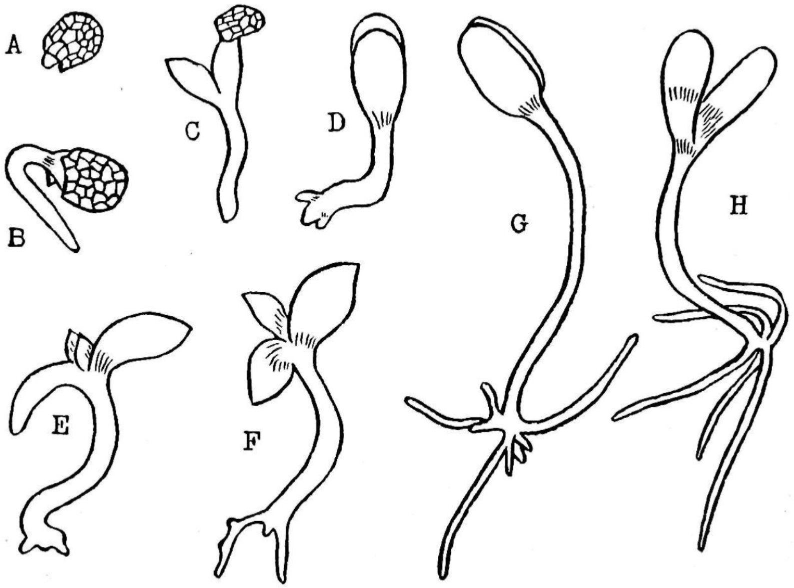
At the end of nineteen days three plates contained each a number of germinating seeds and were absolutely free from micro-organisms.

No trace of fungal or bacterial growth appeared round any of these seeds, about 50% of which germinated at the usual rate.

The early stages of growth of these sterile seedlings are otherwise normal and precisely similar to those of infected seedlings.

The seedlings were transferred singly to special culture tubes fifty-three days after sowing, *i.e.*, about five weeks after germination. Many of them formed several leaves and developed the rudiments of roots while still in the seed dishes (Text-fig. 1, A—F).

The cotyledons and young leaves were green and the seedlings appeared to be perfectly healthy, with the exception that they had made no attempt to send down roots into the culture medium, or to place the axis in a vertical position. For comparison with these sterile seedlings, there are shown, in Text-fig. 1, two seedlings (G, H) germinated in moist air from unsterilized seeds and infected with mycorhiza.



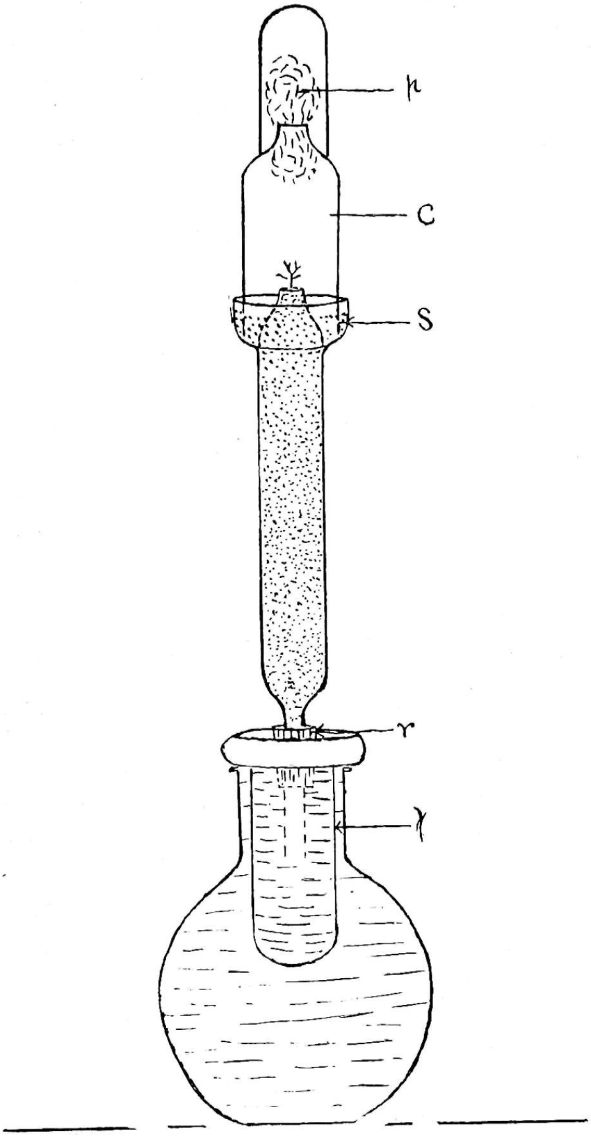

Text-fig. 1. Seedlings of *Calluna vulgaris*. A to F, sterile seedlings germinated under aseptic conditions on agar plates. (A to C, four weeks after sowing; D to F, eight weeks after sowing). G and H, seedlings germinated in moist air, seeds unsterilized, seedlings infected with mycorhiza; eight weeks after sowing. All × 12.

The tube shown in Text-fig. 2 is used for sand cultures; with slight modification similar tubes can be used, without filters, for water or agar cultures. After filling the sterilized tubes with agar media or with fine quartz sand as the case may be, the latter washed and sterilized in the usual way, the whole apparatus is sterilized again and when cool, is ready for planting.

After placing the filters in the solutions, the sand at the surface becomes moist, from capillary rise of the solutions, in about three hours.

The seedlings can be rapidly transferred to the tubes and the covers replaced. By filling the shoulder S with sterilized sand, the tubes can be manipulated without risk of knocking off the covers after sterilizing, and by moistening this sand with sterile water, a moment before the joint is made air-tight with hot paraffin wax, the air inside the cover C is maintained in a moist condition.

Once planted and placed under suitable conditions, little attention is required.




Text-fig. 2. Apparatus for growing sterilized seedlings in sand cultures. Lettering :—*p*, cotton wool plug ; *c*, glass cover ; *s*, shoulder filled with sterile sand ; *r*, rubber cork ; *f*. Massen filter candle, The evidence available as a result of these cultures points to the conclusion that roots are not formed by the seedlings unless infection with a fungus associated with the seed-coat takes place: and that therefore the relations between the plant and its endotrophic fungal partner is an obligate one, under the conditions supplied, *viz.*, a supply of inorganic salts, in a solution favourable to the healthy growth of unsterilized seedlings.

Since roots are not formed, it is impossible to demonstrate with certainty how far the susceptibility of the roots to particular salts and to the concentration and the reaction of the culture media are specific characters of the plant.

It is proposed to deal more fully with these and similar cultures and with the effects of inoculation from a pure culture of the fungus in a subsequent paper.

The evidence is conclusive that the resting embryo of *Calluna* is uninfected; also that, by effective sterilization of the seed-coat, seedlings can be germinated in a sterile condition and can be maintained alive under aseptic conditions for six months or longer. Infection of the plant by the fungus takes place under normal conditions immediately after germination and it is believed that a functional root-system is not formed by the seedling unless such infection occurs.

Hence, the nutrition of *Calluna* is bound up with that of its mycorhizal fungus: and the soil preferences of the plant and the soil conditions which limit its growth are determined *indirectly*, by the ability of the fungus to flourish under the conditions supplied.

In short, the maintenance of a definite biologic relation between the roots and the fungus which infects them acts as a limiting factor to the spread of the plant.

The refusal of *Calluna* to grow in the calcareous soil investigated, and presumably in similar soils elsewhere, is due to a disturbance of these conditions. On this view, the habit usually described as "calcifuge" may be, in this case, characteristic of the fungus rather than of the plant.

Directly connected with this disturbance is the presence of colonies of bacteria closely associated with the roots, especially with the regions where the fungus is usually prominent.

It is suggested that the bacteria are to be regarded either as pathogenic agents, or as indicators of soil conditions unfavourable to the fungus and therefore ultimately to the plant.

There is no doubt that the abnormal growth exhibited by the roots in unfavourable soil is directly correlated with the presence of an investing sheath of bacteria on these roots, but the evidence is not conclusive that the organism concerned is the inducing cause of the unhealthy condition.

## Summary.

1. As shown in a previous communication, small and sharply defined communities of *Calluna vulgaris* occur on the Wiltshire and Berkshire Downs, associated with soils on clay-with-flints.

The distribution of these heather communities is anomalous, in that they have monopolized areas of fertile soil.

Experimental work was undertaken to investigate the nature of the factors which limit the spread of *Calluna* from these areas to the surrounding down soils and indirectly to throw light on the significance of the calcifuge habit as shown by the soil relations of *Calluna* and its allies.

2. As demonstrated by pot cultures in soil from (*a*) a heather area, (*b*) soil overlying chalk, *Calluna vulgaris* grows *normally* in the former, *abnormally* in the latter.

3. Abnormality of growth is exhibited in (*a*) reduced germination capacity, (*b*) retarded germination, (*c*) arrest of root and curvatures of growing region, (*d*) arrest of shoot, (*e*) small size and red colouration of leaves.

4. Intimately connected with these abnormalities is the presence of colonies of bacteria on the roots, especially around the tip, and also a marked diminution of vigour in the growth of the mycorhizal fungus.

5. The abnormalities of growth can be *induced* in seedlings growing in "heather" soil by watering with filtered extracts of the unfavourable soil, if the treatment is continued over a considerable period. The unfavourable factors are presumably of a chemical nature.

6. The result of germinating unsterilized or imperfectly sterilized seeds in agar media, made up with extracts of the two soils named, shows that the development of the fungal and bacterial elements of the micro-flora associated with the seed-coat is determined *qualitatively* by the nature of the soil extract used.

With an extract of the heather soil, the mycelial constituent is predominant; with an extract of the down soil, colonies of bacteria

constitute the prominent feature of the growths associated with the seed-coat.

7. Seedlings of *Calluna vulgaris* are infected by the mycorhizal fungus immediately after germination.

Infection of the primary root takes place by a growth of mycelium from the seed-coat, the latter being infected while still in ovary.

8. The resting embryo and endosperm are free from infection.

9. The fungus isolated from the seed-coat is morphologically identical with one obtained in agar cultures of pieces of healthy root.

10. Seeds can be sterilized and seedlings germinated in a sterile condition, *i.e.*, free from fungal or bacterial infection.

The germination and *early* stages of growth of such sterile seedlings are normal, but in the absence of infection complete arrest of root-formation occurs.

11. The evidence at present available points to the conclusion that the relation between *Calluna* and its mycorhizal fungus is an obligate one, and that successful growth of the plant is ultimately bound up with infection of the roots at an early stage by the fungus, and with the subsequent healthy growth of the latter; hence, the soil preferences exhibited by the plant depend on the maintenance of a biological balance between the roots and the constituents of the microflora which beset them.

12. On this view, the inability of *Calluna* to flourish in the soil investigated is due to a disturbance of the relations maintained under favourable soil conditions between the roots of the plant and its mycorhizal fungus.

The bacterial colonies associated with the roots, more especially with the regions where the fungus is usually prominent, are to be regarded either as pathogenic agents, or as indicators of soil conditions unfavourable to the fungus. The evidence available points to the bacterial colonies as directly correlated with the abnormal growth displayed by the roots, but is not at present *conclusive* that they are the immediate cause of that condition.

13. Water cultures of *Calluna* have not up to the present thrown any light on the significance or not of the relatively high ratio of MgO to CaO in the soil in the area investigated.

They confirm previous observations as to the inability of the plant to thrive in any but very weak solutions.

DESCRIPTION OF THE PHOTOGRAPHS ON PLATE III, ILLUSTRATING MISS RAYNER'S PAPER ON "THE ECOLOGY OF *CALLUNA VULGARIS*."

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Fig. 1a. *Calluna* seedlings from "heather" soil, showing normal root and shoot development; six months old.

Fig. 1b. *Calluna* seedlings from "heather" soil treated with carbon bisulphide vapour; six months old. The more vigorous growth as compared with Fig. 1a is less marked than at an earlier stage.

Fig. 1c. *Calluna* seedlings from "chalk" soil, showing arrest of root and shoot development; six months old.

Fig. 1d. *Calluna* seedlings from "chalk" soil treated with carbon bisulphide vapour; six months old. As compared with Fig 1c, root development is more normal.

Fig. 2a. Apex of a young *Calluna* root from "heather" soil, showing normal conditions.

Fig. 2b. Apex of *Calluna* root of same age from "chalk" soil, showing abnormal condition with bacterial investment.

Fig. 3. Part of root apex shown in Fig. 2b, more highly magnified, to show bacteria in sheath.

Fig. 4a. *Calluna* seedling in "heather" soil watered for six months with an extract of "chalk" soil.

Fig. 4b. Control—similar seedling watered during the same period with distilled water.

Fig. 5. *Calluna* seedling from "chalk" soil, watered for six months with an extract of "heather" soil. Control seedlings in similar soil watered with distilled water all died before the end of the experiment. Fig. 1c shows surviving seedlings of the same age in this soil from another series of experiments.

Fig. 6. Mycelium from fungal colonies surrounding seeds, in agar cultures of "chalk" soil, to show investing sheath of bacteria.

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