Some recent Publications bearing on the question of the Sources of Nitrogen in Plants.

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IN the *Berichte der deutschen botanischen Gesellschaft* for 1885[1] there appeared a paper by Professor Frank of Berlin, entitled '*Ueber die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze*,' in which the author made known to science the results of some investigations of a kind sufficiently astonishing at the time, and which have been considered of late as possibly leading to yet more remarkable results when they are further examined. I propose to give a brief account of the published substance of this and certain other papers, with short critical notes of the views which the facts have suggested.

In the above paper Frank states that researches were being made in order to obtain information as to the culture of Truffles, which have long been known to affect the neighbourhood of living beeches, hornbeams, and oaks. Having regard to the observation[2] that the Truffle-like fungus *Elaphomyces* has its mycelium affixed to the roots of living pines, like a parasite, the question arose whether the true Truffles may not also be parasitic on roots?

[1] pp. 128–144, Pl. x.

[2] Reess, 'Sitzungsber. d. physik-med. Soc. zu Erlangen, 10 May, 1880.'

Investigation yielded the unexpected result that certain trees, especially the Cupuliferae, have almost the whole of their root-system covered with mycelium, the fungus being associated symbiotically with the root: the conclusion is drawn that the fungus-hyphae act the part of the root-hairs elsewhere, and that the whole of the absorption from the soil is due to their action.

The younger roots of any oak, beech, hornbeam, hazel, or chestnut, at any time of the life of the tree, from any of the distant places examined, were found to consist of a double structure—the true root as a sort of core, covered by a close web of mycelium as an envelope. Such an association of root and fungus is to be named a *Mycorhiza.*

In appearance the Mycorhiza resembles some sclerotia, the mycelium forming a sort of pseudo-parenchyma, the outer walls of which become dark brown as it ages. The weft of mycelium covers the root-apex as well as the parts behind, and is at first white: even the root-cap is therefore covered in.

The coating of mycelium varies in thickness, but usually forms a layer several cells deep. Hyphae dip down between the cells of the outer layer of the root proper, and grow around them completely; they do not leave the cell-walls, however, and are not found in the cell-lumina, nor deeper down in the tissues of the root. These 'endophytic hyphae' are very much thinner than those outside.

The outer surface of the mycelial envelope may be clean and smooth, and it will be understood that so complete and continuous a covering prevents the formation or emergence of root-hairs; in many cases, however, free hyphae develope from the outer surface of the mycelial envelope, and radiate out into the soil, growing at their ends, and curiously resembling true root-hairs in many morphological points.

Frank insists moreover that they replace the root-hairs physiologically. They become attached to particles of soil, and can be the only means for absorbing water and substances dissolved in it. Sometimes many of these free hyphae grow out into the soil parallel to one another, and form compound strands in every essential respect like the *Rhizomorphs* (of *Agaricus melleus* for instance); from such strands free hyphae may radiate out into the soil in their turn again. Anastomoses and ramifications of the most varied kind may take place in the surrounding soil, and the regions where Truffles grow have the soil permeated with such systems.

From the study of longitudinal sections, &c., Frank concludes that the development of the Mycorhiza is somewhat as follows. Since the mycelium closely invests the whole root-tip, it must elongate coincidently with the root: as matter of fact the hyphae covering the root-cap are thinner, and show every sign of growth, both by elongation of the existing hyphae, and by the interpolation of new branches between those already formed. The 'endophytic hyphae' do not develope until the growing apex has passed out of the stage of elongation; hence no organic connection between fungus and root is formed at the apex. The complete covering of the apex seems to be causally connected with the very feeble development of root-cap cells—in other respects the root (that of *Carpinus* is figured) conforms to the common type for Dicotyledons; probably, on the one hand, the pressure prevents the fuller development of root-cap cells, and, on the other, the fungus-web has acquired the protective function of a true root-cap.

Although the first stages of germination of the tree are passed through without the appearance of the fungus on the radicle, the lateral rootlets are usually soon attacked. The hornbeam is attacked very early, the young oak may remain a year or two free from mycelium.

In contrast to the roots of plants cultivated without the fungus, the Mycorhiza is shorter and thicker—the number of layers in the plerome and periblem increase: the tendency to branch is also increased, and the lateral roots emerge at points closer together, on account of the slow growth in length. These peculiarities give the Mycorhiza a 'coral-like' shape. As regards endogenous origin, monopodial order of development, &c. of the lateral roots the Mycorhiza behaves like an ordinary root; but of course the emerging young root is covered by mycelium from the first.

Further back, on older parts of the roots, the mycelium turns black and dies off: its life coincides with the activity of the younger parts, and may be longer or shorter according to circumstances.

Sufficient has been said as to the ubiquity of the fungus, and its presence at all ages on all Cupuliferae. The Mycorhiza is (in beech and hornbeam) most abundant in the upper parts of the soil, among the vegetable remains; the Truffles are also most abundant there, and Frank states that the ripe Truffles rest on and in a dense matting of Mycorhiza. As the roots go deeper, the Mycorhiza is rarer: this is quite in accordance with the known fact that the *Saugwürzeln* —*i. e.* young active lateral roots—are more sparsely developed on deeper roots, and the author's point is made on learning that when they are found low down they are in the form of Mycorhiza. The assumption is that the growing root carries mycelium down with it.

It is not superfluous to mention that numerous other plants growing in woods were examined, herbs, shrubs, and trees; but birches, ashes, alders, elms, &c., &c. were all devoid of the fungus. The Mycorhiza is so far a special peculiarity of the Cupuliferae.

Subsequently, Frank states that he has found a Mycorhiza here and there on species of *Salix* and *Populus*; and also on pines, spruces, and firs in the neighbourhood of Berlin. This is noted as remarkable because *Elaphomyces* is not known in the places examined—it will be remembered that Reess had found it on pines.

Frank then discusses the probability of the fungus having been seen by others, and comes to the conclusion that Gibelli[1] has mistaken it for a disease-producing parasite; R. Hartig's *Rosellinia* (*Rhizoctonia*) *quercina*[2] is a totally

[1] 'Nuovo studi sulla malattia del Castagno detta dell' inchiostro.' Bologna, 1883.
[2] 'Unters. aus d. forstbotanischen Inst. zur München,' 1880, p. 1.

different fungus. The reason Frank's fungus has been overlooked is probably that those who investigate roots use the seedlings, water-cultures, &c.

Beeches, hornbeams, oaks, and hazels, removed from the ground in spring, when one to two years old, and already bearing Mycorhiza, can be easily grown as water-cultures, with the result that the roots go on growing free from the fungus—or, rather, that new laterals are formed as water-roots, and the mycelium does not spread on to these. The fungus could not be cultivated.

As to the question, what is the systematic position of the fungus? no clear answer can be given. It is no doubt the mycelium of a subterranean form—one of the Tuberaceae or Gasteromycetes, perhaps. But new forms are discovered every day, and the presence of a mycelium does not necessarily imply the presence of the perfect fungus fructification; mycelia may go on growing and sterile for years.

We now come to Frank's views as to the biological significance of the Mycorhiza. The organic union between root and mycelium, their harmonious growth, and the close physiological relations which must exist between them, all point to this being a new case of symbiosis. From the side of the root, we must regard the fungus as a parasite, which takes from the former food-supplies of the nature of carbonaceous assimilated material: its minerals &c. must be taken by the fungus itself from the soil, the free hyphae acting like root-hairs. We may regard the thickening and other changes produced in the root as similar to the alterations met with in hypertrophy, &c.,—here in a slight degree only—due to a stimulus exerted by the parasite on the host. The roots are by no means killed, however, and that they preserve their capacity to serve the tree is proved by the well-being of the latter. We must conclude that the root-fungus, in the mycelium stage at least, is not injurious to the root and tree.

Under such conditions we must look for a contrary benefit derived from the fungus by the tree, and Frank sees this in the functioning of the mycelium as root-hairs. Since the whole surface of the root is covered by the mycelium, water and dissolved substances can only reach the former through the latter, and the extensive ramifications of the outlying mycelial strands and hyphae in the soil no doubt achieve the work of true root-hairs. Frank also sees in the enlargement of the epidermis-cells of the root and their enclosure in fine hyphae an adaptation which probably works to the same end. We are therefore to look upon the root-fungus as the sole organ for the absorption of water and materials from the soil, in the cases concerned. Frank therefore contrasts the mode of nutrition of Cupuliferae, as *heterotrophy*, with that of ordinary land plants — *autotrophy*.

The comparison with the symbiosis of Lichens is evident, and it need only be remarked that just as the gonidia of a Lichen are not incapable of independent existence, so the roots of oaks, beeches, and other Cupuliferae may be grown independently for years in water-culture.

Whether the Cupuliferae can develope under ordinary conditions, with their roots in the soil, in the absence of the 'nurse fungus,' and whether they would do better or worse simply cannot be decided, because there appear to be no Cupuliferae free from the fungus.

Just as Lichen-fungi will not flourish without the host Alga, so the root-fungus seems to be dependent on the tree: no efforts to cultivate the mycelium artificially have succeeded.

Such is, shortly abstracted, the story of the Mycorhiza as told by Frank in the first instance.

This was soon followed by two more or less critical notes, first by Woronin[1], and then by O. Penzig[2]. Woronin writes to the effect that he had known the 'Mycorhiza' for two years, having found it in Finland when investigating the biology of certain edible *Boleti*, &c.

[1] Ber. d. deutsch. Bot. Ges., 1885, p. 205. [2] Ibid. p. 301.

Woronin's Mycorhiza was found on species of *Salix* and *Populus*, and on Conifers, *Corylus*, and a few other plants. Since Truffles 'do not exist in Finland,' the mycelium in question cannot belong to that fungus, but Woronin thinks it not improbable that a *Boletus* is here concerned.

Woronin then goes on to remark that Kamienski had already discovered the symbiosis of which Frank makes so much, in 1882, in a work on *Monotropa Hypopitys*[1], pointing out the same thing in *Fagus sylvatica* and the Coniferae, whence the priority belongs to this observer.

The note by O. Penzig has reference to Frank's remarks about Gibelli's study of the chestnut-disease, and may be taken as admitting generally the possibility of Frank's conclusions, though protesting against some details which do not concern us at present.

M. Reess also adds a few notes on the subject of *Elaphomyces* and other root-fungi[2], and he too points out that Kamienski's paper contains the germ of the matter. Reess states that the hyphae of *Elaphomyces* not only enter between the outer cortical cells of the pine-root, but drive the cell-wall before them as vesicles into the lumina. As regards common growth, distribution, occurrence, &c. of the mycelium on the roots, Reess states that the anatomical and other facts concerning *Elaphomyces* and pine-roots accord with Frank's facts about the Mycorhiza of Cupuliferae.

Reess also states that he has repeatedly seen fungus-envelopes on the roots of other plants as well as the pine. He has also investigated Kamienski's fungus on *Monotropa*, and finds his observations in some points differing from those of that observer: he believes the *Monotropa*-fungus to be different from *Elaphomyces*, but cannot be sure. Reess admits that these and Frank's root-fungi must take nutriment from the roots; but regards the rest of the conclusions as needing much more careful investigation.

The above criticisms are replied to by Frank in an article

[1] Mem. de la Soc. Nat. des Sc. Nat. et Math. de Cherbourg, T. xxiv.

[2] Ber. d. deutsch. bot. Gesellsch. 1885, p. 293.

in the *Berichte der deutsch. bot. Gesell.* for Nov. 19, 1885[1], in which he insists on his claims to have 'discovered a hitherto unknown biological phenomenon in the nutrition of certain trees,' &c., &c. He points out that Woronin's rejoinder as to Kamienski's having already discovered the nature of the Mycorhiza is premature, since Kamienski only discovered mycelium overlying the roots, and denied the organic union of root and fungus. However, it seems unnecessary to enter into a criticism of the discussion as to priority, as it may be accepted that Frank was clearly the first to generalise, and to suggest in the wide sense the biological significance of the symbiosis.

What is more important is, that Frank himself investigated the *Monotropa* roots anew, and found facts beyond the mere association of fungus and roots described. He discovered that the hyphae of the fungus are not simply on the surface of the *Monotropa* root[2], but enter between the cells: this, as in the case of the Cupuliferae, only takes place on those parts of the root behind the region of growth in length. Hence the Mycorhiza of *Monotropa* is, like that of the Cupuliferae, a case of organic union and symbiosis between the fungus and the root.

Frank then proceeds to state :—

(1) That the phenomenon which he at first thought confined to the Cupuliferae and a very few other trees probably appertains to 'all trees under certain conditions.' He finds a form of Mycorhiza on the roots of the Scotch pine, the Weymouth pine, the spruce, the silver fir, the larch; also in Salicineae, alders, and birches, and in one case even on the lime, and *Prunus spinosa*.

(2) That 'the Mycorhiza is formed only in a soil which contains humous constituents or undecomposed vegetable remains; the development of Mycorhiza increases or di-

[1] Neue Mittheilungen über die Mycorhiza der Bäume und der Monotropa hypopitys, pp. xxvii–xxxiii.

[2] In a foot-note Frank states that he has found a case of this kind on *Andromeda polifolia*.

minishes with the poverty or richness in these constituents.'

(3) 'The fungus of the Mycorhiza conveys to the tree not only the necessary water and the mineral nutritive substances of the soil, but also organic matters taken direct from the humous and decomposing vegetable remains.' Only by the mediation of the fungus is the tree enabled to employ directly such organic matters.

(4) The theory, superseded in the doctrine of the nutrition of plants, of the direct nutrition of green plants by humus, is therefore again brought to the front in the light of the Mycorhiza, although in another sense than formerly, and the significance of the humus and the covering of dead leaves on the soil needs further investigation and consideration.

This is the substance of Frank's renewed and extended theoretical statements. They speak for themselves; and it must be admitted that he proposes to raise a very large superstructure on the foundation of his anatomical investigations, and that, in doing so, he assumes a proportionately heavy responsibility.

The next important communication on the subject is again by Frank, in the *Berichte der deutschen botanischen Gesellschaft* for 1887[1]. In this he expressly sums up once more the chief points already insisted upon, and then proceeds to add other facts.

He finds a peculiar formation of pigment associated with some kinds of Mycorhiza, reminding us of the pigments formed by certain Schizomycetes and Saccharomycetes, and not unknown in connection with higher fungi (no cases of the latter are cited, but the author would no doubt accept the green dye in wood affected by *Peziza aeruginosa* as an example). These pigments stain the membranes and contents of the fungus hyphae as well as the surrounding media.

Frank then proposes to classify all the known forms of Mycorhiza as follows:—

[1] Ueber neue Mycorhizaformen, pp. 395–408.

A. Ectotrophic Mycorhizae (i. e. the fungus is entirely outside the cells of the root).

(1) The ordinary coral-like Mycorhiza of the Cupuliferae, &c.

(2) A long, branched Mycorhiza with hair-like outgrowths found on beech; the outgrowths consist of hyphae only.

(3) A somewhat similar form on *Pinus Pinaster*, but the outgrowths consist of rootlets covered with hyphae.

B. Endotrophic Mycorhizae (i. e. the hyphae enter and live in certain cells of the root).

(4) The Mycorhiza of Ericaceae.

(5) The combination of fungus and roots found in Orchids, and described by Wahrlich[1].

It is not necessary to describe in detail the new forms, but I will state what seem to be the most important points. The form (2) on the beech was found once, and it resembles at first sight an ordinary branched root—i. e. the growth in length is not interfered with, and so the 'coral-like' thickening does not occur. It appeared to be clothed with a dense pile of root-hairs. The microscopic examination showed that it was clothed with a dense thick coat of mycelium—the thickness equal to half the radius of the root—and that the apparent root-hairs were strands of hyphae radiating out from this covering. These free strands were peculiar in the hyphae, being parallel in one plane, and thus forming flat bands. Some were as long as 1½ to 2 mm. The hyphae fuse with the particles of soil as do root-hairs.

The Mycorhiza of *Pinus Pinaster* (3) is superficially somewhat like the last, but the radiating filaments which look like root-hairs are coarser, and in this case turn out to be *true lateral roots*, but so fine and closely packed that they look like root-hairs. Some were 3 mm. long and 0·1 to 0·135 mm. thick. Each of these hair-like rootlets was covered by a relatively very thick felt of mycelium. The above

[1] Bot. Zeitung, 1886.

measurement of thickness includes the mycelial envelope as well as the rootlet proper: a vascular bundle of a few elements runs down the axis. The fungus was on the outside only. No such Mycorhiza could be found on a specimen of *Pinus Pinaster* in the Botanic Garden at Berlin.

As regards the Mycorhiza on the roots of Ericaceae (4) it appears that one or two observers had already found here and there instances of association, more or less regular, between hyphae and roots.

In the Ericaceae the simpler roots may consist only of a few tracheides and sieve-tubes surrounded by relatively huge epidermal cells, each of which may occupy one-sixth of the periphery. There are no root-hairs. Each of these very large epidermal cells is filled with a dense complex of extremely fine, interwoven fungus-hyphae: these are so densely crowded that they form a sort of pseudo-parenchyma. 'In most cases these fungus elements are so fine, that one may be in doubt whether this intracellular mass is to be explained as a fungoid pseudo-parenchyma.' Frank has no doubt of this, however, since he can trace the finest hyphae from certain coarser ones which pass into the cells from the outside. The growing-point of the root of *Andromeda polifolia* is curiously reduced, and the author finds that it possesses an apical cell, triangular in surface view, from the segments of which the other tissues proceed. The dermatogen runs all round: the root-cap is reduced to two or three small loose cells; and the plerome cylinder is also extremely simple. The fungus fills the cells of the dermatogen up to the extreme apex, and the fine mesh-work alluded to above can be detected in all but the youngest cells.

On the surface of the root are loose hyphae, as a rule, and sometimes they cover the root rather thickly; even when these outer hyphae are absent, the intracellular fungus is present. In *Vaccinium Oxycoccus* the author traced the connection between the thicker hyphae outside and the finer ones in the epidermal cells, and also found hyphae running in the rather thick cell-walls. In some cases the superficial hyphae are as delicate as those inside ; they often stretch from the root to the neighbouring turf and humus remains.

Although all plants investigated had the fungus somewhere at the roots, still branches of the roots here and there were devoid of mycelium inside or out. The fungus is to be regarded as constantly present on *Andromeda polifolia*, *Vaccinium Oxycoccus*, *Ledum palustre*, *Vaccinium uliginosum*, *Empetrum nigrum*, and also the American moor-plant *Vaccinium macrocarpum*. Numerous other moor-plants showed no traces.

Further research showed the presence of the fungus on *Calluna vulgaris*, *Vaccinium Vitis idaea*, *V. myrtillus*, and even on some specimens of *Rhododendron ponticum* and *Azalea indica*. On the other hand, the mycelium was not present at the roots of *Pyrola*—a statement which corrects Kerner's short announcement (Sitzung. d. Akad. d. Wissensch. in Wien, 4 Mar. 1886 : see footnote to Frank's paper, p. 401) that he had found the fungus on all Pyrolaceae, Ericineae, and Vaccineae.

The Mycorhiza of *Monotropa* is, as we have already seen, an ectotrophic form, agreeing with the typical form found on the Cupuliferae.

Frank therefore claims to have established a case of root-symbiosis in the Ericaceae, of similar biological significance to that assumed for the Mycorhiza of Cupuliferae. The epidermis-cells filled with hyphae 'constitute the most important organ of the whole root, and the sole apparatus for the absorption of nutritive materials, and abut internally directly on the conducting paths of the root.' If we suppose the cell-walls of the epidermis away, then the fungus alone would remain as the medium for conveying nutritive substances to the root.

Enough has been said to show how Frank has gradually been led to extend his original idea of a Mycorhiza, so as to include not only the type of shortened, thickened, coral-like Mycorhiza of the Cupuliferae, but also any root which has a fungus mycelium definitely associated with it, in such a way that the root and fungus may be regarded as symbiotically related one to another. Of course this paves the way to a still wider definition of the idea Mycorhiza, and a concomitant risk of vagueness ; in fact, Frank has himself had to go much further, as will be seen from what follows.

Frank's second type of endotrophic Mycorhiza is that of orchids. It has long been known that the roots and rhizomes of exotic and native orchids contain hyphae, which live in the cells of the cortex. In 1886 Wahrlich[1] carried out a masterly investigation of the subject, along the well-known thorough lines for which the Strassburg laboratory is so celebrated, and showed that the fungus in question is a *Nectria*. Reference must be made to Wahrlich's paper for details. He examined more than 500 species and all had the fungus. Aerial roots are infected as well as others.

The fungus only affects spots here and there, its hyphae coiling themselves up in certain cells into knots, which as a rule only partly fill the cell and do not destroy the protoplasm but cause the cell to enlarge.

Frank lays stress on the following points: (1) The protoplasm of the cell and the fungus live together, 'without the former being parasitically affected or its vital phenomena disturbed.'

This can only be an assumption, and the impression I gather from the study of what is known of this orchid-fungus is in favour of the view that the fungus *does* disturb or 'parasitically affect' the protoplasm of the cell, and that an outward and visible sign of some such action exists in the hypertrophy of the cells affected, and in the turning yellow of the chlorophyll-grains[2]; moreover, as Frank himself points out, the nucleus of the affected cell is larger. The conclusion that the fungus does not act as a ruthless parasite is warranted by the facts ; but not so the conclusion that the hyphae do not stimulate the cells to increased metabolic activity.

[1] Bot. Zeit., 1886, pp. 481-499.

[2] Wahrlich, l. c. p. 484.

Frank's second conclusion is (2) that the root and the fungus increase together; as the root-cells divide, the fungus passes forward cell by cell. (3) The fungus is strictly connected with that part of the plant which absorbs the food-materials. (4) The orientation of the cells which contain the hyphae is such that they must necessarily act as the go-between for the absorbed substances and the conducting paths of the root. (5) Those orchids which are devoid of chlorophyll—and which therefore depend on the humus of the soil for carbonaceous matters—always have this form of Mycorhiza, and highly developed.

We are therefore to regard the Mycorhiza as a humus-absorbing organ.

It is thus evident that, according to Frank's latest publications, the idea of Mycorhiza is to be extended to all such cases as that investigated by Wahrlich, and it follows in the opinion of several botanists that the root-tubercles of the Leguminosae will have to be included as another example; for, as I have lately shown [1], we have here an exquisite example of symbiosis between a fungus and the root. It is of course not to the purpose to enter here into details about this case, but I wish to point out how decidedly the facts observed are opposed to Frank's view that the fungus acts as root-hairs or absorbent organs to the bean. Of course, it may be replied that on this account it must be excluded from the category of Mycorhizae; if this is allowed, I think the same will follow as regards several of the others. The case of the fungus in the roots of *Juncus bufonius*[2] will also have to be taken into account in this connection, as well as a very remarkable example in *Podocarpus*, which I have lately observed and am at present investigating. And there are other instances also.

The point on which stress is to be laid at present is that in the bean (1) the mycelium of the fungus stimulates the

[1] 'On the Tubercular Swellings on the Root of *Vicia Faba*.' Phil. Trans., 1887, pp. 539–562.

[2] See Bot. Zeit. 1884, No. 24.

root in such a manner that local hypertrophy is brought about, attended with concentration of food-materials, and other signs of extraordinarily active metabolism; and (2) the root-hairs are by no means absent, but on the contrary are very numerous and well developed.

Consequently, those who are inclined to compare all the cases of symbiosis between roots and fungi, will at least be impelled to sharply discriminate between this form and that of the Cupuliferae and similar ones. Of course, this distinction implies much more. It is at least clear that the fungus-hyphae in the leguminous plant do not prevent the root-hairs from acting as the absorbing organs, or dissolving food substances, &c. for the plant.

The view to which my experiments and observations on the root-tubercles of the Leguminosae lead is the following: that the stimulating action of the fungus enables the roots to acquire relatively large quantities of nitrogenous materials from the soil. I purposely avoided raising the question as to whether or not the fungus of the bean-root tubercles affects directly the supplies or preparation of nitrogenous matter in the soil. We may now, however, survey shortly some of the suggestions that have been literally flung about lately as to the possibilities of the case under investigation, or of others like it.

First, however, let it be clearly stated that the questions raised do not affect the results obtained by Boussingault and Lawes, Gilbert and Pugh, as to the non-assimilation of free nitrogen by the higher plants. Plants have no power of directly employing the nitrogen absorbed by their leaves, &c.

But it has become a revived question of late as to whether the acknowledged sources of nitrogenous food of plants really suffice for the large crops taken from the soil, and whether the free nitrogen of the atmosphere is not perhaps 'fixed' in the soil and enabled to combine with other elements and so enrich the soil with nitrogen. The importance of the subject needs no insisting on, and it may simply be mentioned that the Leguminosae especially have repeatedly been cited as carrying away more nitrogen from the soil than could be accounted for.

In illustration of this I may first give an abstract of a paper sent to me a short time ago by Professor Hellriegel, the Director of the Agricultural Experimental Station in Bernberg.

Professor Hellriegel's paper was published in November 1886, in the *Zeitschrift des Vereins f. d. Rubenzucker Industrie des Deutschen Reichs*[1], and deals with the question of the sources of nitrogen in Gramineae and Leguminosae respectively. He was aided by Dr. Wilfarth. The author sums up the well-known points that, while nitric acid, ammonia, and certain complex organic compounds such as urea, uric acid, hippuric acid, proteids, and certain humous constituents, &c. are available as sources of nitrogen for plants, cyanogen and alkaloids and certain other complex organic compounds are useless for this purpose. Moreover, as proved by Boussingault (and he might have added by Lawes and Gilbert), the free nitrogen of the air is unavailable[2].

It is also known that various natural processes lead on the one hand to the conversion of unavailable nitrogenous compounds into available forms, and *vice versa*; and, on the other hand, to displace such compounds in the atmosphere and soil. For example, electric discharges, the evaporation of water, and the activity of certain micro-organisms aid in rendering nitrogen available, and rain, dew, and certain absorptive properties of the soil supplement or aid the processes.

For a long time it has been generally known that the Leguminosae, especially, have what we may term a special aptitude for seizing large quantities of nitrogenous substances from the soil, and this property has become a classical puzzle in vegetable physiology.

Hellriegel has been engaged for some time with this pro-

[1] 'Welche Stickstoffsquellen stehen der Pflanze zu Gebote,' pp. 863–877.

[2] There is a short discussion of this subject in Dr. Vines' 'Physiology of Plants,' pp. 126–129.

blem; and the following is a short summary of his chief experimental results.

When graminaceous plants were sown and allowed to grow in a soil devoid of nitrogen, but to which all other necessary minerals were added in proper quantities, they developed normally until the third leaf appeared and the reserves were exhausted. The experiments were conducted in the open, care being taken that no rain fell on the plants, &c. Then the 'production' ceased suddenly. But the plants did not die—they lived as long as normal plants, only their vegetation was dwarfed. The stunted plant developed stunted and miserable organs (even barren ears), and struggled on through the season: the total dry weight increased very little, and this concerned the non-nitrogenous constituents only.

If nitrates are added at the moment when the above arrest of development sets in, the grasses go on growing normally again, and if sufficient is added the recovery is complete; if insufficient, a *gradual* passage to the starved condition sets in again. Hellriegel also finds that there is a direct proportion between the amount of nitrates added and the yield of grain, up to a certain point of course.

If ammonia salts or other nitrogenous compounds are used instead of the nitrates, the above proportion does not make itself evident, and the author finds that a pause ensues between the addition of these salts and their employment by the grasses—it is concluded that the above-named nitrogenous compounds have to be oxidised to nitrates before they can be used by the grasses. In other words, nitrification must be accomplished in the soil before the grass roots can employ the manure used.

Summing up the above results. The Gramineae are entirely dependent on the soil for their nitrogen: the atmosphere cannot furnish them with nitrogenous food, except in so far as rain or dew carry down nitrogenous compounds to the soil.

The most useful source of nitrogen for Gramineae is a salt of nitric acid, and nitrates supply them easily and completely. They employ the nitrates directly, and the yield of grain &c.

is directly proportional to the quantity of nitrates employed (so long as the maximum is not surpassed).

Moreover, nitric acid is no doubt the only available source of nitrogen for the Gramineae; when other nitrogenous compounds are offered, they only become available so long as they are oxidised to nitric acid compounds. Thus the development of the Gramineae is in direct relation to the quantity of nitric acid present in or manufactured in the soil.

Hellriegel then proceeds to show that experiments with leguminous plants yield totally different results.

If peas are allowed to germinate and grow in soil devoid of nitrogen, the result is astounding. In the same kind of soil deprived of nitrogen, in which grasses always pass into the starved condition above described, the peas *flourished and yielded a large increase*. Thus, from small culture-vessels, 20 cm. high and containing each four kilos of sand, the author got the following results.

In 1884, 13·947 gr. of peas (seed) yielded 28·483 gr. of dry substance above ground.

In 1885, 11·710 gr. of peas gave a yield (above ground) of 27·816 gr.

In 1885, also, 12·426 gr. peas yielded 33·147 gr. of dry substance.

And in 1886, 8·956 gr. peas gave 20·372 gr. dry substance. Moreover, the plants were normally growing, and even vigorous, and Hellriegel points out that such a yield as 33 gr. of dry substance from the same sources could not be obtained with barley even if nitrates were added.

Now comes the question, whence did the peas obtain the nitrogen necessary for this rank growth? 'There is apparently but one definite answer—from the air!' The soil was a pure quartz sand, repeatedly washed; the nutritive mixture contained no nitrogen compound; the distilled water was specially prepared, and free from ammonia or nitric acid. Even if it be supposed that traces of any nitrogen compound did fall into the vessels, the author points out that it would be out of account when we consider the large yield in question; moreover, the grasses cultivated under the same conditions showed that the soil &c. could not have yielded the nitrogen.

Thus we must look to the atmosphere. Now the only conceivable sources of nitrogen yielded by the atmosphere are (1) the free nitrogen, (2) nitric acid, (3) salts of ammonia (carbonate and nitrate). Hence we must either assume that the Leguminosae have an extraordinary capacity for collecting and absorbing the nitrogen compounds from the atmosphere, or we must admit that the Leguminosae are in some way able to make use of the free nitrogen of the atmosphere.

Enormous difficulties stand in the way of direct proof. First, the author asks us to consider the following further observations. When peas are cultivated in a sand devoid of nitrogen as above, two remarkably sharp periods of development are to be noticed.

Up to the period when the reserve-materials are exhausted, the seedlings grow normally, luxuriantly, and with normal colour. But directly the reserves are exhausted, a somewhat sudden change occurs—growth stops, the leaves turn pale, and the plant evidently begins to starve.

Sooner or later, however, the pale or yellow leaves again turn green, and a *second period of growth begins,* and the plants go on growing normally to the end.

The sharply marked starvation is not reconcilable with the view that the peas take their nitrogen directly from the above compounds in the air. When the reserves begin to be exhausted the plants have each about six leaflets; how are we to explain that these six leaflets suddenly and so completely fail, and that just at this particular period the plant becomes unable to use the nitrogen supplied? and further, to explain why and how, after a pause, the plant begins to acquire nitrogen?

We are then asked to note the following observations. When the above cultivation experiment is repeated on a large scale, it is noticed that the development of the individual plants—all under the same conditions—is very unequal. Some, usually few, grow very vigorously as said;

others close beside these do worse; and yet others may never pass through the starvation-period. It occurs not rarely (and this is expressly insisted upon) that of two peas growing side by side in the same vessel, the one starves and the other succeeds in the highest degree.

Now, since it is impossible to assume, in earnest, that peas have the power of growing without nitrogen in the soil, and at one time to succeed and at another to fail, the only explanation is that the above extraordinary behaviour of the control-plants in well-arranged experiments, is that there is, in addition to the known and carefully regulated factors, *some unknown* co-operating factor, which depends on accidentals and which exists outside the culture-vessels.

Hellriegel then proceeds to describe the following experiment. Four vessels were filled with soil devoid of nitrogen, and peas put in and allowed to germinate; the vessels were then placed under four glass bell-jars, enclosed, and joined by tubes, and the whole so arranged that a constant stream of air was drawn through from No. 1 to No. 4. Absorption-vessels were placed between each pair of bell-jars, and matters so arranged that the air passed into No. 1 unaltered, but, before entering Nos. 2, 3, and 4, was deprived of ammonia and nitric acid. The pea-plants were each about 15 cm. high, and had passed successfully through the above-named starvation-phase, and entered into the second lease of existence. This continued under the bell-jars, and, in short, all the plants flourished, and attained an average height of 120 cm., and had entered upon the flowering and fruiting stage when the experiment was stopped. The results were—

No. 1 = Ordinary atmospheric air, yielded 13·6 gr. of dry substance in the straw, and 3·4 in the roots, = 17 in all.

No. 2 = Purified air, yielded 14·6 in straw, and 3·5 in roots, = 18·1 in all.

No. 3 = Purified air, yielded 19·1 in straw, and 3·9 in roots, = 23 in all.

And the author states that the observations lend no probability to the idea that the small traces of combined nitrogen in the air can supply the plants with what they obtain, 'and probably the only assumption which remains is that the Papilionaceae have the power of making the free nitrogen of the air available for their life-purposes.'

Now it has been certainly shown by Boussingault that even the Papilionaceae are unable directly to assimilate the elementary nitrogen; but this does not exclude the possibility that something of the kind may occur indirectly, and we have now to examine a few observations which may point to something of the kind.

Berthelot has shown that free nitrogen may be absorbed by the soil and converted into compounds, probably by means of schizomycetes or micro-organisms of some kind. The roots of Papilionaceae are provided with tubercular swellings full of 'bacteria'.[1]

It has been stated above that in the researches some plants did well and others worse: now, Hellriegel finds that those plants which are still in the starvation-phase have either no tubercles or very few and insignificant ones, whereas the plants which are flourishing have many well-developed specimens on the roots. 'The more plants we investigated, the more we were convinced that the development of the root-tubercles stands in the closest, strictest relation to the growth and assimilation of the whole plant.'

Now, notice the following experiments. On May 25 were taken forty vessels filled with soil devoid of nitrogen, and two pea-seeds placed in each. Then ten of these vessels were watered with soil-washings—the authors say, 'Resting on the fact that in every normal culture-soil micro-organisms exist in abundance, we took some of the fertile soil of our culture-field, stirred it up with five times the quantity of distilled water, and after a short settling gave 25 cc. of this quasi-solution to each vessel.'

Bearing in mind that the experiment began on May 25,

[1] Here the author is following older views as to the nature of the contents of the tubercles: they are not bacteria, but yeast-like gemmules budded off from the mycelium of a true fungus. (See paper in Phil. Trans. 1887, pp. 539–562.)

the early phases were passed through, and in the second week of June the aspect of the plants was changing, and they became pale as the reserve-materials were exhausted.

So far, there was no difference to be observed between the forty cultures.

On June 13, however, a difference began to set in, and by June 18 it was decided—'In the ten vessels supplied with bacteria[1], all the plants had regained their fresh green colour, and commenced to grow vigorously.'

Of the thirty vessels in which the appearance of micro-organisms was left to chance, only two at this time presented a similar appearance, the remainder starving and in part yellow.

By the 30th of June, the plants supplied with bacteria were developing the tenth leaf, and were luxuriant; only one of the twenty individuals was behindhand, and the deep green colour showed this was not from want of nitrogen—later examination showed that its tap-root was injured.

Of the sixty plants not supplied with bacteria, about ten were nearly as flourishing as the above, and five were nearly dead: among the remaining forty-five were all stages between these extremes.

At this time the plants from two of the vessels infected with bacteria and those from five of those not so infected were taken up and examined, and showed the above-described relation between the growth of the sub-aërial parts and the development of the root-tubercles.

Of twenty-two plants to which no bacteria were added, only five yielded more than 15 gr. dry substance, as follows—

$$\left.\begin{array}{ll} \text{No.} & 2 = 15{\cdot}053 \\ \text{"} & 26 = 15{\cdot}950 \\ \text{"} & 29 = 17{\cdot}142 \\ \text{"} & 18 = 17{\cdot}305 \\ \text{"} & 1 = 20{\cdot}372 \end{array}\right\} \text{gr.}$$

The yield of the remaining seventeen plants was between 1·640 gr. to 13·190 gr.

[1] Here again it is of course an assumption that 'bacteria' were the agents.

On the other hand, the yield of the plants in four vessels supplied with the 'bacteria' (and these were not the best) was in every case more than 15 gr. dry substance :—

$$\left.\begin{array}{l}\text{No. } 31 = 15{\cdot}789 \\ \text{,, } 32 = 18{\cdot}768 \\ \text{,, } 36 = 19{\cdot}743 \\ \text{,, } 40 = 16{\cdot}200\end{array}\right\}\text{gr.}$$

Hellriegel then points out that the uniformly and decided large yield in the latter cases must have been due to the co-operation of the micro-organisms, for since in each case the 25 cm. of fluid added contained less than 1 mg. of nitrogen, we cannot suppose it due to that.

Two cultivations were made in soil without nitrogen, to which nutritive materials and 25 c.cm. of the above soil-washings were added, and then the whole sterilised by heating, then the seeds sown, and then covered with a layer of sterilised wadding. All went well until the development of the sixth leaflet, and the setting in of the starvation-phase. The plants never recovered, however; all died. 'No trace of tubercles was to be found on their roots.'

Passing over other experiments, which lead to the same general result, I may sum up Professor Hellriegel's results in his own words :—

'The Papilionaceae, in contrast to the Gramineae, are not dependent on the soil for their nitrogenous nutrition; the sources of nitrogen afforded by the atmosphere have for these plants the highest importance, and are alone sufficient to bring them to normal and even luxuriant development.'

. . . .

'It is seen that not one of these observations supports the idea that the sources of nutriment of plants are to be sought in the small quantities of combined nitrogen which are found in the atmosphere, and thus probably the only remaining assumption is that the Papilionaceae have the power of making use of the free nitrogen of the air.'

. . . .

'To the nutrition of the Papilionaceae, and especially to the assimilation of nitrogen by them, the so-called tubercles and the micro-organisms which dwell in them stand in the closest active connection.' . . . .

The following remarks may be made respecting this paper. It is above all unfortunate that the authors do not give us more details as to the analysis of their crops: in the absence of exact numbers, their conclusions as to the increment of nitrogen can scarcely be criticised. It must also be pointed out that the tubercles referred to do not contain bacteria, but that the 'bacteroid' bodies are minute yeast-like gemmules budded off from the hyphae of a true fungus which enters the root-hairs, crosses the cortex, and branches &c. in the tubercles.

Before making any further observations, I may quote the following.

In August 1886, a paper was published by Frank, on the sources of nitrogen of plants[1], in which the author points out that Schultz-Lupitz and others have shown that Leguminosae will grow for years, without any marked decrease in productiveness, on a soil which is barren, provided all other needful salts are supplied except the nitrogenous ones; also that crops of Leguminosae preceding Gramineae on a given piece of land, enrich the latter in nitrogen. He then points out that three kilos per hectare is the most that could be supplied annually from the combined nitrogen washed down by rain from the air, whereas a normal yield corresponds to about fifty-one kilos of nitrogen per hectare.

In experiments with finely sifted soil consisting of sand and humus, the following results were obtained. A quantity of the soil was analysed: a second lot was put in vessels, and seeds of leguminous plants sown in them; a third lot was allowed to stand in pots alone.

All were exposed to the air, and watered with distilled water, and protected against insects. In the control-pots, all weeds were carefully removed as they sprang up.

[1] Ueber die Quellen der Stickstoff-nahrung der Pflanzen, Ber. d. deutsch. bot. Gesellsch., 1885, p. 293.

Before giving his own results Frank summarises those obtained by some other observers.

Dietzell[1] found with peas and clover that there was a loss in nitrogen during the progress of experiments similar in principle to these.

Berthelot[2] found that the soil itself can fix free nitrogen in combination, and that the process may depend on the activity of micro-organisms. The increase was not in the form of ammonia or nitrates, but as organic compounds. Sterilisation destroyed this power on the part of the soil.

Joulie[3] found similar results. He cultivated plants in pots, and the nitrogen increased.

Frank's results are shortly as follows. In the soil with no plants there was a gradual loss of nitrogen; in those in which the plants grew there was an increase in many cases. Frank concludes that 'the increment of nitrogen here observed can only be looked upon as a fixation of uncombined atmospheric nitrogen, unless we assume that this large quantity of ammonia has been seized from the air by the plant.'

Frank further concludes that two processes occur side by side in the soil—one which results in the freeing of nitrogen from its combinations in the soil, and another which consists in the fixation of nitrogen from the air—'the latter is favoured by the presence of living plants.'

Some experiments made by Dr. Vines in 1887, and communicated to the British Association at Manchester, also bear on this subject. Dr. Vines cultivated beans in a medium devoid of nitrogen, and found that they went on growing much as if nitrogenous food-materials were present at the roots.

The following paper is quoted simply to give an example of publications bearing on another aspect of the same question.

In 1873 M. Dehérain published a paper in the *Annales des*

[1] Sitzung der Section fur landw. Versuchsw. d. Naturf. zu Magdeburg, 1884.

[2] Compt. Rendus, 1885, p. 775.

[3] Ibid. p. 1010.

Sciences Naturelles[1] on the subject of the relations of the atmospheric nitrogen to that of plants. After pointing out that a forest, regularly exploited, loses annually when the trees are cut certain quantities of nitrogen, and that large pastures &c. do the same, and this goes on year after year without any apparent restitution further than what is afforded by the manure of animals, decay of organisms, &c., he then proceeds to show that the opinion gains ground that the soil seems to lose more combined nitrogen than it receives, and the only explanation of the anomaly is that the atmosphere's free nitrogen intervenes.

The author then considers the question of the losses and gains in nitrogen of cultivated soils.

Regarding, first, the losses: they are as follows:—

(1) Losses of combined nitrogen, due to—

(*a*) Excess of nitrogen carried off in crops.

(*β*) Washed away by rain from soil.

(*γ*) Lost in drainage through subsoil.

(*δ*) Loss of ammonia diffused into the air.

It is only necessary to note that various observers have shown that in a rotation of crops more nitrogen is carried off in the total crops than was contained in the manure, supposing the latter completely utilised. The examination of streams and of drainage-waters gives some idea of the loss by superficial and subterranean water: quoting one case only, the Rhine and Seine were calculated to carry off about 200,000 kilograms of nitrates annually.

With respect to ammonia diffusing into the air; not all the ammonia of the soil is oxydised to nitrates, but some forms volatile compounds—e.g. the carbonate.

(2) Dehérain then proceeds to examine the loss of free nitrogen.

It appears that whenever decomposition of organic matter occurs, there is, in addition to ammonia, free nitrogen also evolved: the chief condition necessary is active oxidation.

[1] Vol. xviii. Ser. 5, 'Recherches sur l'intervention de l'azote atmospherique dans la vegetation,' p. 147.

The second part of the paper is devoted to the consideration of the gains in nitrogen of the soil. First, we have the gain in combined nitrogen :—

Ammonia and nitric acid found in the atmosphere during electric discharges, and carried down by rain, snow, &c. This can be measured and shown to be too small to account for the nitrogen acquired by plants in addition to that in the soil, &c. : this is admitted fully since Boussingault, and was clearly evident in the experiments of Lawes, Gilbert and Pugh [1].

We then come to the chief points in Dehèrain's paper :—the gains due to the fixation of free nitrogen.

It is unnecessary to discuss the question of the 'assimilation' of nitrogen by the plant direct : it is allowed on all hands that the experiments of Boussingault, and of Lawes and Gilbert, settled that point for ever—no free nitrogen is assimilated by the leaves.

Dehèrain experimented with various combustible—i.e. easily-oxidisable bodies, such as carbo-hydrates, old wood, &c., in contact with certain bases. Such mixtures exposed to the air were found to absorb and 'fix' not only oxygen but also certain quantities of free nitrogen.

The explanation first suggested was that some of the oxygen and nitrogen of the air unite to form nitric acid at the moment of combustion, just as they do when hydrogen is detonated with air ; but it turned out that this was not the case, and the compound formed was some other combination of nitrogen—possibly a lower oxide of nitrogen, possibly cyanogen, or ammonia.

Dehèrain then made experiments to determine the fixation of atmospheric nitrogen by vegetable substances. He agrees with Lawes and Gilbert in rejecting the view that ammonia is formed in damp soil simply by union of hydrogen evolved by putrefaction and the nitrogen in the confined spaces afforded by soil.

[1] Contained in their well-known paper in Phil. Trans. 1860.

The results of experiments with saw-dust, humus, roots, &c. led to the inference that free nitrogen is fixed, and that if oxygen is absent the hydrogen disengaged—being unable to form water—unites with the nitrogen to form some compound.

This led to experiments under other conditions, and nitrogen was passed over warmed mixtures of glucose and soda, and the results confirmed the authors' expectations, but are chiefly of interest as leading to other suggestions.

Experiments based on these led to the conclusion that if nitrogen is passed, in the cold, over saw-dust or glucose, alone or mixed with alkalis, some of the nitrogen is retained, 'fixed' in combination. It is thus demonstrated that, 1st, 'the nitrogen of the atmosphere may be fixed by vegetable substances, even in the cold and under conditions analogous to those which are met with in cultivated soils ; 2nd, this fixation is singularly promoted by the absence of oxygen.'

Thus when organic matter decomposes in an atmosphere deprived of oxygen, or nearly so, giving rise to carbonic acid and to hydrogen, the nitrogen of the atmosphere is absorbed and unites with the hydrogen to form ammonia.

It appears that Thenard and others have shown that in the soil there are, as it were, two atmospheres—one, an oxydising atmosphere in the upper layers, the other, a reducing atmosphere lower down. Dehérain points out that 'the energy of slow combustion is much greater than is usually supposed: germinating seeds in a closed space absorb the oxygen, even to the last trace, in a few days; aquatic plants kept in water in the dark take from it all the oxygen it contained. If the composition of the air confined in a heap of manure is determined, there is found only nitrogen and carbonic acid mixed with a slight proportion of combustible gas, oxygen is absolutely wanting. This is an experiment which we have repeated at Grignon for several years without variation.'

Thus there is in the soil, at a certain depth, an atmosphere devoid of oxygen—the decomposition of organic substances may give rise to hydrogen—the latter may meet with nitrogen and form ammonia.

I now pass to a summary of several other papers recently published, and bearing on the general question : they must, of course, stand on their own merits. In 1885 Berthelot[1] showed that the amount of combined nitrogen in pots of soil, exposed for some months to the atmosphere, continually increased; this was proved to be due to the absorption and 'fixation' of free nitrogen, and much in excess of any nitrogen compounds that could be supplied in rain, &c. Berthelot showed also that this action does not occur if the soil is sterilised by heat, and concludes thence that the action is due to the intervention of living organisms. The process, moreover, comes to a standstill in the winter, and is at its best when vegetation is most active.

The author concluded that in six months more than 26–32 kilos of nitrogen per hectare would be absorbed in his experiments.

In 1886, M. Berthelot[2] published further results, showing that nitrogen is continually absorbed from the air, even when no plants are being grown in the soil. The amount absorbed is in all cases very much greater than the quantity of nitrogen existing as ammonia or nitrogen oxides in the air or rain. Much of the absorbed nitrogen is converted into nitrates.

In the Comptes Rend., T. 104, p. 625, Berthelot again publishes results on this subject, especially referring to soil in which plants are being grown, and finds that less nitrogen is fixed than was the case with fallow soils. A further paper appears by the same chemist in the same volume, showing that, independently of the other processes, ammonia is continually being evolved from vegetable soils. This double action—fixation of nitrogen on the one hand, and the escape of ammonia on the other—has been noted by other observers also.

In the 'Proceedings of the Royal Society' for 1887[3], Messrs.

[1] Compt. Rend., T. 101, p. 775. [2] Ibid. T. 104, p. 205.

[3] Proc. Roy. Soc., p. 108, 'On the present position of the Question of the Sources of Nitrogen of Vegetation, with some new results, and preliminary notice of new lines of Investigation.'

Lawes and Gilbert give a resumé of the question of the sources of nitrogen in plants, and especially of their further results. In their earlier paper they concluded that, except the small annual increment of combined nitrogen washed down by rain, the source of nitrogen was substantially the stores in the soil. The compared growth of gramineous crops and of leguminous crops under parallel conditions resulted in the conclusion that more nitric acid accumulated in soils under Leguminosae, indicating increased nitrification.

Attempts to explain the increase of nitrogen under Leguminosae as due to the subsoil, or to the action of acids in the roots, &c., failed.

References are then made to the experiments of Berthelot and André, and of Loges, showing that the insoluble nitrogenous substances in soils are of the nature of amides.

Experiments are then adduced showing that green plants can take up soluble complex nitrogenous organic bodies in water-cultures, and possibly they can take up amides in the soil.

Frank's researches on Mycorhiza are then referred to: of course only the earlier paper is quoted. Then comes in the question of the participation of free atmospheric nitrogen, and the authors reserve their opinion, pointing out, however, that the soil contains enormous quantities of combined nitrogen, and that there is 'obviously still a wide field for enquiry as to whether or not, or in what way, the very large store of already existing combined nitrogen may become available to growing vegetation.'

In the above citations it is not by any means to be implied that a complete survey of the literature has been given or attempted; several papers have been passed over as either generally known, or too technical for the present purpose, and of course there is still much discussion on many points—e.g. as to the *modus operandi* of nitrifying organisms[1], as

[1] See Schlœsing and Müntz in Comptes Rendus, 1879; Warington, Chemical Soc. Journal, 1879 onwards; and Berthelot, Comptes Rend. 1876 onwards.

to the view that nitrogen is fixed by organic substances in the soil during slow electric changes, and so on.

The chief points to be summarised seem to be these. There is a general tendency to the view that the Leguminosae at least take more nitrogen from the soil than can be accounted for if the only sources are (1) the combined nitrogen of the atmosphere washed down into the soil, and (2) the combined nitrogen of the soil found by analysis of samples. It is therefore surmised that the free nitrogen of the atmosphere is 'fixed' under such conditions that it can combine with other elements, and so supplies the deficiencies.

In favour of this are quoted the experiments of Berthelot, Frank, Hellriegel, and others. As a point against the *necessity* of this—not as against the facts of such fixation—Messrs. Lawes and Gilbert especially remind us that sub-soils may and do contain large quantities of combined nitrogen, and it is still questionable how far these can be carried up into the soil, or reached by the roots of deep-rooted Leguminosae.

It should be noted that the water of the sub-soil (containing dissolved substances) may rise for long periods in dry summers, when the plants above are transpiring, by capillarity; hence the adduced increase of nitrates in the upper parts of the soil during active vegetation is not in itself a proof of absorption from the air. Of course this does not apply to pot-plant experiments.

Then comes the consensus as to nitrification by means of organisms in the soil. But it must not be overlooked that the usual case consists in the oxidation of nitrogenous compounds already present in the soil.

The startling point in Hellriegel's experiments—more cautiously entertained by Frank and Vines—is that organisms co-operate in the fixation of *free nitrogen* under such conditions that it then enters into combination. That we are here face to face with a difficulty must be clear to every one.

In conclusion, it seems that we cannot, as yet, clear up the question as to whether the fungus of the Leguminosae aids in the fixation of free nitrogen, and we cannot regard it as proved that the fungi of Frank's Mycorhiza take any part in providing the plant with nitrogenous elements, however probable it may appear. Moreover, I may suggest that the cases are not quite similar: in Frank's observations the fungus may merely hurry the decomposition of organic remains. With respect to the alleged absorbent function—or root-hair function—of Frank's fungi, it is only necessary to point out that it is difficult to imagine how a fungus hypha with its low and peculiar organisation can assume the remarkable and by no means simple functions of root-hairs: the anatomical facts are in Frank's favour, so far as they go, in reference to the Cupuliferae, but of course it is always hazardous to attempt to explain physiological problems simply on anatomical evidence.

With respect to *Vicia Faba*, there are no reasons for supposing that the fungus replaces the root-hairs functionally in any way; the experiments of Hellriegel, Frank and Vines, point to the possibility of its aiding in rendering nitrogen available, in some way as yet unexplained; and my own observations point to the probability that it stimulates the roots to absorb and use whatever nitrogenous materials are present with extraordinary avidity. One consequence of this is, no doubt, increased respiration,—i. e. a more rapid rate of absorption of the oxygen in the soil; but whether we can go further than this needs investigation, though it may have a bearing upon Dehérain's suggestion.

As regards the Leguminosae, therefore, we are still face to face with two distinct problems, quite independent of the old one as to the parasitic nature of the tubercles, which has been solved by my discovery of the causal fungus entering the root-hairs and stimulating the root-cortex locally. These two problems are: (1) Does the fungus in question directly co-operate in the absorption of food-materials from the soil, nitrogenous or otherwise? and (2) Does the fungus take any part in the preparation of nitrogenous substances, or the absorption and fixation of free nitrogen, so as to render them available to the plant?

If Hellriegel's results are confirmed, the last question is answered generally, the further enquiry narrowing itself into, How can the fungus act in the fixation &c. of free nitrogen? But the previous question will still remain to be answered, the evidence at present being distinctly against the view that the fungus aids directly in absorbing food-materials, and in favour of the supposition that it stimulates the plant to greater metabolic activity. It is only fair to add that the possibility that the combined fungus and stimulated cells—i. e. the root-tubercles—may act, as a whole, as a compound organism possessing the power of making use of the nitrogen, is not to be set aside as absurd so long as the question of a nitrifying organism can be entertained at all.