tion Prof. Dendy puts forward as to its function is quite a reasonably probable one.

GEO. E. NICHOLLS.

King's College, December 17.

Nitrogen-fixing Bacteria and Non-Leguminous Plants.

I REGRET that owing to Mr. A. D. Hall's letter of July 12 (NATURE, July 22) appearing during vacation, I had not an opportunity of seeing it until my return to college at the commencement of term. I trust that, in spite of the long delay, I may yet be allowed to reply to some of Mr. Hall's questions.

Mr. Hall commences by stating that my conclusions are based "on experiments to show that *Pseudomonas*, the bacterium associated with the leguminous plants, will fix more nitrogen, &c." This is not correct, for it is definitely stated in the abstract that both the *Pseudomonas* and the *Azotobacter* used for the nitrogen determination results quoted were obtained from a non-leguminous plant—the root tubercles of *Cycas*.

There are four non-leguminous plants possessing root tubercles which contain nitrogen-fixing organisms. In all four a species or variety of *Pseudomonas* is present, but in *Cycas* only is *Pseudomonas* found living outside the cortical cells, in the algal zone, and in *Cycas* only is *Pseudomonas* found in association with *Azotobacter*. As stated in my paper, the nitrogen determinations quoted had reference only to these organisms from *Cycas*, and were made in order to determine to what extent, if any, *Cycas Azotobacter* assisted *Cycas Pseudomonas* in assimilating free nitrogen.

"*Pseudomonas*, the bacterium associated with the leguminous plants," and the use of "a reasonably active culture" of *Azotobacter*, to quote Mr. Hall, had nothing to do with the determination experiments. On what grounds, then, does Mr. Hall state that "the only conclusion that could be drawn from Prof. Bottomley's figures would be that *Pseudomonas* injuriously affects the power of *Azotobacter* to fix nitrogen"? Naturally, a determination of the amount of nitrogen fixed by the *Azotobacter* alone was made (for Mr. Hall's information I may say that it was very small, -0.56 mgr.), but as the immediate object of the experiment was to determine the effect of *Cycas Azotobacter* on *Cycas Pseudomonas*, it was not thought necessary to quote the figures for *Cycas Azotobacter* alone, especially as this will be dealt with in a future paper.

Again, when speaking of the experiments with oats, Mr. Hall says that the mean error of ± 10 per cent. found in Rothamsted experiments "would more than cover the differences observed by Prof. Bottomley's experiment with oats." The figures given for oats are:—average weight per plant, untreated, 0.42 gm.; treated, 0.74 gm.; increase, 0.32 gm., or 76 per cent. Surely Mr. Hall cannot have read the abstract carefully or he would not have stated that a mean error of ± 10 per cent. more than covers an increase of 76 per cent.! As regards probable experimental error, the abstract states that the oats were "grown in sand, dressed with phosphates, potash and lime." Why, then, should Mr. Hall assume that they were grown "in soil which presumably already contains both organisms"?

As regards the field experiment with barley, duplicate samples were not taken from different parts of the untreated plot, but the total yields from treated and untreated plots were kept separate, and the treated showed an increase of 13.6 per cent. The sample for estimation of nitrogen content was taken from the bulk in each case.

The treated bulbs gave an increased yield of 18.6 per cent. They were not selected as being specially "suitable for experiments on nutrition," but to determine the effect of the mixed culture of bacteria on a totally different kind of plant from any of the other experiments. The bulbs were already planted when the experiment commenced, hence the original weight of the bulbs is not available. As, however, the bed contained 500 bulbs, and was divided

into two equal halves with 250 bulbs in each, one may assume that the original weight in each half was approximately equal.

As regards the culture solution "being a considerable factor in any beneficial effect experienced," it is difficult to comprehend how $4\frac{1}{2}$ grs. potassium phosphate and $\frac{1}{2}$ gr. magnesium sulphate dissolved in a gallon of water for the culture solution, and this afterwards diluted with fifty gallons of water before applying, could possibly produce any appreciable effect on growing crops. Assuming that the bacteria had not used up any of the culture salts in their growth to produce the culture solution, can one imagine any benefit to plants by watering them with a solution containing 1/50,000 part of potassium phosphate and 1/450,000 part magnesium sulphate?

Mr. Hall appears to consider his questions "somewhat critical." True and fair criticism is always welcome in scientific investigation, for it is only thus that one can get at the truth of things, but misstatements of fact or misrepresentations of results exceed the bounds of criticism. So far as Mr. Hall correctly states the experiments and results given, one welcomes his criticisms.

W. B. BOTTOMLEY.

King's College, Strand, W.C.,
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IN Prof. Bottomley's paper in the Proceedings of the Royal Society I cannot read that he makes any claim that the *Pseudomonas* and the *Azotobacter* he isolated from *Cycas* were in any way different in kind from the usual forms of these organisms. When I used the expression "*Pseudomonas*, the bacterium associated with leguminous plants," it was as a sort of explanatory label for the lay reader, but if it has confused the issue at all I would still repeat my former criticism on Prof. Bottomley's basis that the *Pseudomonas* and *Azotobacter* he used are special and unlike all others. The question was if "the association gave an increased power of assimilating free nitrogen," and Prof. Bottomley's figures are:—

Control...	...	...	0.48	mgm. nitrogen
<i>Pseudomonas</i> alone ...	...	...	0.91	" "
<i>Pseudomonas</i> + <i>Azotobacter</i> ...	...	...	1.24	" "

and I asked if the action of *Azotobacter* alone ought not also to be known before any answer is possible. Prof. Bottomley now tells us that *Azotobacter* alone fixes 0.56 mgm., so that I still conclude that *Pseudomonas* and *Azotobacter* together (1.24) are less effective than when grown separately (0.91+0.56), were I not more inclined to think that all the differences are within the limits of experimental error.

To pass to the experiments with plants, by an error which the context rendered sufficiently obvious I wrote "oats" instead of barley when dealing with Prof. Bottomley's first-quoted experiment with soil. The oat experiment is beside the point; it only demonstrates fixation of nitrogen by *Azotobacter* and *Pseudomonas*—a fact on which we are all agreed; but when Prof. Bottomley claims increased crop production due to inoculation with the two organisms in the open ground, we do want the means of judging what weight to attach to the results. He gives for the first barley experiment a gain of 13.6 per cent.; in barley 2 the increase cannot be estimated; the *Galtonia* experiment shows 18.6 per cent. increase, the parsnips 21.7 per cent. Such percentage differences are not outside the limits of error for a single plot experiment worked on a large scale with every condition in favour of accuracy; when they are differences between lots of 250 bulbs or 68 parsnips I have no hesitation in regarding them as without any significance whatever. I have just had pulled and weighed two lots of fifty successive roots of mangolds growing side by side in neighbouring rows on the same plot, inside rows perfectly similar to the eye and on a very uniform plot, yet one lot weighed 220 lb., the other lot 176 lb., a difference of 25 per cent. The assistant who gathered the roots was quite unaware of the question involved; his instructions were to begin inside the plot and take fifty consecutive roots along a row, then another fifty