

done by biologists, especially in cases in which there was no possible excuse for it.

Professor Grave has fortified himself against confirmation of my views by assuming the position that even if no reversal of the beat of cilia is to be observed when my methods are employed, "it seems clear that it was due to the fact that the animals on which he made his observations were, in every case, in a mutilated condition." I removed the shell, "and," he says, "in its removal the adductor muscle was cut and the visceral ganglion, which is imbedded in this muscle, was necessarily severely injured. Under such a condition of shock normal behavior is not to be expected, especially in the case of activities that may be subject to nervous control."

Here is another pure assumption, made without observation, or even the opinion of some one else to substantiate it. I have no reason to believe that there is any element of truth in it; and I have several reasons for believing that it is not true that cilia of the palp, gill or mantle tracts are in any way under the control of the nervous system (such as the continued and unchanged beat on fragments of any of these organs, and also on isolated single cells, facts that can not be presented here).

Now the action of gill and mantle cilia are precisely the same in normal and in "mutilated" *Pectens*, and in some other lamellibranchs that open the shell valves widely, a condition that I have observed very many times. Why should Professor Grave not naturally expect these cilia tracts, as well as those of the palps, to behave abnormally from the detachment of the end of the adductor muscle? For he must know that gills and mantle receive large nerve trunks from the visceral ganglion, while the palps do not. The palps are so situated that they can not be examined without removing the shell valve, or using great force to pry the valves far apart by stretching the adductor muscles, and I have not seen their currents otherwise. I would like to ask Professor Grave if Engelmann was careful not to mutilate the lamellibranch on the palp of which he discovered a reversal of the cilia beat?

Finally, the cause of my mistakes in observation, we are told, was that when the end of the adductor muscle was separated from its shell attachment, the visceral ganglion "was necessarily injured." I venture to offer the information that, when one actually tries the experiment, it will be found that a shell valve may quite easily be removed from any lamellibranch without touching the visceral ganglion, or any of the nerves arising from it; and that to say that it is necessarily injured in the process is but to add another to the list of these entirely unsupported assumptions. This *a priori* method of arriving at truth ought to be even more out of place in present-day biology than the employment of analogies. Very likely, the use of the binocular dissecting microscope, which I did not have because it was not yet invented, will show that I made mistakes; but years were spent in making the observations before they were published, and perhaps I may be pardoned for objecting to their summary dismissal, in some cases with a very small show of reason, and in others with none at all.

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#### CHLOROSIS OF PINEAPPLES INDUCED BY MANGANESE AND CARBONATE OF LIME

It has been recently found by M. O. Johnson at the Hawaiian Experiment Station that the chlorosis of pineapples occurring on highly manganiferous soils can be cured by spraying the leaves with ferrous sulphate.<sup>1</sup> As the chlorosis of pineapples growing on strongly calcareous soils in Porto Rico can also be cured by the application of iron salts, some have the idea that the two forms of chlorosis are the same. Although the phenomena are remarkably similar in many respects, and although the cure is the same, it is not yet clear that they are identical. It seems advisable to point out certain differences that seem to exist in the two kinds of chlorosis.

<sup>1</sup> *The Pacific Commercial Advertiser*, Honolulu, Hawaii, July 21, 1916, and a personal communication.

The chlorotic pineapples in Hawaii occur on acid or neutral soils that average 5.0 per cent.  $\text{Mn}_2\text{O}_3$  and 0.5 per cent.  $\text{CaO}$ .<sup>2</sup> The chlorotic pineapples in Porto Rico occur on soils containing from 2 to 80 per cent. carbonate of lime and no manganese. That the chlorosis in Porto Rico is induced by the carbonate of lime was proved by direct experiment. Soils which normally produced healthy pineapples were made to produce chlorotic plants by the admixture of carbonate of lime from different sources.<sup>3</sup> We may thus speak of one as a manganese-induced chlorosis and the other as a lime-induced chlorosis.

The lime chlorosis was shown to be due to a lack of iron in the plant, caused by the carbonate of lime diminishing the availability of iron in the soil. At first it was not known whether the chlorosis was due merely to a lack of iron or to a lack of iron combined with a large amount of lime in the plant. Recent work seems to show that it is merely due to a lack of iron.

Now the manganese chlorosis may be similar to the lime chlorosis if the manganese acts similarly in merely diminishing the availability of iron in the soil. The recent discovery of Johnson shows that this may be possible. Previous work by Kelley,<sup>4</sup> and Wilcox and Kelley<sup>5</sup> suggests, however, that the manganese chlorosis may be more complicated. In the work of these investigators there is some evidence of a direct toxic effect of the manganese, although they do not ascribe the chlorosis to this. It is possible that the manganese chlorosis is due to a deficiency of iron combined with a direct toxic effect of the manganese. From the results so far obtained it can not be said whether the two kinds of chlorosis are essentially the same. Certain differences in the appearance and behavior of

pineapples affected with manganese and lime chlorosis give reason for supposing the two forms may be more or less distinct.

The manganese plants are described as having roots with swollen tips and a generally poor root system, while the lime plants have good root systems, differing from normal plants only in the roots being longer.

In the development of "manganese yellows" a purplish color is spoken of as preceding the yellowish-white. This purplish color was not observed in the lime-induced chlorosis, although leaves sometimes had red splotches.

The manganese chlorosis is described as being most intense during the winter months when we may assume normal plants were growing less rapidly. The lime-induced chlorosis we found to develop fastest in plants growing most rapidly and to be more intense the more sunlight they received.

The application of ferrous sulphate to the leaves apparently has a more permanent effect on the manganese plants than on the lime plants. From the reports so far it appears that a few sprayings permanently cure the "manganese yellows," while application of iron salts to pineapples, rice, or sugar cane affected with lime-induced chlorosis effects only a temporary cure. Repeated trials showed that the treatment must be made frequently to maintain the plants in a green and vigorous condition. With rice growing on a strongly calcareous soil it was necessary to spray sixteen times with ferrous sulphate to maintain a normal growth. This difference in the amount of treatment necessary to cure the two forms of chlorosis may merely indicate a difference in the extent to which manganese and carbonate of lime depress the availability of iron.

The differences pointed out lend ground for supposing that manganese chlorosis may be due in part to a deficiency of iron in the plant, induced by the action of manganese in the plant or in the soil, and in part to a direct toxic action of the manganese. Lime-induced merely the result of a lack of iron in the plant, due to carbonate of lime diminishing the availability of iron in the soil. It is of

<sup>2</sup> Kelley, W. P., Hawaii Agr. Exp. Sta. Press Bull. No. 23.

<sup>3</sup> Gile, P. L., Porto Rico Agr. Exp. Sta. Bull. No. 11, 1911.

<sup>4</sup> Kelley, W. P., Hawaii Agr. Exp. Sta. Bull. No. 26, 1912.

<sup>5</sup> Wilcox, E. V., and Kelley, W. P., Hawaii Agr. Exp. Sta. Bull. No. 28, 1912.

course possible that the two kinds of chlorosis may be found to be essentially the same except for certain secondary effects produced by an undue absorption of manganese.

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#### RELATIVE IMPORTANCE OF FUNGI AND BACTERIA IN SOIL

TO THE EDITOR OF SCIENCE: In a recent number of SCIENCE,<sup>1</sup> Waksman discusses the question: "Do Fungi Live and Produce Mycelium in Soil?" He answers the question in the affirmative. I have been interested in the same question for some time and have arrived at conclusions slightly different from those to be inferred from Waksman's article.

Waksman has tested for the presence of mycelium by placing a lump of soil about 1 cm. in diameter in the center of a plate of agar (containing Czapek's solution<sup>2</sup> for nutrient material). After 24 hours at 20–22° C., he finds that mold hyphæ radiate out into the medium from this lump of soil. If instead of soil he uses a drop of water in which mold spores are suspended, the amount of mycelium produced in 24 hours is very much smaller. From these findings he concludes that such a lump of soil contains living mycelium.

This conclusion is undoubtedly supported by theoretical considerations. The soil is continually showered with mold conidia from the air and without question contains moisture enough to allow them to germinate even if conditions are not favorable for their long-continued growth. The presence of mushrooms, moreover, in woodland soil and even in fields and lawns, proves beyond doubt that conditions do favor the growth of certain Basidiomycetes, at least. Their mycelium undoubtedly penetrates the soil sufficiently to be present in a lump as large as that used by Waksman. The question of real importance,

<sup>1</sup> SCIENCE, N. S., 44, pp. 320–322.

<sup>2</sup> Waksman does not publish the formula of this solution, but it has been obtained from him in a personal letter. It is:  $\text{MgSO}_4$  0.5 g.,  $\text{K}_2\text{HPO}_4$  1 g.,  $\text{KCl}$  0.5 g.,  $\text{FeSO}_4$  0.01 g.,  $\text{NaNO}_3$  2 g., Sucrose 30 g., to one liter of water.

however, is whether the mycelium is abundant enough in the soil to compare in its activities with the soil bacteria. Waksman does not discuss this question; although from his statement that the plate method gives figures as high as 1,000,000 fungi per gram soil, the natural implication is that they must be nearly as important as bacteria. His actual data, however, show nothing of the sort. He merely shows that mold hyphæ can be found in lumps of soil 1 cm. in diameter. A lump of soil that size contains many millions of bacteria. Compared to their activities, those of a few mold hyphæ would be quite insignificant.

I have tested several soils by Waksman's method, and have generally obtained results similar to his; but because the information furnished by it is not quantitative, I have modified the method by the use of smaller quantities of soil (crumbs weighing about 10 mg.), with quite different results. Such a crumb of soil should contain, according to the plate method—which is generally acknowledged to give figures that are much too low—perhaps 100,000 bacteria. If fungi are of anything like the same importance as bacteria in soil, one of these crumbs should certainly contain mold hyphæ. Their presence, however, has been indicated only in the case of soil to which large amounts of organic matter (manure or grass roots) have been added. When crumbs of soil to which no organic matter has been added have been used, the development of mold hyphæ in the agar has been slower than in the case of drops of water containing nothing but mold conidia. This certainly suggests that no mycelium is present in these small crumbs and that molds are relatively insignificant in soil; but as the crumbs of soil were always surrounded at the end of 24 hours by vigorously growing bacteria, it is possible that the development of mold hyphæ may have been suppressed. For this reason, Waksman's method is considered inconclusive.

It seems as if the question could be conclusively answered only by the use of a microscope. The microscope would furnish direct instead of presumptive evidence on the sub-