5. The complete removal of the ammonia from the Kjeldahl mixture should be tested with Nessler's solution. The precaution of Van Slyke to run the aeration slowly or at half speed for the first minute or two may be an advantage.

Addendum.

After sending in this paper for publication I learn that recently a number of other investigators have tried the aeration method and found it accurate. I. K. Phelps and H. W. Daudt, from the Bureau of Chemistry, Washington, D. C., reported favorably on the method at the Urbana meeting of the Society. B. S. Davisson, E. R. Allen and B. M. Stubblefield[1] were able, with a powerful aeration, to remove and absorb small amounts of ammonia from large volumes of solution accurately, using only magnesium hydroxide as an alkali.

ALBANY, N. Y.

[CONTRIBUTION FROM THE SPRECKLES AGRICULTURAL RESEARCH LABORATORY.]

PRESENCE OF NITRITES AND AMMONIA IN DISEASED PLANTS. ITS SIGNIFICANCE WITH REGARD TO CROP ROTATION AND SOIL DEPLETION.[2]

BY P. A. BONCQUET.

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The present study was made independently from the discussion now at large concerning the presence of nitrites in plants.[3] It is not the intention of the writer to decide the above contentions, but only to make known some facts which may throw new light on the controversy.

Nitrites in Diseased Beets.—During previous work *Bacillus morulans*, Boncquet,[4] was found to be an inhabitant of the sieve tubes of sugar beets affected with Curly Leaf.[5] Moreover it was established that the organism was not confined to the disease of beets called Curly Leaf, but that it was connected with a great variety of irregular foliage types representing various forms of leaf wrinkling, curling and distortions.[4] The same organisms were also isolated in cultures from the interior of leaves of beets

[1] *J. Ind. Eng. Chem.*, 8, 896 (1916).

[2] The writer is greatly indebted to the Spreckles Sugar Company for the splendid facilities placed at his disposal in the realization of this investigation.

[3] Klein, *Bot. Centbl. Beihefte, I Abt.*, n 1, **30**, 141–166 (1915); *E. S. R.*, **33**, 627; Oso and Sekine, *Ibid., I Abt.*, **32**, 146–147 (1914); *E. S. R.*, **33**, 627; Maze, *Compt. rend. soc. Biol.*, [Paris] **78**, 98–102 (1915); *E. S. R.*, **34**, 627.

[4] Boncquet, *Baccillus morulans n. sp.* A bacterial organism which inhabits the sieve tubes of sugar beets and related plants. Its characters and significance. A thesis presented for the degree of Doctor of Philosophy at the University of California.

[5] Ralph E. Smith and P. A. Boncquet, "New Light on Curly Top of the Sugar Beet," *Phytopathology*, **5**, 103–107 (1915).

affected with the diseases called Mottled Leaf, Black Leaf, and Black Tip of beets.[1]

As the biochemical investigations of *B. morulans* proved this organism to be a most vigorous nitrate reducer,[2] it was in logical sequence to test for nitrites any plant tissue in which the organism was found as a facultative parasite. These investigations were crowned with success every time the slightest irregularity revealed the presence of bacteria in the plant tissues. On the other hand, absolutely smooth and sterile tissue gave, without any exception, a perfectly negative result. Not even a trace of nitrite could be detected in perfect, normal leaves. In some cases, however, even a slight irregularity in the vein distribution indicated traces of nitrites which could be detected in the leaf juices.

Ammonia in Diseased Beets.—The reducing power of *B. morulans* Boncquet is not confined to the reduction of nitrates to nitrites, but the bacillus is found able to reduce nitrates as far as ammonia. In consequence of this, the same tissues which were found to contain nitrites were also tested for ammonia. The presence of the organism likewise decided whether the leaf juice contained ammonia or not. Under no circumstances was ammonia detected, where the leaf was found strictly nonpathological. Under some circumstances some leaves contained more ammonia than nitrites in comparison to other beets. This was especially true if the leaves had been separated from the crown of the plant, or if the plant had been taken from the soil and left to wilt. The reduction was sometimes found so far advanced that only ammonia could be detected, all nitrites having been reduced.

Nitrites and Ammonia in Tobacco Leaves Affected with the "Mosaic Disease" (filtrable virus).—An exceedingly small streptococcus was found to be connected with this disease. Its biochemical functions proved to be vigorous in reducing power. Nitrates in the test tubes were easily converted to nitrites and even ammonia. Thereupon the juices of diseased leaves were also tested for nitrites. It was found that normal tobacco leaves did not give the test for nitrites. Conversely, the juice of all diseased leaves gave a very strong nitrate reduction; ammonia was also detected.

Nitrites in Potato Leaves and Tubers.—A streptococcus called for the present *Streptoccocus solani n. sp.* was found uniformly in certain potato vines and leaves. The fact that the micrococcus was found to be a most active nitrate reducer naturally suggested the presence of nitrites in the plant juices. It was found that all potato plants of the leaf type containing the micrococcus give a vigorous nitrite reaction in their expressed

[1] Ralph E. Smith and P. A. Boncquet, "Connection of a Bacterial Organism with Curly Leaf of the Beet Sugar," *Phytopathology*, 5, 335–342 (1915).

[2] Boncquet, *Loc. cit.*

leaf sap. Slight traces of ammonia were also detected. On the other hand, leaves and tissues of plants which did not possess any abnormalities and of a totally different leaf type, give negative results. This pathological condition of the potato has not been described distinctly from the pathological standpoint, owing to the fact that the plant appears normal in the broadest sense. The writer has at present a great deal of evidence that this condition is connected with the so-called "brown streak" and with the "blossom abortion" of potatoes, all of which appear to be different symptoms of one and the same disturbance, brought about by *Streptococcus solani*. The plants which gave the most pronounced nitrite test were growing very luxuriantly. The leaves were dark green, crinkled, however, and light revealed irregular patches of lighter green all over the leaf; more or less of the nature of those patches to be observed in the mosaic disease of tobacco. The tubers, however, were not affected with the regular "brown streak" but rather with a disturbance better called "brown or petrified heart." This petrifaction consists of the cells of the heart of the potato being slightly incrusted with a light cement. Patches of necrotic cells are distributed irregularly throughout the hard mass. Numerous conglomerates of streptococci were abundantly observed in and around the brown tissues. Although the nitrites were very abundant in the leaf tissues, the affected tubers gave only traces of nitrites. This absence of nitrites with the presence of an abundant bacterial flora is easily understood when chemical analysis revealed only traces of nitrates present in the tubers.

Nitrates in Other Plants.—Several other plants which showed abnormalities in leaf or stem structure where examined for their nitrite content. It was found that some individual alfalfa, bean and Malva rotundifolia plants gave a decided nitrite reaction. The intensity of the reaction ran parallel with the severity of the sickly conditions in which the plants were found. However, a yellow color was not always a sign that the plant contained nitrite or ammonia. It was rather the abnormal plant development from a structural standpoint, like the deformities of leaf, dwarfing, curling and distorting that manifested the disturbance.

All plant juices from plants where these abnormalities had been found, although obtained aseptically, were able to reduce nitrates to nitrites when inoculated in nitrite-free peptone tubes. At the same time a considerable growth of bacteria was obtained, which proved on subsequent inoculation to be very active nitrite reducers.

Significance of the Presence of Nitrites in Plant Tissues.

Relation of the Internal Bacterial Flora to Soil Depletion and Crop Rotation.—The presence of nitrites in plants, at least in all cases which the writer had under investigation, is due to the reducing power of the internal bacterial flora in the tissues. Nitrites have not been detected in any plant tissue which was normal in the strictest sense of the word. The pathological disturbance is brought about by a partial and local nitrogen starvation of the tissues, and further by the mechanical laceration of local foci, where the bacteria in some cases, by their active growth, were forming metastatic ulcers.

The local and partial nitrogen starvation in the leaves around nitrate-reducing foci explains the mozaic nature of the leaf diseases in which an abundance of nitrite is detected. The foci of infection, found at the headgate of some secondary leaf veination, explains just why that region in the leaf which is under the control of the infected headgate, suffers most from starvation and why it is pale yellow while other regions of the leaf, not controlled by a diseased headgate in the veination, are abundantly supplied with nitrates and consequently develop the heavy green color so characteristic of nitrogen abundance.

The mechanical laceration of infected foci is the cause of a local arrest of growth in the veins and this arrest of growth brings about leaf distortion. In some other cases the internal bacterial flora is uniformly distributed throughout the plant tissues. In consequence of this the symptoms are also uniform, and result in dwarfing and arrested growth as in "little leaf" diseases.

The pathological condition due to the disturbance of a nitrate-reducing bacterial flora in the tissues, may even be without any visible symptoms. Nevertheless, in these cases the nitrite-containing juices extracted aseptically from diseased plants when inoculated in nitrite-free peptone tubes, always brings about a vigorous nitrate reduction, with an abundant growth of nitrate-reducing bacteria. Tissues free from nitrate on inoculation give a sterile tube with no tract of nitrite to be detected.

Significance to Soil Depletion and Crop Rotation.—Further studies and field observations have led the writer to believe that the nitrite content of plants and consequently the internal bacterial activity which brings the nitrate-reduction about, has a fundamental bearing on certain cases of soil depletion. This is especially believed to be true in potato fields. A field was observed which had been planted with potatoes for more than fifteen years. Nearly every vine was affected with nitrogen starvation due to internal bacterial reduction.

On the other hand, the soil in which the potatoes were growing was abundantly supplied with nitrates. The analysis showed more than sixty parts per million of soluble nitrates. In this abundance of plant food the potatoes gave a poor yield. It seems, especially from observations on beet fields, that the virulence of nitrate-reducing bacteria as invaders of plant tissues is increased by lack of crop rotation and consequently reduces the yield in some cases to such a point that the reduction is attributed to soil depletion.

Summary.

Nitrites in plants in the cases observed were due to the reducing power of the internal bacterial flora. This reduction is the cause of nitrogen starvation of plants affected with some peculiar diseases. This nitrogen starvation may occur even when plants are growing on a soil where nitrates are abundant. The internal bacterial flora of plants with its nitrogen-reducing power may be a direct factor in soil depletion where crop rotation is not practiced, owing to the increased virulence and invading power of reducing bacteria. It is believed that lack of crop rotation provides soil organisms a better opportunity to establish themselves in the veins and tissues of plants by means of adaptation.

SPRECKELS, CAL.

NEW BOOK.

Annual Reports of the Progress of Chemistry for 1915. Issued by the Chemical Society. Vol. XII. London, 1916. Pp. viii + 268. Sold by D. Van Nostrand Company. $2.00 net.

In 1905 the Chemical Society of London inaugurated the publication of this series of annual reports of the progress of chemistry. Issued usually in the late spring, they aim at giving an epitome of the most important work of the preceding year. For the busy chemist who would know what is taking place in other branches of chemistry than his own special field, they are invaluable; within his field he will find them at least interesting and suggestive. Prepared so soon after the close of the year, they naturally lack perspective, and they may often give undue prominence to topics for which the reviewer has a particular bias, but this detracts little from their value. The reviewers of the different fields are for the most part specialists and investigators, which lends interest and value to their work. The contents of the present volume are: General and Physical Chemistry, H. M. Dawson. Inorganic Chemistry, E. C. C. Baly. Organic Chemistry: Part I, Aliphatic Division, J C. Irvine; Part II, Homocyclic Division, F. L. Pyman; Part III, Heterocyclic Division, A. W. Stewart. Analytical Chemistry, G. Cecil Jones. Physiological Chemistry, F. G. Hopkins. Agricultural Chemistry and Vegetable Physiology, N. H. J. Miller. Mineralogical Chemistry, T. V. Barker. In time past the American Chemical Society has published occasional similar reviews in THIS JOURNAL. Such duplication of preparation is unwise to say the least, but it is a pity that some arrangement cannot be made whereby such reviews in the English language may be put in the hands of every member of both societies, and we should add that the Society of Chemical Industry ought also to coöperate in the work.

JAS. LEWIS HOWE.