extractor. On evaporation of the extract the peculiar crystals already referred to were obtained, and then purified by recrystallization.

Analysis of the product gave the following results:

	Found.			Calc. for $C_6H_{13}NO_2$.
C	54.78	54.34	55.14	54.92
H	9.88	9.50	9.99	9.99
N	10.78	10.78	10.85	10.69
NH_2-N (Sörensen)	10.79	10.79	.....	10.69

The figures agree closely with those calculated for leucine. The substance was then further identified by its specific rotation and by the properties of its benzoyl derivatives.

A 5% solution of the substance in 20% HCl (sp. gr. 1.10) was examined in the polariscope. The average of six readings was +2.3° V.

$$[\alpha]_D = +15.9°.$$

The specific rotation of *l*-leucine in HCl as reported by Fischer is +15.8°.

The benzoyl derivative melted at 109° and contained 5.89% N; theory for benzoylleucine, 5.96% N.

In the samples of sweet clover silage examined, the amount of leucine recovered ranged from 0.4 to 1.0% of the dry material. Leucine has not been obtained by the writer from any sample of corn silage, nor has its occurrence in silage been reported by any previous investigator.

AMES, IOWA.

PRESENCE OF NITRITES AND AMMONIA IN DISEASED PLANTS.

II. OXIDASES AND DIASTASES; THEIR RELATION TO THE DISTURBANCE.

BY P. A. BONCQUET AND MARY BONCQUET.

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In a previous paper[1] it was said that in certain plant diseases of the so-called physiological type such as Curly Top,[2] also called Curly Leaf of sugar beets, Curly Dwarf of potatoes, Mottled Leaf of the potatoes,[3] Mosaic disease of the tobacco, nitrites and ammonia were detected in the extracted juices. The origin of this nitrite and ammonia was supposed to be the absorbed nitrates from the soil. The reduction of these nitrates in the plant tissues was supposed to be caused by bacterial action from the fact that the presence of nitrate-reducing bacteria in the plant tissues run parallel with the presence of nitrites and ammonia in diseased plants. In this paper some more facts about the same diseases are re-

[1] THIS JOURNAL, **38**, 2572 (1916).

[2] E. D. Ball, U. S. Dept. Agr., Bur. Ent., *Bull.* 66 (1909); C. O. Townsend, U. S. Dept. Agr., Bur. Plant. Ind., *Bull.* 122 (1908).

[3] W. A. Orton, U. S. Dept. Agr., Bur. Plant Ind., *Bull.* 277; F. C. Stewart, New York Agr. Expt. Sta., *Bull.* 422.

ported; they all seem to corroborate the idea of nitrogen starvation of plants by the bacterial reduction of the nitrates to nitrites and ammonia, after they have been taken up by the roots. In all these diseases about the same phenomena occur in the parts affected. This similarity is especially noticeable if the cell is considered as a unit of life. The response to the stimulus is so strong as to manifest itself in biochemical, physiological and even morphological changes. All these responses and adaptations apparently have one single aim in view: the supply of the necessary nitrogen to the plant cell. This increase of biological activity however is in itself a pathological phenomenon. As the disease progresses, the symptoms of nitrogen starvation become more and more apparent till the plant finally makes no further progress in growth, completes its life cycle in a nanified condition or dies before having reached maturity.

Phenomena of Stimulation.

Oxidases.[1]—The biochemical phenomena appear to be the first to respond to the stimulus of the invader. Among these, the first noticeable are the decrease and subsequent increase of the oxidases. Indeed a strong reducing force is introduced in the tissues of the plant by the invasion of reducing organisms. These tend to destroy some well-defined oxidases. (In the cases we have examined we have found that the α-naphthol reaction was totally absent. The benzidine reaction however had hardly been affected.) Subsequently an increase in oxidizing enzymes is noticed, especially in the new leaves, which have responded positively to the stimulus, of the invasion.

It has been found upon microscopical investigation that the response of the oxidases to the reducing stimulus of the bacteria is first negative and can be observed in some cases even before any cell lesions or protein hydrolysis can be detected in the phloem sieve tubes. In normal leaves of beets and many other plants the cells, the cell walls and the intercellular spaces of the bundle sheath appear to be the generating source and storage place of powerful oxidizing enzymes. The inside of the bundle, the xylem and the cambium region appear to be free. In the phloem on the other hand small streams of enzymes are originating from the sheath and circulate between the cells. Upon infection, the oxidases of the bundle sheath first decrease and even disappear completely, but later increase considerably and migrate in thicker streams towards the infected cell. This abnormal increase is especially noticed in the new born leaves; old leaves sometimes do not respond at all positively and remain without any oxidases till they wilt and die. In the beginning, the increase in oxidases in the bundle sheath is not general, but starts around one or two cells and expands subsequently to the other cells of the sheath. The place where the increase starts will be found to be the sheath cell

[1] Bunzel, *J. Agr. Res.*, 2 (1914); U. S. Dept. Agr., Bur. Plant Ind, *Bull.* 277.

closest to the infected sieve tube. The increase in flow of the oxidases from the cell wall and the intercellular places of the sheath seems to take place through the intercellular spaces of the cell walls of the phloem, so as to take the shortest way to the infected region. They surround the infected cell and penetrate its walls, but have not been observed to enter the lumen of the diseased sieve tube itself. When the disease progresses the protein content of the infected phloem cells disorganizes. It gives a positive reaction with neutral red, which resists more to washing out than the surrounding healthy tissue. Living organisms are distributed loosely among these disorganized proteins; nitrites and ammonia are found in the plant sap. The cellulose wall of the infected tube does not collapse however; the nitrate-reducing bacteria in the plant do not seem to hydrolyze celluloses, nevertheless when several adjacent cells of the phloem are infected, their common wall soon collapses, perhaps through subnutrition and the formation of a lesion or phloem necrosis becomes apparent. It seems that the oxidases surround the whole infected area and follow the infection increasing in density. At this time they seem to stream in more abundantly from all sides out of the bundle sheath between the cell walls and through the intercellular spaces of the sieve tubes which still remain healthy. These observations have been made in the diseases of Curly Leaf of the sugar beet, and also in the leaves of potatoes affected with Leaf Roll. These facts seem to indicate that the increase of oxidases in plants affected with bacterial nitrogen starvation is a direct effort of the physiological functions of the plant to overcome the reducing forces of the bacteria. As no method for the histological detection of the oxidases seems to have been described up to the present, it may be advisable to describe here briefly one of the methods used. The material to be examined is collected and immediately put in 60% alcohol (100 cc.) to which 5 cc. of hydrogen peroxide has been added. The specimens are left in this solution for some time, until the catalases are exhausted. Sections are subsequently cut and put on the slide in a drop of α-naphthol solution, one gram in 200 cc. of 80% alcohol. The reagent is left to dry on the slide till the α-naphthol begins to form a white cloudiness on the sides of the drop. Then a drop of hydrogen peroxide is added to the section, with also a small quantity of $6/N$ NaCl solution. The slide is passed through the flame of a Bunsen burner until vapors appear and is then left to cool slowly. In from one to two minutes the reaction will be complete. The section is then passed without previous washing into a drop of glycerine and is ready for examination. The sections must be examined at once, as secondary adsorptions tend to shift the color reactions to places where they were not originally formed.

Diastases.—Equal in importance to the behavior of the oxidases in plants affected with nitrogen starvation, caused by an internal reducing bacterial flora is the increase and behavior of the diastases. The location of their increased activities has been the subject of precise investigations by M. Boncquet. After overcoming many technical difficulties, it was found that all tissues under the influence of the bacterial stimulus developed a most vigorous increase in starch-splitting power. Tissues which contained the abnormal heavy green color, were always found to be five to six times faster in their reaction than normal tissues. Conversely, tissues which were yellowing and showed visible symptoms of nitrogen subnutrition were sometimes found to be devoid of starch-hydrolyzing power.

Formaldehyde in Beets Affected by Curley Leaf.—A most remarkable fact, repeatedly observed, is the presence of an increased amount of formaldehyde in the juices of diseased beet leaves. This most extraordinary phenomenon however, has not been studied enough to permit the drawing of conclusions. It may be suggested, however, that it represents only an increase in the CO_2 fixing power of the stimulated tissues.

Starch Formation.—The leaves or parts of leaves, which contain the greatest amount of diastases were precisely the tissues that were found to be most active in starch formation and starch storage. These tissues, especially the heavy green abnormal palisade formations of tobacco leaves, affected with Mosaic, were found to be full of starch after exposure to sunlight. On removal of the plants to darkness, the full store of starch in these cells quickly disappeared. Conversely, cells from tissues which were yellow or less green, even if taken from the same leaf (Mosaic of tobacco), were found poorer in starch formation. But the removal of the few grains of starch present was found to be slower on exposure to darkness. Sometimes the contrast was so marked that the cells of the less green tissue contained still some starch grains when all the starch had been removed from the heavy palisade cells.

Sugar Storage.—Sugar beets affected with Curly Leaf have, in the main, a far higher sugar content in the roots than normal beets growing under the same conditions. This increase in sugar, however, is only noted in beets which have answered by their increased chlorophyll production to the stimulus of the invading nitrate-reducing bacteria. Beets which have not responded to this stimulus, and had from the time of their infection with Curly Leaf decreased their chlorophyll content, were found to be poorer in sugar in the roots. The formation of great quantities of starch in the stimulated tissues of the leaves is therefore in all likelihood followed by an increase of sugar or other carbohydrate in the roots. The development of greater quantities of diastases seems to be the necessary response of the plant to the need of an increased hydrolysis of starch and subsequent transfer of the product to the storeplaces in the roots.

Ash Content and Water Absorbed Per Unit of Dry Matter.—Plants apparently affected with nitrogen starvation prove always to have a higher percentage of ash than normal plants, on the basis of dry matter. This phenomenon has been observed in Curly Leaf of beets, in the Mosaic disease of tobacco and in Curly Dwarf of potato. This increase in ash content, however, seems to follow definite rules. It was always found to be at its maximum in those plants where the response to the nitrate-reducing bacterial stimuli had been most positively answered, where the heaviest green color had developed in the leaves. This increase in ash is noticed in all parts of the plant—in the roots, in the stem and in the leaves. That the soil was not the factor determining this increase in ash was easily demonstrated when healthy and diseased beets growing side by side were tested. It was also proved that the transpiration coefficient of diseased dark green beet leaves was higher than that of normal leaves per unit of area. This seems to prove that diseased beets under the stimulus of the Curly Leaf disturbance absorb more water from the soil per unit of dry matter than healthy beets.

The Increase of Root System observed in such diseases as Curly Leaf of sugar beets, caused by nitrogen starvation, also seems to prove that the plant has answered to a stimulus for increased water absorption. This increase of water absorption per unit of dry matter would easily explain the increase in ash if no backflow of salts occurs in healthy plants.

Total Nitrogen in Plants Affected with Nitrogen-Reducing Bacteria.—Repeated investigations have demonstrated that the total nitrogen, in all diseased plants, calculated on the basis of ash is always inferior to the total nitrogen found in healthy plants. As for equal amounts of ash, about equal amounts of total nitrogen should be found in the plants, supposing no backflow occurs, if equal amounts of water are taken up per unit of dry matter, it is to be concluded that on finding an increase in ash and a decrease in total nitrogen, a great loss of the nitrogen element must have occurred. The presence of nitrate-reducing bacteria in the tissues and the presence of nitrites and volatile ammonia in the plant juices seem to explain how the waste of nitrogen has occurred. Experiments to establish the volatilization of ammonia from the leaves of diseased plants are now under way, and thus far are uniformly confirming that plants infected with nitrate-reducing bacteria lose ammonia in the air with their transpiration water. As much as from 0.15 to 0.19 g. loss of ammonia per square meter of leaf surface has been observed in ten hours' sunshine. Perfectly healthy plants (in this case we used potato seedlings) apparently give no ammonia in their transpiration water.

Morphological Adaptations.—The disease brought about by nitrate-reducing bacteria in all likelihood is a case of starvation of the protein-forming element. To this powerful stimulus a response for the conservation of the individual seems to occur in the biochemical and physiological functions of the affected cells. In some cases the stimulus is negative and the starvation of the cell seems to take place on account of subnutrition. Other cells are stimulated positively, palisade tissues are formed and the changes in morphology tend to a general economy of the nitrogen supply in the building of plant tissue. This economy is observed in the decrease in size of the secondary organs produced subsequently to infection. In beets, after they have been infected by the *Eutettix tenella*, the new leaves have shorter petioles in proportion to leaf surface; the plant has a tendency to roset formation, making the greatest amount of leaf surface with the smallest amount of material. Potatoes affected with Curly Dwarf have the internodes of their stems and the petioles of their leaves much reduced. A disease that seems to show all the morphological adaptations of nitrogen starvation is the little leaf of the vine[1] where the short internodes are a characteristic example of economy. Plant disturbances where abnormal economy is exhibited in tissues of secondary importance to the life of the plant may be suspected to be due to nitrogen starvation, if other factors such as water content of the soil, humidity of the air, exposure to sunlight and other factors of the habitat, such as insect injury, cannot explain the new adaptation.

Summary.

The biological, physiological, morphological phenomena in some plants infected with nitrate-reducing organisms, seem to work in harmony for the preservation and increase of the nitrogen content of the plant tissues. Preservation seems to be the aim and the object of the oxidizing enzymes which tend to neutralize the reducing forces of the bacteria. The morphological changes, such as the reduction in the size of secondary organs, that occur after infection, seem to economize the nitrogen and preserve it for the more essential parts of the plant, such as roots and leaves. The increased tendency to supply the plant with the necessary nitrates for the building up of the tissues seems to be demonstrated by the fact that the plant uses more water per unit of dry weight and also by the increase of the root system. The consequence of this is, in all likelihood, an increase in ash-content which, if the plant were normal, would mean most probably an increase in total nitrogen. The fact, however, that no matter how well the plant has succeeded in absorbing a surplus of water from the soil, a deficiency of total nitrogen is found in the tissues suggests the idea that the bacteria in reducing nitrates to nitrites and ammonia have wasted this necessary element bringing about a disease of nitrogen starvation.

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[1] Bioletti and Bonnet, *J. Agr. Res.*, 8 (1917).