

*Citation:*

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2. By a large number of bacteria, universally spread in nature, fats may be broken off anaërobically, oxydised aërobically, or if nitrates or nitrites are present be denitrified.

3. All these processes are caused by the secretion of "lipase" by microbes; glycerin and fatty acids separated by the action of this enzyme are then further converted by the organisms.

4. Several fat-splitting organisms produce two lipases,  $\alpha$ - and  $\beta$ -lipase; the former diffuses more rapidly than the latter and splits fat as well in an acid as in an alkaline medium;  $\beta$ -lipase is formed in an acid medium but does not decompose fat in it; it may however, become active again after neutralisation of the medium.

5. Lipase diffuses through water-free fat; but the diffusion velocity is very small.

6. The aërobic fat-splitting bacteria thrive well in culture media containing exclusively fat as source of carbon and ammonium chloratum as source of nitrogen.

7. To the already known fat-splitting bacteria we can add: *Bacillus nutriticus* (BIENSTOCK), a representative of the *mesentericus* group, *B. Stutzeri*, and *B. denitro fluorescens non-liquefaciens*.

8. Milk is a favourable medium for fat-splitting microbes.

9. In spontaneously infected milk, kept under circumstances usual in practice, growth and destruction of fat-splitting bacteria and lactic acid ferments occur about simultaneously; they are chiefly dependent on the acid production.

10. The injurious influence of fat-splitting microbes on the quality of dairy products is chiefly owing, besides to their lipolytic properties, to the formation of bitter tasting and badly smelling products from proteids and casein by these microbes.

This subject will be more elaborately treated in the "Centralblatt für Bakteriologie".

**Botany.** — "*On the cause of dimorphism in Oenothera nanella.*"

By H. H. ZEIJLSTRA FZN. (Communicated by Professor HUGO DE VRIES).

In 1905 I occupied myself with an investigation of the dimorphism of *Oenothera nanella* which in consequence of many other duties before my departure to India, could not be completed. Although I intend to continue this inquiry next year, I think it desirable already to communicate the following preliminary results.

In "die Mutationstheorie" DE VRIES in a description of the species of *Oenothera* arisen by mutation has made us acquainted with a

dwarf-form, which already occurred at the beginning of his experiments in 1888. It has since shown itself each time that a sufficiently large number of plants has been worked with.

DE VRIES named this plant *Oenothera Lamarckiana nanella*, or rather, briefly, *Oenothera nanella*, because, although the *nana*-characteristic can occur in species of the most diverse orders, yet this *Oenothera* with regard to its constancy on sowing can in no respect be distinguished from an elementary species.

Not only from *Oenothera Lamarckiana*, but also from *Oenothera laevifolia*, *O. scintillans*, *O. leptocarpa*, and from hybrids of *Oenothera Lamarckiana*, with the new species, *Oenothera nanella* appeared quite uniformly. On the average  $\frac{1}{2}$  % of the plants showed the dwarf-type.

The *nanella* can already be recognized as a seedling by the two first leaves, which are broad and have a short petiole. Then follow 2—4 leaves with long petioles, which resemble more the *Lamarckiana*-type; DE VRIES regards their appearance as an atavism.

As a rule *Oenothera nanella* is an annual. In this case the ascending stem grows out at once; in plants which will be biennials, several broad radical leaves with short petiole are developed, so that the plant hibernates with a dwarf rosette.

The fully grown stem has remarkably short internodes. This, in addition to the broad shape of the leaves, gives the plant a very squat appearance.

In spite of the small size of the plant, its flowers and fruits are hardly smaller than those of *Lamarckiana*. It sometimes happens that a plant bears these flowers when the shoot is no more than 10 cm. high.

This very characteristic form showed itself through mutation about 400 times in 80,000 plants. Its constancy was carefully and repeatedly tested.

In 1893 some *nanellas*, of which the ancestors had arisen in 1889 as mutants from *Oenothera laevifolia*, were pollinated with their own pollen. The seed yielded 440 plants, all of which bore the *nanella*-characteristic.

In 1895 twenty *nanellas* were treated in the same way, they themselves having occurred as mutants from *Lamarckiana*. They yielded 2463 descendants: all were *nanellas*.

The experiment was performed in 1896 with 18000 plants and the same favourable result was obtained. Three seedlings showed simultaneously with the *nanella* characteristics, those of *Oenothera oblonga* and one those of *O. elliptica*.

From these experiments the conclusion was drawn that *Oenothera nanella* when it appears through sowing is at once perfectly constant.

In 1905, among the *nanellas*, some plants were found, which, although dwarfs, differed from the form just described by possessing elongated internodes and narrow petiolated leaves. At the same time there were plants which, in addition to these elongated shoots, bore one or more compact branches.

In comparison with the new form, the first has an unmistakably malformed appearance. The question therefore rises to the forefront whether possibly the dimorphism of stem and leaves must be attributed to the influence of organisms in the body of the plants. An aberration which often occurs in the floral buds of *Oenothera nanella*, and the course of which is accurately described in "die Mutationstheorie", gives powerful support to a belief in a parasite, which cannot kill the plant, but which nevertheless greatly hinders its development.

"Auf einjährigen Exemplaren sind die Blüten vielfach unvollständiger Ausbildung ausgesetzt. Aber meist nur eine oder wenige Blumen pro Pflanze. Bisweilen fehlt der Blütenstaub oder er ist nur in spärlicher Menge entwickelt; ziemlich oft können die Narben sich nicht öffnen und bleiben somit zu einem vierseitig-conischen Gebilde zusammengefügt. Dieses Gebilde ist oft nur sehr klein und so schwach, dass es vor der Bestäubung sich schwärzt und vertrocknet. Oder der Griffel ist zu kurz, bisweilen kaum aus der Blütenröhre hervorragend.

"Sehr auffallend ist der schiefe Stand der Blütenknospen auf den Kelchröhren. Die Kelchzipfel mitsamt der Krone sind dann an ihrem Grunde gebogen; im ersteren Fall derart, dass sie senkrecht auf der Röhre stehen. Das Öffnen des Kelches ist dadurch erschwert und geht in abnormaler, oft mangelhafter Weise vor sich. Die Blumenblätter entfalten sich unvollständig und die Geschlechtsteile sind meist mehr oder weniger steril.

"Alle diese Abweichungen sieht man namentlich an den untersten Blüten der Traube, zumal wenn die Pflanze bereits bei einer Stengelhöhe von 10—15 cm. zu blühen anfängt. Aber auch bei der gewöhnlichen *Lamarckiana* misslingen oft mehrere von den untersten Blüten. *Wächst die Nanella durch diese Periode hindurch und wird sie dabei auffallend kräftiger, so bildet sich nach einer kürzeren oder längeren blütenlosen Zwischenstrecke in der Traube meist eine volle und schöne Krone grosser Blumen aus. Diese erhebt sich auf dem dünnen, wenig beblätterten blütenlosen Stengelteil hoch über die*

*untere Hälfte der Inflorescenz empor. Aber bei Weitem nicht alle Individuen werden hinreichend stark, um solches zu erreichen.*

“Handelt es sich somit darum, die Zwerge in möglichst schöner Entwicklung und voller Blütenpracht zu cultiviren, so empfiehlt es sich, sie stets durch späte Aussaat zu zweijährigen Exemplaren zu erziehen”.<sup>1)</sup>

It can be seen from the italicised portion of the above quotation that, considered objectively, the squat-shaped *Oenothera nanella* behaves as if in its development it has a resistance to overcome. If it succeeds in this, a normal apex is formed on the inflorescence.

If the stem of a malformed *nanella* is cut through, one finds in part of the section the elements in groups filled with a black mass. This is most plainly shown in the elements of the secondary wood; these are often in a transverse section wholly black internally. This filling is found not only in the wood fibres, but also in the medullary ray cells; the wood-vessels are not as a rule wholly filled, but a similar substance lies here along the wall in the shape of a segment. The same applies to the cells of the pith, the cortex-parenchyma, the collenchyma and the epidermis. Nevertheless wholly filled cells can also be found in the cortex-parenchyma.

This phenomenon appears in the same way in the root of *Oenothera nanella*.

On looking at a section under a high power the black mass resolves itself into a large number of black spots which are evidently imbedded in a gelatinous matrix. With sufficiently strong magnification, especially where the mass of cells which have been cut through, has spread more or less over the preparation, places are readily found, where the black dots lie so far apart that they can be made out separately.

Every individual is surrounded by a lighter zone. The individuals are partly arranged in pairs or in fours, and are not of equally dark colour; the greater number are however opaque in the strongest illumination.

The external characteristics leave no doubt that the organism seen in the tissue of *Oenothera nanella* is a bacterium of the genus *Micrococcus*. It does not differ from the *Micrococcus* in tooth-mucilage either in shape or size, though it is as a rule darker in colour.

<sup>1)</sup> Die Mutationstheorie, Bd. I. p. 267 and 268.

In the English edition is added Vol. I. p. 376: “These and other malformations of the dwarfs are often due to a disease and as such to a large degree dependent on outer circumstances; (note of 1908)”.

This note is based on the investigation here published.

It would appear that the *Micrococcus* forms a zoogloea, which fills the whole fibre or cell. There is, as a rule, in the preparations, a space between the cell-wall and its contents, though the latter has often so exactly the same polygonal outline as the cell, that there can be little doubt that the contents only retracted from the wall in the making of the preparation.

Other organisms than the *Micrococcus* have not up to the present been found.

Although therefore on the one hand *Oenothera nanella* with its contracted shoots has the appearance of a diseased plant, and on the other hand there is found in its tissues an organism, that may very well be the cause of the disease proof remains to be given, that *Micrococcus* is indeed the agent producing the malformation. At the same time the question waits for a further answer, how it can be explained that on the origin by mutation of *Oenothera nanella* the diseased form always occurred and that the first normal plant was only obtained many years later.

The appended figure of an *Oenothera nanella* with two kinds of shoots was prepared from a photograph made on the 27<sup>th</sup> of Sept. 1905 in the Botanic Garden at Amsterdam from a fully grown plant.

The shoot on the extreme right was 33 c.m. high. In the middle a diseased shoot is seen, with closely packed leaves. The top of this shoot is added from another specimen drawn from nature, in order to show the difference between normal and abnormal flower buds.

In the diseased shoot, the calyx-tube is curved, so that the calyx and the corolla make almost a right angle with the axis of the ovary, whilst in the normal shoot the different parts of the floral bud lie in one straight line.

The diseased shoots have much shorter and thicker internodes than the healthy shoots.

The leaves of the true *Oenothera nanella* completely resemble those of *O. Lamarckiana*, except for the size. Those of the aberrant shoots are on the contrary provided with very short brittle petioles and broad short laminae.

Healthy shoots have up to the present only been observed exceptionally. The reason is partly to be found in the fact that the normal *nanella* wholly resembles a dwarf *Lamarckiana*, and in the method employed, of examination as young seedlings, can be mistaken for the latter.

On the other hand we must take into account, that attempts to obtain seed from healthy shoots have been unsuccessful. These shoots generally appear later on the plant and bloom too late for their



Fig. 1.

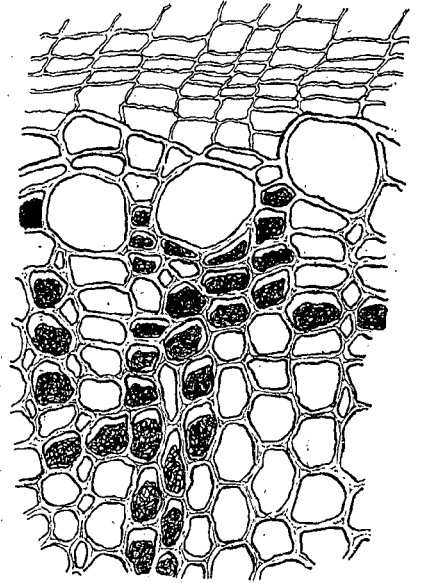


Fig. 2.

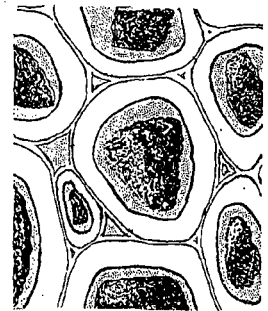


Fig. 3.

fruits to mature. The *nanellas* obtained from seed have therefore all had diseased parents, and the possibility is not excluded, that they may have received from their parents, if not the *Micrococcus* itself, yet the susceptibility to the attacks of this organism.

As long as we have not succeeded in growing perfectly healthy *nanellas*, we cannot determine by infection experiments whether the *Micrococcus* is indeed the cause of the pathological phenomenon.

## EXPLANATION OF PLATE.

Fig. 1. *Oenothera nanella*, dimorphic plant ( $\times \frac{2}{3}$ ).

Fig. 2. Transverse section through the stem, near the cambium ( $\times 555$ ).

Fig. 3. Transverse section through the stem ; a few wood fibres near the primary wood ( $\times 1730$ ).

**Chemistry.** — “*The synthesis of as. heptachloropropane from tetrachloro-ethylene and chloroform with the co-operation of aluminium chloride.*” By Prof. J. BOESEKEN and Dr. H. J. PRINS. (Communicated by Prof. S. HOOGEWERFF).

In a previous research by one of us (Recueil XXIX p. 109 (1910)) it was found that dichloroacetyl chloride is decomposed by aluminium chloride in two directions. Firstly it decomposed into chloroform and carbon monoxide; secondly, besides carbon monoxide and hydrochloric acid a beautifully crystallised product was obtained melting at  $32^{\circ}$  to which, provisionally, the composition  $C_2Cl_6$  was assigned. In the meanwhile we have succeeded in conducting the decomposition in such a manner that a large quantity of this substance was obtained, thus enabling us to study some of its decomposition products. From this research it follows that the substance is identical with the as. heptachloropropane obtained by FRITSCH (Ann. **297** [1897] pg. 312) from pentachloroacetone and phosphorus pentachloride as shown by the subjoined table.

While we were engaged in studying the question as to how heptachloropropane was formed from dichloroacetylchloride, one of us (Pr.) succeeded in obtaining a very good yield of this chloride by direct addition of chloroform to tetrachloroethylene under the influence of aluminium chloride. If these chlorides are boiled together for some time in a reflux apparatus, the ethylene perchloride is almost entirely converted into heptachloropropane.

It should be observed, that neither chloroform, nor ethylene tetrachloride, nor the end product  $C_2Cl_6H$  combine with the aluminium