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Gummosis of *Prunus Japonica*, Thunb.

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No. 144.]

DECEMBER.

[1898.

DCXXXI.—GUMMOSIS OF PRUNUS JAPONICA,
THUNB.

(*With Plate.*)

During the past two years a considerable number of examples of the beautiful flowering shrub, *Prunus japonica*, Thunb., growing in Kew Gardens, have been killed or much disfigured by a parasitic fungus belonging to the genus *Cladosporium*.

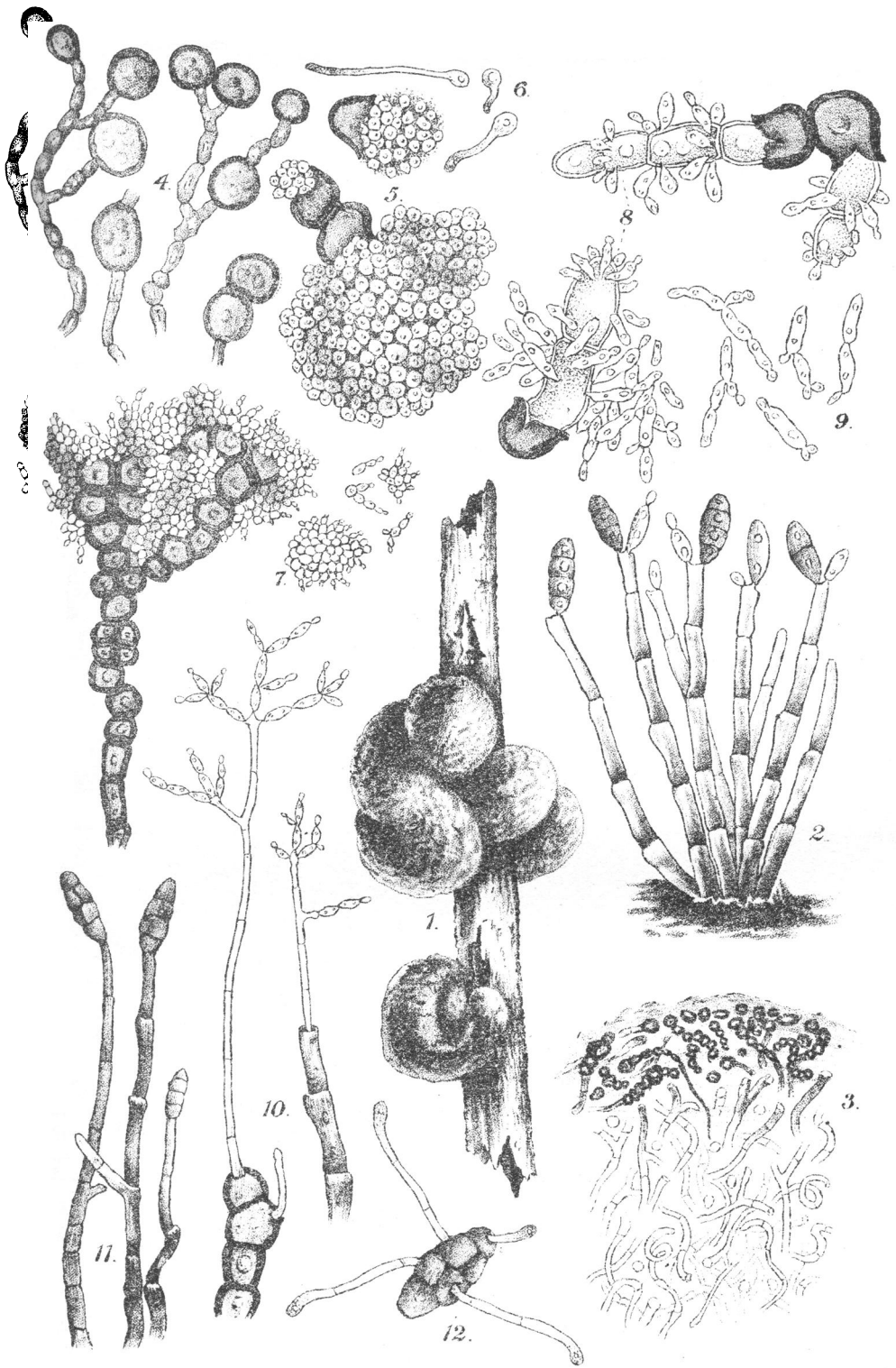
The disease is first indicated by the appearance of tear-like drops of almost colourless gum on the branches. These drops are sometimes solitary, in other instances numerous and more or less crowded.

The drops continue to increase in size for some time, often forming masses varying in size from a marble to that of a walnut, and when two or more originally distinct drops coalesce the resulting mass usually becomes irregularly nodulose and contorted.

During damp or rainy weather the masses of gum are quite soft and gelatinous, with just sufficient consistency to hold together, or sometimes during a heavy rain drip away by degrees. In very dry, warm weather the mass shrinks very considerably in size and becomes horny, expanding again when moistened.

As previously stated the mass of gum is almost colourless at first, becoming steel-grey as it increases in size, and finally black. The black colour is however confined to a surface layer, the central portion remaining colourless. This is most evident on cutting through a mass that has been hardened in spirit.

In the end these outflows of gum are always washed to the ground by rain, where they eventually dissolve and disappear.



Gummosis of *Prunus japonica* , Thunb

When the black masses are removed irregular canker-like wounds, sometimes extending to the pith, are present on the branches ; if such wounds are numerous, and occur on different sides, the branch dies at once ; whereas if the wounds are confined to one side of the branch, most frequently the under side, it may continue a feeble existence until the following season, when it almost invariably succumbs, owing to the formation of new disease spots.

The fungus is a wound-parasite gaining access to the living tissues through small wounds in the bark, broken branchlets, and more especially at those points where leaf-buds or flower-buds have been broken off, and as birds remove these buds rather freely, probably in searching for insect larvæ, the opportunity for infection is ample.

The following account of the life-history of the fungus is founded on observance of the sequence of development and microscopic examination of material resulting from artificial inoculation of previously healthy specimens.

Whether inoculation is effected by means of spores or conidia, the first product is invariably a *Cladosporium*, which morphologically appears to be in exact accordance with the ubiquitous species, *C. epiphyllum*, Fries, although physiologically, the two are widely separated ; neither am I aware that any known species of *Cladosporium* has been described capable of promoting the disease known as gummosis, as is the case with the form under consideration.

Inoculation was effected by placing spores in a small wound made in the bark, or on the surface of the wound caused by breaking off a leaf-bud. Oiled silk was immediately tied round the branch at the points inoculated, and allowed to remain for ten days, as a preventive against complications that might possibly have arisen from undesired inoculation by foreign, floating spores.

The fasciculate sporophores appeared at the points of inoculation at periods varying from sixteen to twenty days after infection took place, and remained for about another fortnight, all the while producing spores, after which they gradually disappeared ; their position becoming occupied by a small drop of gum.

If a section is taken through a disease spot at this stage, it will be seen that the hyaline, slender, septate, and much branched mycelium has extended to a distance of about 2mm. on all sides, from the point of infection, and has also passed down to the cambium. Towards the centre of the diseased spot the tissue is completely disintegrated, the transformed material oozing to the surface as the drop of gum already alluded to. Towards the periphery of the infested portion of tissue, the slender hyphæ can be seen in the cells, having perforated the wall, probably by means of a ferment secreted by the tips of the hyphæ. There is very little discolouration of the tissue, just a tinge of brown in the contents of cells recently attacked.

Returning to the small drop of exuded gum—the increase in size of which may be taken as an index of the activity of the

hyphæ of the fungus in disintegrating and converting into a gummy substance the tissues of the host—which usually retains a more or less spherical form and a hyaline or very pale amber colour until about the size of a pea, when it becomes tinged with grey on the surface. If a globule at this stage of development is hardened in spirit, and afterwards examined in section, its substance will be seen to be permeated by innumerable, slender, hyaline, septate hyphæ, agreeing in all essentials with the hyphæ of the fungus present in the tissues of the host. These are in reality an extension of the hyphæ of the parasite into the external mass of gum for the purpose of producing secondary reproductive bodies.

The hyphæ radiate from the base of the globule of gum, and continue to grow until within a very short distance from its periphery, but never protrude beyond the matrix.

At the stage indicated, the grey tinge observable on the surface of the mass of gum is due to the formation of larger, thick-walled cells, arranged in a moniliform manner, and of a pale smoky-grey colour, at the tips of those branches of hyphæ situated nearest the outside of the gum matrix.

The mass of extruded gum continues to increase in size, and the included hyphæ keep pace with this extension, always keeping just within the circumference. At the same time the terminal moniliform strings increase in number, size, and depth of colour, imparting to the mass of gum the black colour indicating what may be termed the period of maturity.

If during the period of formation of the mass of gum, the weather has been continuously moist, the included hyphæ radiate in comparatively straight lines from base to circumference of the mass. On the other hand, if, after a period of damp weather, the mass of gum loses its moisture and contracts, owing to heat, the hyphæ assume a spiral or cork-screw form as the means of accommodating themselves to the altered conditions, and when the mass again expands after being moistened, the coils of hyphæ do not unfold, the tips resuming growth in a straight line so long as conditions are favourable for so doing; consequently when during the formation of a gum-mass, spells of rain and dry hot weather have alternated, this is indicated by the alternation of spiral and straight zones of hyphæ in its interior.

If, after hardening in spirit, a section of a black mass of gum is examined, the internal portion is seen to be crowded with delicate hyaline hyphæ, all trending towards the circumference, as already explained, and possessing no feature of special interest, but as we approach the periphery of the mass, these slender hyphæ become tinged with grey, and gradually widen out into the strings of dark-coloured, thick-walled cells previously mentioned.

These terminal chains of dark cells are very irregular in structure, sometimes consisting of strings of elliptic or sausage-shaped cells, much constricted at the septa, frequently branched, and bearing at the tip of each branch, sometimes also intercalary, very large, subglobose, thick-walled cells. Other strings consist of large quadrate or irregularly angular cells, which at times coalesce to form solid masses of tissue resembling micro-sclerotia.

A black mass of gum that had been collected and allowed to dry and contract for several days, was then hardened in spirit, and on examination it was found that most of the large cells described above had germinated, and produced innumerable very minute hyaline sporules, many of which were reproducing themselves by a process of budding, *Torula*- or *Saccharomyces*-fashion.

After this discovery another mass of black gum was collected and allowed to become perfectly dry and horny. After remaining in this dry condition for several weeks, a portion of the material was examined, and the same process of germination and reproduction of sporules by gemmation was seen to have occurred. After another interval of some weeks, during which the material remained perfectly dry, a fragment was placed in a hanging drop of sterilized water, and reproduction of sporules by gemmation was soon as active as if the process had never been interrupted, the matrix of gum presumably serving as nutrient material.

Germination of the large brown cells, and continued reproduction of the sporules by gemmation in a dense matrix of gum comparatively devoid of air, suggested the idea that under certain conditions the fungus could exist as an anaerobic organism. For the purpose of testing the validity of this idea, two flasks of nutritive solution, consisting of thoroughly sterilised colourless masses of the gum exuded during the early stage of the disease and dissolved in water, were prepared according to Kitasato's method, which practically consists in excluding the air by a layer of paraffin poured on the surface of the nutrient solution.

Seven days after inoculation the contents of the flasks were turbid, and microscopic examination showed this turbidity to be due to the presence of myriads of sporules, mostly arranged in chains of two to four cells.

When removed to hanging drops of the same nutrient solution those anaerobic sporules refused to grow, and inoculations of the host plant with them produced no sign of the disease.

Grown in hanging drops or in flasks as aerobic organisms, the large brown cells gave origin to a very stout, hyaline mycelium composed of two to four cells, constricted at the septa. When full grown, these hyaline cells give origin from every portion of their surface, but most abundantly near the septa, to numerous small elliptical sporules, which generally form chains consisting of two or three cells by acropetal growth. The sporules soon fall away from the parent mycelium, and continue to reproduce themselves by gemmation, soon rendering the nutrient solution turbid by their immense numbers.

The product of germination just described corresponds to what has been described as *Dematium pullulans*, well known as a phase in the life-cycle of *Cladosporium*.

The *Dematium* sporules or conidia readily produce the disease when placed on a wounded surface of the host.

Fragments of the sporophores of *Cladosporium*, when placed in water, also give origin to the *Dematium* form of reproduction.

PREVENTIVE MEASURES.

The disease, which spreads rapidly, was checked by spraying with a solution of potassium sulphide.

Diseased branches should be removed, as the mycelium is probably perennial in the tissues, and would consequently give origin to the disease the following season.

Quicklime should be placed on the soil under diseased plants for the purpose of destroying the sporules produced from the fallen masses of gum.

SUMMARY.

Gummosis of *Prunus japonica*, Thunb., is caused by a species *Cladosporium*, morphologically indistinguishable from *Cladosporium epiphyllum*.

The masses of extruded gum are permeated with the hyphæ of the *Cladosporium*, which bear large, thick-walled, dark brown cells, or masses of cells resembling micro-sclerotia at their tips, situated just within the periphery of the mass of gum, and imparting to it a black colour.

These large cells and micro-sclerotia, when caused to germinate in the absence of air, give origin to myriads of very minute sporules, which reproduce themselves by gemmation; under these conditions hyphæ are not formed.

Grown in a nutrient solution in the presence of air, the form of reproduction once known as *Dematium pullulans* is produced.

Inoculation with the *Dematium* sporules produces the disease. No results were obtained from infections with the sporules of the anaerobic condition.

Bacteria were entirely absent from the masses of gum during every phase of development.

DESCRIPTION OF THE FIGURES.

- Fig. 1. Portion of a branch of *Prunus japonica*, Thunb., bearing two masses of gum; nat. size.
- „ 2. *Cladosporium*-form of fruit; $\times 400$.
- „ 3. Section of a portion of the periphery of a black gum-mass, showing the hyphæ of the *Cladosporium*; $\times 80$.
- „ 4. Dark coloured tips of hyphæ from the periphery of a gum-mass, bearing large, thick-walled, brown cells; $\times 400$.
- „ 5. Large thick-walled, brown cells germinating in a nutrient solution in the absence of air, and producing yeast-like cells, which reproduce themselves by gemmation; $\times 400$.
- „ 6. Stray cells which are emitting a germ-tube, seen in the material described in 5.

Fig. 7. Micro-sclerotia germinating under conditions similar to those described under 5, and producing similar sporules; $\times 400$.

- „ 8. Large brown, thick-walled cells germinating in a nutrient solution with free access of air, and producing the form of fruit known as *Dematium pullulans*; $\times 400$.
- „ 9. Sporules of the *Dematium* increasing by gemmation; $\times 400$.
- „ 10. Fragments of sporophores of *Cladosporium* producing a slender form of *Dematium pullulans*; $\times 400$.
- „ 11. A form of *Macrosporium* often appearing on old canker-spots caused by the *Cladosporium*. No genetic connection between the two could be established; $\times 400$.
- „ 12. Spore of the *Macrosporium* germinating; $\times 750$.

G. MASSEE.

DCXXXII.—THE ADVANCES MADE IN AGRICULTURAL CHEMISTRY DURING THE LAST TWENTY-FIVE YEARS.

An important address has been recently delivered by Professor MAERCKER, of Halle, to the German Chemical Society (*Ber.* 1897, p. 464), summarising the advances which have been made in agricultural chemistry during the last twenty-five years. Professor Maercker pointed out that the term Agricultural Chemistry meant more at the present time than the mere application of chemistry to agriculture, as shown by the fact that the agricultural chemist, in his efforts to assist the farmer, was often more concerned with the biological sciences than with chemistry; while, in addition to his purely scientific work, he was required to take account of economic questions of the day possessing special interest to agriculturists. The following account of the most important parts of the address is given under the following heads:—I. Plant-food; II. Soils and Manures; III. Artificial Selection. It is reproduced here by the kind permission of the Editor of the *Imperial Institute Journal*.

I. PLANT-FOOD.

In supplying nourishment to plants we must know what substances are necessary, and in what form and quantity they should be provided. Little progress was made in our knowledge of the subject till the quite recent introduction of the method of water-cultures of Sachs, Knoop, and Nobbe and the method of sand-cultures of Hellriegel permitted of the conduct of experiments in pure media, and thus rendered it possible to ascertain not only what substances are essential for plant life, but also the