

The 'Spot' Disease of Orchids.

BY

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THE disease known as 'spot,' which appears under the form of brown spots or blotches on the living leaves of orchids, is unfortunately too familiar to cultivators and admirers of these plants, and although the health of the plant is not materially affected, except when the spots are unusually numerous, nevertheless the unsightly blotches on the leaves detract greatly from a full appreciation of the beauty of orchids when in bloom.

The disease first appears under the form of minute pale spots, one to two millimetres in diameter, on the upper surface of the leaf, which vary considerably in number and arrangement, being in some instances numerous and crowded, in others few in number and scattered. Every portion of the leaf is equally susceptible to the disease, and the fact that very young leaves of diseased plants frequently show 'spot' has been considered by some as strong evidence in favour of the disease being due to some parasitic organism; this, however, is not the true explanation, the disease proving conclusively to be of a non-parasitic nature, and with proper

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precautions to be entirely under the control of the cultivator. My previous note¹ announcing that 'spot' was due to the presence of an organism called *Plasmodiophora orchidis*, was based upon the absolute agreement with the microscopic details of two diseases of vine leaves described by Viala and Sauvageau², and attributed to the presence of two organisms called *Plasmodiophora vitis* and *Plasmodiophora californica* respectively. Returning to the orchid disease; the minute pale spots already alluded to, which, it may be remarked, are not at all conspicuous, and are likely to escape observation unless specially looked for, soon assume a pale brown colour and gradually increase in size, retaining an irregularly circular outline, until they attain a diameter varying from four to eight millimetres. When the spots are numerous and near together they frequently run into each other, forming irregular blotches of variable size and form. As the disease progresses the spots become darker in colour, and owing to the collapse of diseased cells beneath the epidermis, the surface of the spot becomes depressed below the level of the surface of the leaf (Fig. 1). In many instances the disease passes completely through the leaf, forming a corresponding brown depressed spot on the under surface.

Microscopic examination shows the following details. The first indication of disorganization is plasmolysis, followed by the complete disappearance of the chloroplasts from the palisade-cells of the leaf, hence the pale colour of the spots during the first stage of the disease. This is immediately followed by the appearance of a highly refringent, hyaline, oleaginous looking sphere in each cell. During the early period of formation, the centre of the sphere presents a granular appearance, and when broken up and treated with a solution of iodine, the granules prove to be minute starch grains, which were liberated from the disintegrated chlorophyll grains, and engulfed during the formation of the sphere

¹ Annals of Bot., Vol. ix, p. 170 (1895).

² La Brunissure et la Maladie de Californie. Journ. de Bot., Tom. vi, pp. 355 and 378, Pl. XII (1892).

(Fig. 2 *b*). Within three days from the first appearance of the spheres, the enclosed starch grains have become completely dissolved and their substance added to the common mass. If at this stage a section through a diseased spot be placed in water and examined under the microscope, the spheres will be seen to undergo vacuolation, gradually changing from the previous solid condition into hollow vesicles, the walls of which become irregularly perforated or reticulated, the configuration of the network slowly and constantly changing after the fashion of the movements presented by the vegetative phase of *Plasmodiophora Brassicae*, but differing in the movements being of a purely physical nature, the perfectly homogeneous membrane becoming thinner in proportion as the vesicle increases in size, and in constantly retaining a rounded, even contour. Increase of size usually continues until the vesicle fills the cell in which it is contained (Figs. 2 *c*, and 4). Vacuolation takes place exactly as stated above when sections are placed in a one per cent. solution of osmic acid instead of water, which, taken alone, is sufficient proof that the spheres in question are not of an amoeboid nature. In some instances, instead of one, several spheres are formed in a cell, each undergoing vacuolation, but remaining comparatively small in size.

The composition of the spheres is proved by the action of reagents to be complex, and although I have sometimes spoken of them as tannin-vesicles, it must be clearly understood that I do not intend to convey the idea that they consist entirely of tannin, although agreeing in many respects with the structures called tannin-vesicles by Klercker¹.

That tannin is present is shown by the following reactions. Potassium bichromate produces a bright brown precipitate, insoluble in water; an aqueous solution of cupric acetate causes a dingy brown colour, which changes to green when subsequently treated with an aqueous solution of ferrous sulphate; a 1 per cent. solution of osmic acid blackens the spheres, but as previously stated, does not prevent vacuolation. Finally,

¹ Studien über die Gerbstoffvakuolen. Tübinger Inaugur.-Dissert. 1888.

the accumulation of methylene blue by tannin-bearing cells, as pointed out by Pfeffer¹, held good, the vesicles becoming stained deep blue after remaining in an exceedingly dilute aqueous solution of methylene blue for twenty-four hours; iodine-green may be substituted for methylene blue with good results. All the above reactions are most decided before vacuolation takes place; in fact, when the vesicles are fully distended, but little colouration is produced by any of the reagents mentioned. On the other hand, the presence of proteids in the spheres is suggested by the rapid staining of the mass, on the application of such reagents as eosin, carmine, iodine, &c. Carbohydrates are also in all probability present.

The spheres originate in the cell-sap, and their presence depends entirely on plasmolysis of the cells, which occurs during the earliest phase of the disease.

Contemporaneously with the formation of the tannin-vesicles the cytoplasm becomes turbid, the primordial utricle at the same time becoming tinged brown, and undergoing important changes. In some cases the inner surface of the latter becomes uniformly covered with minute, spherical masses, and in this condition resembles, superficially, cells filled with the spores of a *Plasmodiophora*; here, however, the resemblance ends, as the minute spheres are found to form only a single layer lining the primordial utricle, and not completely filling the cell, as in *Plasmodiophora*; furthermore, reagents show that the spheres consist of tannin, and not protoplasm (Fig. 7 *b*). In other cases the inside of the epiplasm, and sometimes also the cell-wall—which, along with the other parts, undergoes disintegration—is covered with tubes or variously branched, very slender rods of a brown colour. Usually, however, the epiplasm or primordial utricle becomes entirely disorganized, drops of tannin accumulate at various points in its substance, accompanied in many instances by minute crystal-like bodies. These eventually disappear,

¹ Ueber Aufnahme von Anilinfarben in lebenden Zellen, Unters. a. d. bot. Instit. zu Tübingen, Bd. II, p. 179.

leaving holes in the membrane, which, along with others previously present, produce an irregular reticulation, the whole being of a brown colour (Fig. 7 *a*). The nucleus of the cell frequently remains unchanged throughout the entire cycle of disease, as shown in Fig. 7 *a*, *x*.

In Viala and Sauvageau's account of the vine disease previously alluded to, vacuolated tannin-vesicles and the reticulated primordial utricle have been respectively interpreted as constituting the vegetative phase of their supposed *Plasmiodiophora vitis*; Figs. 2 and 4 illustrating their monograph representing the former, and Fig. 1 the latter.

The investigation of the disease under consideration was at first pursued along lines suggested by the preconceived idea that a fungus was the cause of the mischief, and it was only after numerous and varied experiments had failed to demonstrate the existence of the hypothetical fungus, that a search was made for bacteria, but with a like result. Finally, failing to induce the disease in healthy plants by inoculation with the expressed juice from diseased spots, even when introduced under the epidermis, thus proving the absence of an enzyme or organic ferment, which would have been due to the presence of fungi or bacteria, this was accepted as corroborative evidence of the absence of these organisms.

At this stage Mr. W. Watson, Assistant Curator, Royal Gardens, Kew, whom I take this opportunity of thanking for numerous practical hints during this investigation, suggested a sudden chilling of the plants as a probable cause of the disease. Acting on this suggestion, the following somewhat drastic experiment was undertaken.

A young healthy plant of *Habenaria Susannae*, R. Br., perfectly free from 'spot,' and which up to the date of the experiment had been growing in a house having a temperature ranging between 75 and 80° F., was selected for experiment. Minute particles of ice were placed at intervals on the uninjured epidermis of the upper surface of the leaves, the plant—along with the pot in which it grew—was then placed in a sink and covered with a bell-jar, and cold water

from a tap allowed to flow over the bell-jar for twelve hours, during which time the temperature inside the jar ranged between 41 and 45° F. Twenty-four hours after the experiment, the points on the surface of the leaves originally covered by particles of ice were pale in colour, and on examination under the microscope, plasmolysis of the cells of the palisade-tissue, and degeneration of the chloroplasts were found to have taken place. The remaining spots were examined at intervals, and within four days every phase of the disease was observed, agreeing in every respect with the features already described.

The foregoing experiment showed that a sudden fall of 30° of temperature could not induce 'spot' on the dry surface of the leaf, but only at those points where it had been moistened by the melted ice. That the chill caused by contact with the ice itself was not necessary for the formation of 'spot' was proved by a second experiment with the same species of plant, all the conditions being as nearly as possible counterparts of those in the first experiment, excepting that minute drops of water at a temperature of 45° F. were placed on the leaves instead of particles of ice. A diseased spot appeared at each point previously occupied by a drop of water, and showed all the microscopic characteristics of true 'spot.' Numerous additional experiments, with the object of determining the minimum depression of temperature necessary to produce the disease, showed that the formation of 'spot' could not be induced by a fall of less than 9° F. from the average temperature in which the plant had been previously growing. One other point in regard to temperature was clearly demonstrated by the experiments, viz. that plants which had previously grown in a high temperature became diseased at a much smaller reduction of temperature than plants previously accustomed to a comparatively low temperature.

In conducting the experiments described above, irregularity in the appearance of the spots in different specimens of the same species, even when conducted under precisely similar

conditions as to temperature, showed that some other undetermined factor exercised an influence. After repeated experiments this proved to be the relative amount of moisture present in the plant. After a pseudo-bulb with its accompanying leaf had been removed from a plant and allowed to remain for three days in a dry place, it was found impossible to produce spot by the method mentioned above, whereas with a similar specimen removed from the same plant, and having the pseudo-bulb placed in water at once, fully developed 'spot' could be produced in four days. Similar results were obtained when experiments were made with entire plants; those copiously supplied with water at the root, and grown in a high temperature, 'spotting' readily; whereas plants in a resting condition, scantily supplied with water and kept in a low temperature, usually resist all attempts to produce 'spot' artificially.

It may be mentioned that, other conditions being equal, 'spot' can be produced with the greatest certainty, and in the shortest amount of time, when the experiment is conducted in an atmosphere saturated with moisture. This agrees with the experience of gardeners, who state that 'spot' is most prevalent in foggy weather.

Experiments show that 'brunissure,' or browning of vine leaves, when the plants are grown in the open air, can be caused by the following combination of meteoric conditions. A copious deposition of dew and rapid fall of temperature, following heavy rain. Similar conditions produce the disease in the leaves of tomatoes, which has been described by Abbey¹ as due to an organism named by him *Plasmiodiophora tomati*.

SUMMARY.

The orchid disease known as 'spot' is of non-parasitic origin; the initial cause being the presence of minute drops of water on the surface of the leaves at a time when

¹ The 'drooping' disease in Tomatoes. Journ. Hort., Ser. 3, Vol. xxx, p. 360 (April 25, 1895).

the temperature is exceptionally low, and the roots copiously supplied with water.

The effect of the chill produced by the drops of water under the above-mentioned conditions, is to cause plasmolysis of the cells of the leaf underlying the drops ; this is followed by the precipitation of tannin and other substances, and eventually the complete disintegration of the cells.

'Spot' in the broadest sense of the term, which would include the effects of exceptional meteoric conditions on the living parts of plants, more especially the leaves, when growing in a state of nature, is, in the case of cultivated orchids, mainly if not entirely caused by the three following conditions :— (1) too high a temperature ; (2) too much water, and not sufficient air in contact with the roots ; (3) watering or spraying with a falling instead of a rising temperature.

EXPLANATION OF FIGURES IN PLATE XV.

Illustrating Mr. Massee's paper on the 'spot' disease of orchids.

Fig. 1. Leaf of *Eria rosea* showing the appearance produced by the 'spot' disease. Nat. size.

Fig. 2. Section through portion of a diseased spot on the leaf of *Eria rosea*. The cells at the periphery of the diseased spot have the protoplasm only slightly tinged brown, and the tannin-vesicles are still small and not at all vacuolated, as at *a* ; at *b*, the tannin-vesicles are larger, granular at the centre, and appear as if radially striate, due to the commencement of a fine-meshed vacuolation. At *c*, the tannin-vesicles have reached the extreme stage of vacuolation, their substance being reduced to a thin film which soon collapses. In the preparation, owing to being mounted in water containing only a trace of glycerine, the brown protoplasmic contents of the cells, which were plasmolysed and free from the walls, have in many instances become again expanded so as to fill the cells. The tannin-vesicles are stained with a saturated aqueous solution of potassium bichromate. $\times 450$ diam.

Fig. 3. Tannin-vesicles in various stages of development in leaf of *Eria rosea*. The other cell-contents are omitted. $\times 450$.

Fig. 4. Tannin-vesicles in cells of leaf of *Masdevallia Reichenbachiana*; at *a* the vesicles are small and not much vacuolated, hence take the stain readily; at *b* the vesicles have become much vacuolated, and nearly fill the cell, hence the membrane is very thin and does not stain, or very slightly. The other cell-contents are not shown. $\times 450$.

Fig. 5. A cell from a diseased 'spot' from the leaf of *Eria rosea* showing the primordial utricle or epiplasm containing drops of tannin, stained with potassium bichromate; the small angular dark patches consist of some substance soluble in water, and when these and the tannin drops disappear, the perforated epiplasm has been mistaken for the plasmodium of *Plasmodiophora*. $\times 450$.

Fig. 6. Section through portion of a 'spot' in the leaf of *Bulbophyllum Carey-anum* showing the primordial utricle presenting the appearance of the plasmodium of *Plasmodiophora*. $\times 450$.

Fig. 7. Section through portion of a 'spot' in leaf of *Eria rosea*; at *a* the epiplasm has contracted, become brown, and presents the appearance that has been mistaken for the plasmodium of *Plasmodiophora*; *x*, the nucleus of the cell. At *b*, the epiplasm is completely covered with minute, uniform globules of tannin, which superficially resemble the spores of *Plasmodiophora*. $\times 450$.